The impact of female sex hormones on concussion

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DOCTOR OF PHILOSOPHY

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Abstract

The thesis aim was to understand the influence of female sex hormones on concussion via investigating: 1) How much does baseline symptomology change across the menstrual cycle in female athletes?; 2) Do salivary concussion biomarkers change across the menstrual cycle for females in the absence of injury?; 3) Does the hormone profile at point of injury predict prolonged recovery from a concussion?; 4) Does hormone profile at point of injury affect salivary concussion biomarkers in females? The thesis included a systematic review, a feasibility study, a cross-sectional study, a single-case study and a prospective cohort study. The systematic review and meta-analysis (chapter 2) highlighted sex and gender differences in sports-related injuries including a higher incidence of concussion in female athletes. Chapters 3 to 8 aimed to better understand concussion in females. In assessing concussion knowledge and attitudes (Chapter 3) via an online survey, over 30% of male and female football players indicated they would continue playing whilst experiencing symptoms of a concussion. This suggested that the observed gender difference in concussion incidence is unlikely to be only due to a reporting bias in females. Investigating the magnitude of change in self-report symptoms across the menstrual cycle (chapter 4) in the absence of injury, identified a meaningful association between menstrual cycle day and symptom score; this may need consideration when evaluating baseline and post-concussion symptom assessments in females. The correlation of salivary hormone measures to blood measures were investigated in chapter 5 in eight females to test feasibility for use in future studies. Progesterone was positively correlated between blood and saliva (rm=0.996, p<0.001). The findings of chapters 4 and 5 informed the protocols for chapters 6 to 8. In chapter 6, concussion biomarkers (salivary cortisol and miR-27a-5p/miR-30a-3p) were monitored in one player across three consecutive menstrual cycles in absence of injury during a football season. The biomarkers showed cyclical variation of miR ratio within a range of 0.7 to 1.1. The third menstrual cycle was shorter and showed significantly (p=0.031) lower miR ratio in the pre-menses compared to the menses phase. Morning cortisol stayed within a normal reference range (2-22 nmol/L) and showed a statistically significant mean difference between menses and pre-menses phases. Larger studies are needed to elucidate a clinically relevant threshold for miR ratio and must include reliable measures of hormone profile. Chapters 7 and 8 outlined the Female Ribonecleic Acid in Concussion (FeRNAC) study that sought to understand whether salivary miR-27a-5p/miR-30a-3p, symptoms and recovery time were associated with hormone profile in 36 females. Progestin only contraception (PROG) and the oral contraceptive pill (OCP) were significantly associated with a shorter time to RTL/W (HR = 2.5; 95% CI: 1.0 to 6.1; p=0.048 and HR = 2.7, 95% CI: 1.1 to 6.4; p=0.027 respectively). There was no statistically significant mean difference between groups for initial symptom score, $F_{(2, 33)}$=1.755, p=0.189). Only 14 (39%) of saliva samples provided a full miR ratio, the mean miR ratio was 0.84± 0.06 (0.75 to 0.92) and there was no statistically significant difference between groups for miR-27/miR-30 ($F_{(2, 11)}$=0.519, p=0.609). Chapter 9 discussed valuable areas of focus for future concussion research in females including recommendations for in-field research protocols. This final chapter provided narrative on reflective learning throughout the thesis.

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Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor used artificial intelligence tools or generative artificial intelligence tools (unless it is clearly stated, and referenced, along with the purpose of use), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Chapters 2, 3, 5 and 7 of this thesis represent stand-alone papers that have been published and chapters 4, 6 and 8 have been submitted to a peer-reviewed journal for consideration for publication. My contribution and the contribution by the various co-authors to each of these papers are outlined at the beginning of each chapter and in the “candidate contribution to co-authored papers” table. All co-authors have approved the inclusion of the joint work in this PhD thesis.

Natalie Hardaker

30 July 2025

Candidate contribution to co-authored papers

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Sims	Writing - Review and editing
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Sims	Methodology, Writing - Review and editing
King	Methodology, Writing - Review and editing
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Manuscript Title:	**The Female RNA in Concussion (FeRNAC) study**
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Basu	Conceptualization, Methodology, Resources, Investigation, Formal analysis and data curation, Writing - Review and editing

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Massive thanks go to my incredible team of supervisors. I hadn't truly appreciated until the end how privileged I am to have worked with and learn from a team with such diverse experience and backgrounds as applied practitioners and as researchers. Patria, Stacy, James and Doug, I can't thank you enough for your patience and professionalism throughout. Patria, without your belief in me and your passion for lifting others up the opportunity wouldn't have been possible. Thank you.

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My last special mention is to Grandma B. The real inspiration for undertaking this PhD.

Ethics approval

Ethical approval for studies were reviewed and accepted by the Auckland University of Technology Ethical Committee (AUTEC) and the Health and Disability Ethics Committee (HDEC). The validity date and approval code are provided for each approved ethics application. Approval letters are provided in the Appendices.

1. Hume, P. A., N. Hardaker, S. Sims, D. King, J. Selfe and T. Stewart (2022-2025). **AUTEC #22/192** Assessing knowledge and attitudes towards concussion in amateur football players and coaches. In progress: Completed 26/07/2023-28/01/2025. For **Chapter 3.**
2. Hume, P. A., N. Hardaker, S. Sims, F. Merien and T. Stewart (2021-2024). **AUTEC #21/167** The validation of measures of female sex hormones in saliva and the relationship between salivary sex hormone profiles and symptoms across the menstrual cycle in healthy eumenorrheic females: A feasibility study. Completed 09/07/2021-09/07/2024. For **Chapters 4 and 5.**
3. Hume, P. A., N. Hardaker, S. Sims, F. Merien, D. King, W. Chang, J. Selfe, T. Stewart, S. Grice, A. Dixon-McIver and A. Morris (2022-2025). **AUTEC #22/135** Hormones, biomarkers and concussion in female athletes; A case series study. Completed 12/05/2022-28/01/2025. For **Chapter 6.**
4. Hardaker, N., P. A. Hume, S. Sims, J. Selfe, F. Merien, W. Chang, T. Stewart and D. King (2021-2023). **HDEC #2022 EXP 11904:** Hormone profiles and biomarkers in concussion in female athletes; A case series study. In progress: Completed 03/05/2022-27/01/2025.For **Chapter 6.**
5. Hardaker, N., P. A. Hume, S. Sims and D. King (2021-2023). **HDEC #2021 EXP 11655**: The impact of the female sex hormones on the incidence of and recovery from concussion. **In progress:** until 24 May 2025. For **Chapters 7 and 8**
6. Hume, P. A., N. Hardaker, S. Sims, F. Merien, D. King, W. L. Chang, J. Selfe and T. Stewart (2022-2024). **AUTEC #22/110** The impact of the female sex hormones on the occurrence of and recovery from concussion. In progress: until 24 May 2025. For **Chapters 7 and 8.**

Preface

Context of COVID-19 impacting data collection

Before introducing the topic area and the scientific background for this PhD, it is important to provide the context in which the studies were conducted. The research came with challenges and often studies did not go as planned.

COVID-19 global pandemic impact on data collection and sample sizes for studies

I encountered substantial challenges throughout this PhD due to the COVID-19 global pandemic, which compromised the quality of the final output primarily due to small sample sizes. In response to these challenges, I made decisions to optimize what could be done given the circumstances and the timeframes of the PhD combined with working full-time. I am based in Wellington (~500 km south of Auckland), and prior to the pandemic had planned to collect data in both cities. Dual city data collection was possible for one study, but it was not feasible to continue this approach in the post-COVID environment. Moreover, all lab-based analysis had to be conducted in Auckland as there are no facilities with the necessary equipment in Wellington.

After securing ethics approval and planning to commence data collection during the 2020 winter sports season, New Zealand entered a period of complete lockdown on March 21, 2020, and a four-tier alert level system was introduced to manage the COVID-19 outbreak. Given that people could not leave home other than to access essential services, all sports fixtures were cancelled, clinics operated online, and emergency departments were restricted to those in genuine need. Consequently, no data collection was feasible for the foreseeable future. I decided to take a six-month leave of absence from my PhD. This initial lockdown period lasted until May 27, 2020, but regionalized alert level changes were also employed and were unpredictable; the Auckland Region entered lockdown twice, in August–September 2020 and February–March 2021. I extended my leave of absence for another six months and resumed my studies in March 2021.

In the post-COVID environment, I modified my research approach, necessitating amendments to ethics. The amendment process with the Health and Disability Ethics Committee (HDEC) and the university ethics committee took longer than expected. Additionally, ongoing travel restrictions in New Zealand required careful planning for travel to Auckland for lab analysis. Sports fixtures continued to be impacted by shorter 'snap lockdowns', causing competition cancellations. When sports events could proceed, additional requirements related to vaccination policies, mask-wearing, and social distancing were necessary. In August 2021, New Zealand entered a second nationwide lockdown with community cases reported in Auckland and Wellington. Due to rising cases, the government prioritized the country's vaccination rollout, impacting healthcare environments. Auckland remained in some form of lockdown until December 3, 2021, when the new COVID-19 protection framework ("traffic light system") was implemented. Data collection from March

2020 to December 2021 was not feasible due to the prioritization of COVID-19 response efforts in sports and healthcare settings.

Between February and May 2022, the Government gradually eased border restrictions, public gathering limits, and vaccine mandates, leading to a slow national return-to-sport. During early 2022, I developed a relationship with a local Wellington-based football club to collect data throughout the 2022 season. As restrictions lifted and group gatherings resumed, COVID-19 transmission continued within the community, affecting the availability of participants for a planned case-series study. I also secured locality ethics approval to begin data collection in the emergency department early in 2022. The Government COVID-19 Protection Framework remained in place until September 12, 2022. Although some data collection occurred in the ED study through 2022, it was slower and inconsistent. The COVID-19 pandemic had a lasting impact on the hospital environment, requiring a balance between sensitivity to the situation and the need to drive research. I engaged staff in research activities and gradually continued data collection in 2023, maintaining relationships for future studies. Community and participant engagement is crucial for research, and while NZ is now considered in a post-COVID environment, the broader effects remained evident, particularly in community-based research. Increased work hours led to less time for participation in additional activities such as sports and research, affecting recruitment. This trend has been observed in other healthcare-based studies I have been involved in, such as the CRANIAC and BIONIC 2 studies, struggling to reach target participant numbers as research participation is viewed as an additional time burden.

The unpredictable nature of the pandemic complicated research planning. I considered broadening participant recruitment for the case series study by working with multiple football teams/clubs but decided against it due to the time required to build new relationships, the seasonal nature of football, managing logistics alongside full-time work, and the timeframe of the PhD. Instead, I reduced the study to a single-case time series observational design to ensure optimal data collection during the 2023 season. Recruitment for the ED study continued in 2024 and 2025. As a result of the constraints caused by COVID-19, my thesis includes 144 participants across a cross-sectional survey (n=97), a feasibility study (n=8), a single-case study (n=1), and a prospective cohort study (n=36).

My contributions versus others for planning, recruitment, data collection and analyses

The topic area and research questions were my own ideas, with refinement in discussions with my supervision team. As lead researcher across the PhD studies, I was responsible for gaining ethical approval, participant recruitment and ongoing engagement, data collection, management, transfer, analysis and interpretation. I received considerable support throughout this PhD utilising a teamwork approach to manage the logistical constraints and time pressures. I allocated some of my PhD budget to employ research officer Juno Barnett-Collins (trained phlebotomist) to support some aspects of data collection.

Study set-up

I set up and tested the online data management aspects for all studies. This included putting the RoCKAS onto the Alchemer survey platform for Chapter 3 and building the relevant tools and logic onto REDCap for the Feasibility (chapters 4 and 5), single-case design (chapter 6) and the FeRNAC study (Chapters 7 and 8).

Study recruitment

For recruitment of the concussion survey (chapter 3) I made a deliberate decision to work with New Zealand Football (NZF) to recruit players through the regional federations and clubs. I provided NZF with the email template, study information and direct link to the survey on the Alchemer platform to send out to their membership database. This was a strategic decision to ensure that the findings of the research could be implemented and inform future work around concussion in community football. I recruited 30 participants for the feasibility study (chapters 4 and 5), this included a 15-minute introductory session with each participant either online or in person. Five of these participants were based in Auckland and three were based in Wellington.

For the single-case design study (chapter 6) during the 2022 season I attended early season training sessions (still somewhat limited due to sport slowly returning from COVID restrictions) to develop my relationship with the coaches and the players and I gave some short talks about my topic area and intended research. I attended six pre-season training sessions in 2023, all 14 games and one evening training session each week for 14 weeks. It was during the 2023 season that data collection was possible, therefore I recruited the case study participant, delivered the introductory session and set up the weekly survey links. Recruitment for the FeRNAC study (chapters 7 and 8) occurred in the emergency department (ED). Given that I am not and ED clinician, it was not feasible for me to be in the ED, particularly post-COVID when additional people were discouraged from being present due to the risk of infection. As it is a very busy environment, for optimal efficiency it was agreed that it would be better for the ED staff to screen and recruit the participants. Recruitment therefore relied on a teamwork approach and was facilitated by my co-supervisor Dr Doug King. I attended three meetings with senior ED staff and I delivered two in-service sessions to the wider ED staff on concussion and outlined the proposed research. Doug and the ED team then recruited 38 participants at the Hutt Valley Hospital ED for the prospective study.

Data collection, management and processing

For the RoCKAS study (chapter 3) Juno Barnett-Collins cleaned and analysed the data according to the plan that I developed. For the feasibility study (chapters 4 and 5), I managed and processed all app and survey-based data. I collected all six blood samples from the three Wellington based participants and interacted with all eight participants on a weekly basis (12-20 weeks each) to receive their self-collected saliva samples for storage in the -80^{o}C laboratory freezer (in Wellington) or co-ordinate sample drop off in Auckland. Pre-COVID

I planned regular travel to Auckland to coincide with receiving the saliva samples from the first two participants in this study. For the three recruited Auckland based participants recruited post-COVID all saliva samples were received by staff at the Auckland laboratory for storage. Blood samples from all five of the Auckland based participants were collected by Juno Barnett-Collins.

All data including the saliva samples, app and survey completions for the single-case study were self-collected by the participant. I checked in with the player every week at training to receive the saliva samples for 14 weeks. I then transferred the samples to the -80^{o}C freezer in the medical laboratory (in Wellington) for storage. All biomarker analyses (blood and saliva) were conducted in the Auckland laboratory. I flew to Auckland to be upskilled in the use of the Cobas analyser in the AUT Roche lab which included the required processing of the blood and saliva samples and programming of the analyser. I analysed the first set of 322 saliva samples and eight blood samples for the first four participants completing data collection in the feasibility study (chapter 5). Once I was confident in the techniques, the additional 306 saliva samples and eight blood samples for the remaining four participants in the feasibility study and the 86 saliva samples from the single-case design study (chapter 6) were analysed by research officer Juno Barnett-Collins (further outlined in the table 0.1 below). I cleaned and collated data collected from all participants in these two studies in preparation for data analysis. For the FeRNAC study, for the duration of the data collection period, I drove out to the hospital to receive the stored saliva samples (temporarily stored in the hospital freezer) and transported them back to the medical laboratory for storage at -80^{o}C, checked in with the staff and delivered morning tea as a small thank you for supporting the research. When participants consented to take part in this study, I then followed up via email to send out the links to the weekly surveys. All information for this study was collected and managed on REDCap.

The miRNA analysis of the 86 saliva samples from the single-case design study (chapter 6) and the first 10 samples from the FeRNAC study (chapters 7 and 8) were done by Indira Basu in Awanui labs. The miRNA analysis of the remaining 28 miRNA samples from the FeRNAC study were done by me with upskilling and support from Indira Basu.

For the studies (chapters 5, 6 and 8) that included blood and saliva sample collection in Wellington, when all samples were collected for each respective study, I organised transfer of the samples from Wellington to the Auckland laboratory for subsequent analysis. This required careful planning and co-ordination to ensure the quality of the sample was maintained.

Data analysis and interpretation

For the RoCKAS study (chapter 3), I developed the data analysis plan, checked the data and outputs and interpreted the results. Tom Stewart (AUT biostatistician) provided support and advice in the development of statistical analysis plans across chapters 4, 5, 7 and 8. Tom carried out the statistical analysis in chapter 4 while spending time teaching me why the polynomial regression was the best approach to answer the research question. Tom also introduced me to the repeated measures correlation (rmcorr) analysis used in

chapter 5 which I then decided to use. For the single-case design study (chapter 6) I analysed all data. I cleaned, collated, analysed all the data in the FeRNAC study (chapters 8). I interpreted the data and drafted the manuscripts for all studies and then finalised with my co-authors prior to submitting to the target journals. A summary of the studies combined is provided in table 0.1.

Table 0.1: Summary of studies showing participants recruited and contributions by collaborators.

Study	**Chapter 3 - RoCKAS Survey: players >16 years**	**Chapters 4 and 5 - Feasibility study: Healthy physically active females 16-45 years** (*participants collected their own saliva samples)	**Chapter 6 - Single-case: Female football player** (*participant collected their own saliva samples)	**Chapters 7 and 8 - FeRNAC study: Female patients with concussion**
Participants recruited % - Total	97	8 (30 recruited)	1	38
Participants recruited % - Natalie		100	100	
Participants recruited % - Juno				
Participants recruited % - Doug ED team				100
Participants recruited % - New Zealand Football	100			
Data collection - blood samples collected # - Natalie		6		
Data collection - blood samples collected # - Juno		10		
Data collection - saliva samples collected # - Doug ED team				38
Samples analysed (blood and saliva) # - Natalie		322 saliva samples, 8 blood samples		28 saliva samples
Samples analysed (blood and saliva) # - Juno		306 saliva samples, 8 blood samples	86 saliva samples	
Samples analysed (blood and saliva) # - Doug ED team				
Samples analysed (blood and saliva) # - Indira Basu - Awanui Labs			86 saliva samples	38 saliva samples

Chapter 1: Introduction

1.1 The value of sport

The health advantages of sport and physical activity are well documented and include social [1], psychological [1, 2], physical [3] cognitive [4] and in the prevention of disease [5, 6]. In addition, sport has been shown to increase confidence, resilience and leadership skills [7, 8] particularly in women and girls [7, 8]. There has been a global increase in the participation of women and girls in sport since the introduction of title IX in the USA in 1972. Title IX protects people from discrimination based on sex in education programs or activities that receive federal financial assistance and states that, "*No person in the United States shall, on the basis of sex, be excluded from participation in, be denied the benefits of, or be subjected to discrimination under any education program or activity receiving Federal financial assistance*", this was pivotal in opening up more opportunities for girls in sport [9]. This can be observed at the highest levels of sport with female athletes participation at the modern Olympics has increased from 26% of total participants at the Seoul Olympics in 1988 to 49% at the Paris Olympics in 2024. The Paris Olympics were the first gender equal Olympic Games by participation and parity of medals available for both male and female athletes [10]. There has also been record breaking participation and attendance at recent world tournament events (FIFA world cup 2023, Ruby World Cup 2023, Cricket World Cup 2023) and increasing professionalism and pay parity for female athletes across a range of sports including football, athletics, triathlon, Rugby. The increased visibility of female athletes as role models highlighting sport as a viable career option, combined with the global drive to increase physical activity levels and engage more women and girls in sport has increased participation at every level.

1.2 Research disparity

Despite the increasing number of females participating in sport and exercise, females are under-represented in the sports science and biomedical literature [11-15]. In some areas females are still considered a "special population" in many related teaching curriculums. The highest volume of sport science and exercise medicine (SSEM) research has been done on cis-gender 18–24-year-old males and data from these studies has been extrapolated to other populations [11, 16, 17].

In a review [11] of 1,382 exercise medicine studies published between 2011 and 2013 across three of the top sports medicine journals, it was reported that females accounted for less than 40% of total study participants in original and epidemiological research. Approximately 34% of the studies included males only, while less than 13% of studies being for females only. A similar review [16] of 188 studies from two academic journals capturing 254,813 participants revealed similar findings where females made up less than half (42%) of the total participants. Further, 27% of the studies were inclusive of men only, but only 4% of studies were exclusively carried out on females. A more recent review of studies from 2014-20 [12]

highlighted that this trend hasn't improved; from a total of 12,511,386 participants, females were present in 69% of studies but made up only 34% of the total participants. It must be acknowledged that along with the apparent investigator bias, there is likely a degree of volunteer (self-selection) bias and females may be less willing, or available, to participate in some research studies despite meeting inclusion criteria [18].

In a report [16] addressing the data disparity between male and females in sport, this incorporated a further breakdown of specific areas of research and females were most well represented in social studies (included in 100% of studies and made up 60% of the participants) and most poorly represented in studies relating to sports performance (included in 4% of studies and made up only 3% of participants) and sports injuries (only 2% of original injury related studies have focused on females versus 20% specifically in males). It is important that female data contributes to the evidence base so that training, injury prevention, recovery, nutrition and medical interventions and recommendations can be developed on relevant data and support optimised health and performance.

A protocol for a more thorough and consistent 'audit' of the existing literature has been developed [19] and applied to different areas including, evidence-based supplements [20], hip labral tears [21], fifth metatarsal fracture [22], Achilles tendon rupture [23] and rotator cuff repair [24]. Female representation across these audits was between 16-55%, highlighting that females are still underrepresented in the SSEM data. Another theme evident in these audits was that female specific studies tended towards health outcomes whereas male focused studies included health and performance outcomes.

1.3 Sex specific injury profiles

Although the overall benefits of participation in sport are positive, there is a risk of injury. Sports related injures can vary in severity. Serious injuries can have a long-term impact on athletes' physical capacity, for those athletes higher up the participant classification framework (tier 4-5) this may lead to early retirement and for those lower down (tier 1-3) it could lead to disengagement from sport and physical activity. In parallel with increasing participation of females in sport, a disproportionate increase in Anterior Cruciate Ligament (ACL) injury has been observed in sports where rapid deceleration, change of direction or jump-landing movements occur [25]. Female athletes have four-to-ten times an increased risk of non-contact ACL injury occurring [26, 27]. There is a substantial body of evidence supporting the understanding of ACL risk profiles in female athletes [25, 27] and therein the development of effective primary and secondary prevention strategies.

Divergent changes occurring at puberty combined with the gendered environment that are thought to contribute to an increased susceptibility to ACL injury [28] may also create other sex specific injury profiles across sport. Therefore, understanding injury patterns in female athletes is essential for the development of effective, targeted interventions to reduce the incidence of sports injuries and to minimise the severity of those injuries that do occur.

Sex differences develop in utero (Male XY or Female XX chromosomes) then, at the onset of puberty the upsurge of sex hormones (testosterone in boys and estrogen and progesterone in girls) leads to the development of secondary sex characteristics which in males includes, growth of pubic and facial hair, deepening of the voice and appearance of the Adam's apple. The secondary sex characteristics in females includes, breast development, widening of the hips and growth of pubic hair. In females the upsurge increase in sex hormones also leads to the onset of the first menstrual cycle [29]. Other physical changes that occur during puberty are, changes in body composition, altered centre of gravity, rate of strength development, altered biomechanics, attainment of peak height velocity and accrual of bone mass. Males typically gain height, weight and lean mass (muscle) simultaneously, whereas females gain height, weight (fat mass), muscle sequentially and in that order [29]. From a SSEM perspective it is important to recognise that these divergent changes may impact on athletic performance and injury risk.

1.4 Hormone profiles

Estrogen and progesterone are the primary female sex hormones synthesized by the ovaries [30]. These hormones work together and rise and fall in a cyclic manner – the menstrual cycle. One full menstrual cycle is defined as the time from the onset of menses to the day before the next onset of menses [31]. The menstrual cycle typically lasts between 21-35 days and can be considered in 3-5 distinct phases. During the early follicular phase estrogen levels are low, then rise to a peak in the late follicular phase leading to ovulation; in the early luteal phase estrogen levels drop and then rise again with progesterone, reaching a peak in the mid-luteal phase before finally dropping away again in the late luteal phase [32].

The menstrual cycle is governed by the brain – neuroendocrine control – and includes a series of feedback loops with other hormones [31]. This begins with the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, which stimulates the pulsatile release of follicle stimulating hormone (FSH) from the pituitary gland [31] to initiate follicle development. As estrogen rises to a peak that activates a bolus release of luteinising hormone (LH) and this is the hormonal trigger for ovulation [33, 34] stimulating the synthesis of progesterone [31]. Approximately seven days after ovulation is the time of peak progesterone concentration [31]. If pregnancy does not occur, progesterone concentration decreases and this removes the negative feedback to the pituitary gland [30, 31] and subsequently, FSH begins to rise, and the cycle repeats again (Figure 1.1) [35].

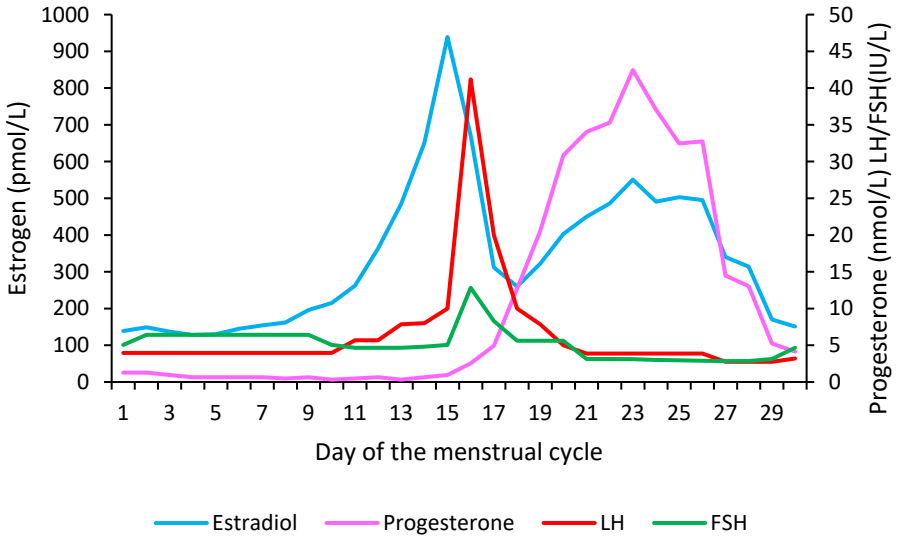


Figure 1.1: Cyclic fluctuations of estrogen, progesterone, LH and FSH across the natural menstrual cycle (based on data presented by Stricker [35]).

The fluctuations in ovarian hormones are thought to trigger a variable cluster of psychologic and somatic symptoms frequently associated with the menstrual cycle (i.e., breast tenderness, fatigue, irritability, bloating, anxiety, etc.) [36, 37]. These symptoms begin during the late luteal phase of the menstrual cycle and disappear after the onset of menses. Most females have some level of discomfort and experience at least one or more emotional or physical symptoms in the week before menstruation [36, 37]. As a result, it is evident that symptoms can affect how female athletes feel during training and performance [38, 39]. There is variation within, and between, females in each menstrual cycle from cycle length to the number and type of symptoms experienced [38, 40, 41]. There are also varying degrees of menstrual dysfunction from oligomenorrhea (irregular cycle length) to amenorrhoea (loss of the menstrual cycle) that result in an altered hormone profile [31] outlined in figure 1.2.

Across studies of female athletes (tier 5 to tier 1) it is estimated that ~50% are using some form of hormonal contraception [42, 43] either for birth control, management of cycle related symptoms or to control the timing of the menstrual cycle in relation to competition. Hormonal contraceptives contain exogenous steroid hormones that act via a negative feedback loop to supress the secretion of gonadotropins and this, in turn, downregulates the endogenous ovarian hormones [44]. There are multiple different forms of hormonal contraception all of which can be considered within one of two formulations. These are:

1) The combined contraceptive that contains both an estrogen and a progestin (e.g., oral contraceptives [OC], transdermal patch, vaginal ring); or
2) Progestin-only contraceptive (e.g. OC [minipill], intrauterine device [IUD], injection, implant) [44].

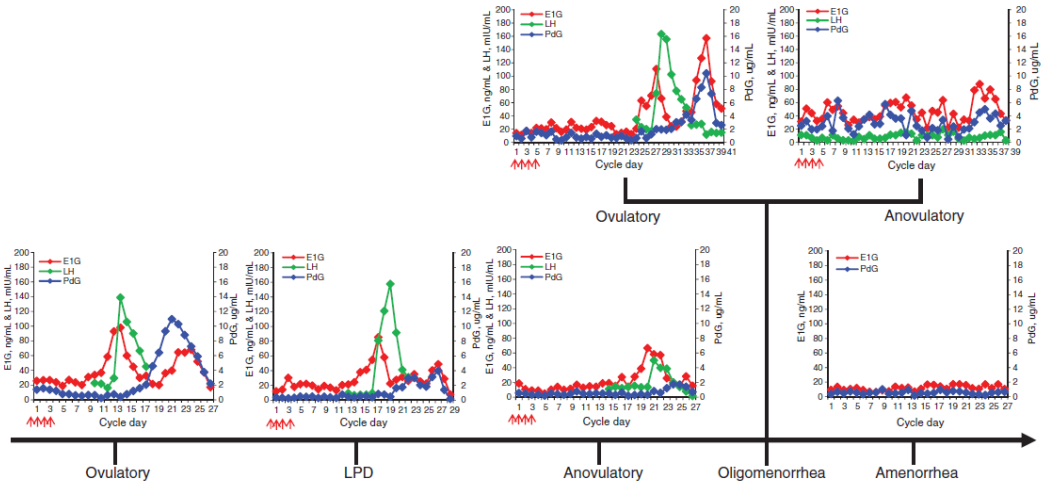


Figure 1.2 Representative example of the spectrum of menstrual function and dysfunction in pre-menopausal women

The mean daily E1G and PdG concentrations are displayed in eumenorrheic ovulatory, LPD, and anovulatory menstrual cycles, oligomenorrheic ovulatory and anovulatory menstrual cycles, and in amenorrheic monitoring periods of 28 days. LPD=luteal phase defect; E1G=estrone- 1-glucoronide; PdG=pregnanediol glucuronide; (arrow symbol)=menses. Figure taken from Allaway et al. [31] with permission.

Hormonal contraceptives are a highly effective form of birth control with a reported 98% efficiency [43]. Data investigating the impact of hormonal contraceptive use on other health and performance parameters in the short and long term are limited [45].

1.5 Low energy availability/ Relative energy deficiency in sport – health and performance

Amenorrhea is the absence of the menstrual cycle [31] and can be classified as either primary or secondary. Primary amenorrhea refers to the absence of the first menstrual cycle by age 16. Secondary amenorrhea refers to the absence of three or more menstrual cycles after having regular periods. Menstrual function and dysfunction occurs on a spectrum from regular ovulatory cycles to amenorrhea as highlighted [31]. Endocrine disruption leading to secondary amenorrhea can be caused by stress including that caused by chronic low energy availability (LEA) [46].

Energy availability (EA) is defined as the dietary energy left over and available for optimum function of body systems after accounting for the energy expended from exercise. Energy availability is expressed as kcal/kg FFM/day:

EA [Energy Availability] = (EI [Dietary energy Intake (kcal)]- EEE [Exercise Energy Expenditure (kcal)]) / FFM [Fat-Free Mass (kg) / day]

Low energy availability (LEA) occurs when there is insufficient energy available to support both physiological function and exercise energy expenditure [47]. This is typically caused by an increase in training or exercise, a reduction in energy intake, or a combination of both, leaving the energy equation unbalanced. LEA occurs as a continuum from short term (adaptable) LEA, and this is mild and quickly reversible to longer term chronic (problematic) LEA that can disrupt normal endocrine function resulting in

negative health consequences. Problematic LEA underpins Relative Energy Deficiency in Sport (REDS); a syndrome of impaired physiological and/or psychological functioning experienced by both female and male athletes [46, 48]. REDS can lead to poor health and performance outcomes such as decreased energy metabolism, reproductive function (specifically loss of the menstrual cycle), musculoskeletal health, immunity, and increased injury risk [46, 48].

There is a higher prevalence of LEA/REDS in female athletes, and this is thought to be due the higher *relative* energy requirement of females to maintain basic physiologic function (~30kcal/kgFFM versus ~15kcal/kgFFM in males) [46]. This higher prevalence in female athletes may be in part due to the sociocultural factors that influence body image and drive disordered eating in female athletes [49, 50]. The reported prevalence of LEA for females varies across sport; gymnasts (45%), football players (33 to 59%), ballet dancers (22%), volleyball players (20%), synchronised swimmers (100%), recreational gym goers (45%) [51-53].

1.6 Female research methodology

To ensure reliability and the validity of the results of research in females, the methodological approach to female specific or sex differences research needs to consider the hormone profile of participants. This is necessary to control for different concentrations of fluctuating estrogen and progesterone within and between females. These differences may be due to menstrual cycle (MC) phase, the use of hormonal contraceptives (HC), any level of menstrual dysfunction or peri- post menopause. Recommendations for study design have been consistently documented [42, 44, 54]. For example, phase verification and the timing of testing throughout the menstrual cycle are critical in menstrual cycle-based research questions [54]. A serum progesterone measure (>16pg/ml) is essential to confirm ovulation, and this is the key criteria for eumenorrhea. The additional time and cost to the researchers and increased participant burden due to the logistics and time commitment can present recruitment challenges for these studies. The requirement for blood samples can also be a barrier for some participants.

Other methods of phase *estimation* include counting methods; the forward counting method takes the first day of bleeding as day one and counts forward to estimate cycle phase based on a 28-day cycle i.e., day 14 is expected ovulation [55]. Another counting method (the backward counting method) counts backwards 14 days from the last day of the cycle (i.e., the day before bleeding starts) to estimate when ovulation occurred and then counting forward seven days from there to estimate timing of the progesterone peak [55]. The backward counting phase projection method can be more useful where data from multiple menstrual cycles has been collected so that within participant patterns can be detected. However, the counting methods are limited due to the lack of physiological measurement.

1.7 Purpose of this research

The central aim of thesis was to understand the influence of female sex hormones on concussion. To address this aim, the specific questions were:

1. How much does baseline symptomology change across the menstrual cycle in female athletes?
2. Do salivary concussion biomarkers change across the menstrual cycle for female athletes in the absence of injury?
3. Does hormone profile at the point of injury predict prolonged recovery from concussion in females?
4. Does hormone profile at point of injury affect the expression of salivary concussion biomarkers in females?

The research design undertaken in the thesis includes a feasibility study, a cross-sectional study, a cohort study and a single-subject design study.

1.8 Significance of the research

The results of the research will give a more informed view of the specific needs of females with concussion and will provide a greater understanding of the potential influence of sex hormones on symptomology and recovery. The research will discuss the interaction of hormone profiles with recovery and symptomology following concussion and evaluate the potential for novel use and measurement of biomarkers associated with brain injury to improve outcomes for females with concussion. The findings of this thesis will also indicate valuable areas for future research and contribute to closing the data gap.

1.9 Structure of thesis

This thesis is comprised of nine chapters (Figure 1.2). Chapter 1 provides and introduction to the key concepts that are relevant throughout chapters. In Chapter 2 is a systematic review and meta-analysis evaluating the existing evidence of published research for the differences in injury profiles between the sexes. Chapter 3 describes the differences in concussion knowledge and attitudes between male and female football players using the ROCKAS concussion knowledge assessment tool. In Chapters 4, 5 and 6 the feasibility of a suite of data collection tools and protocols were tested and developed for future use in applied research methodologies with female athletes (including a single subject design study of concussion biomarkers across three consecutive menstrual cycles during a competition season in a female football player in the absence of injury). Chapters 7 and 8 present the protocol and data from the Female miRNA in Concussion (FeRNAC) study, a prospective study of females with concussion recruited from the Emergency Department environment. Based on the findings across the thesis Chapter 9 discusses valuable areas of focus for future concussion research in female athletes including recommendations for a methodological approach to future concussion research in females, allowing consideration for the effects of, and impact on

the menstrual cycle. This final chapter also provides narrative on reflective learning and application of that learning throughout the thesis.

The chapters in this thesis were written in the format of the respective journal to which they were submitted, except in the case of the first and ninth chapters. A preface explaining how each chapter is linked in the larger narrative of the thesis, has been written between *Chapters two-to-eight*. Due to the nature of the thesis structure (pathway two, thesis by publication), there may be some repetition between thesis chapters.

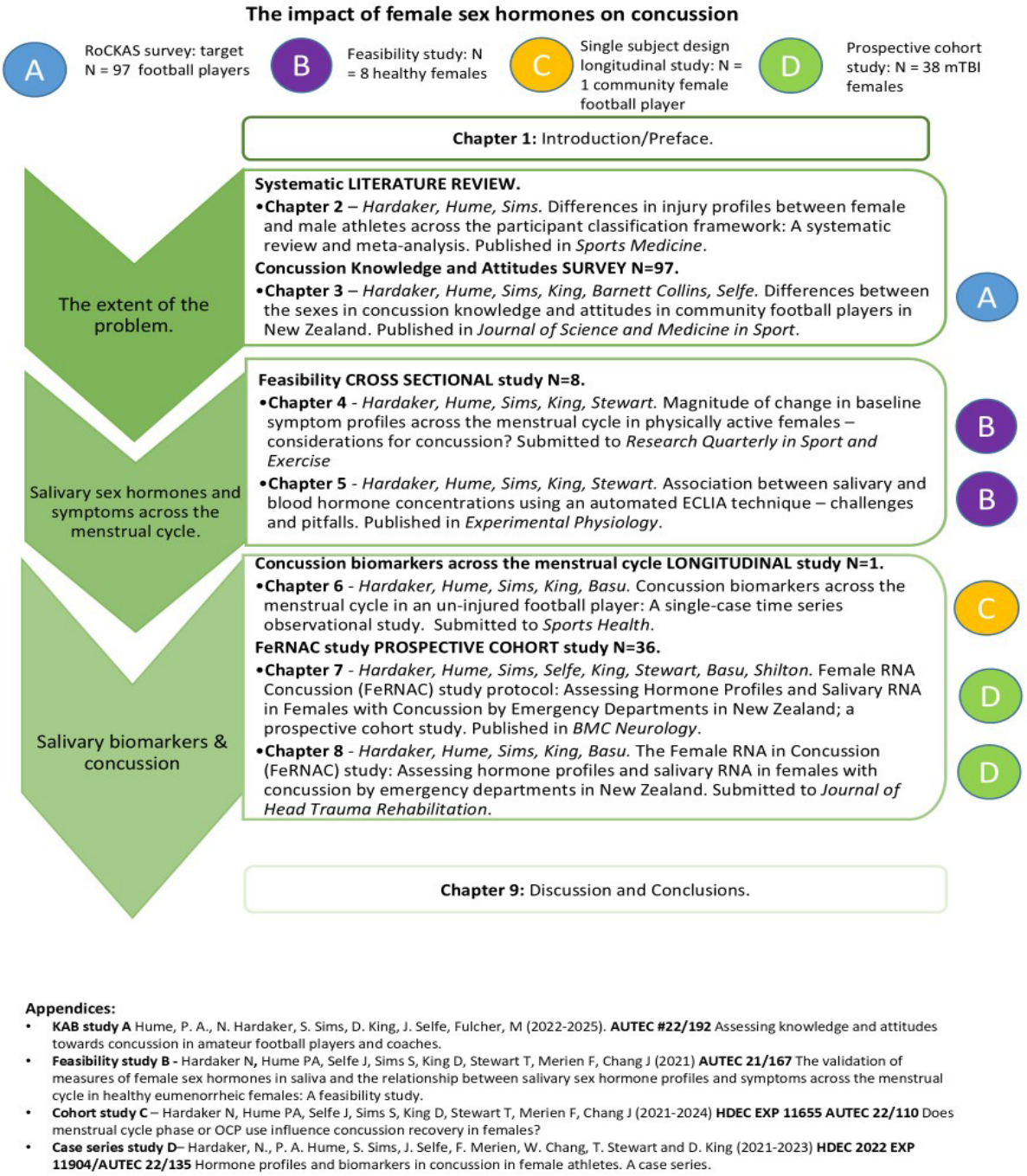


Figure 1.2: Outline of the structure of the thesis and chapters.

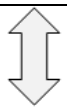
1.10 Chapter content and link between studies

For each thesis chapter the questions, rationale, approach, findings and key contribution, with the linking between chapters is shown in Table 1.1.

Table 1.1: Research key points and links between chapters.

Chapter and Title	Chapter Content - Question / Rationale / Findings
1 **Introduction and rationalisation.**	**Main Question of the Thesis:** 1. What is the impact of the female sex hormones on concussion? **Secondary Questions of the Thesis:** • How much does baseline symptomology change across the menstrual cycle? • Do concussion biomarkers change across the menstrual cycle in the absence of injury? • Is hormone profile associated with symptom severity and/or prolonged recovery from concussion? Is hormone profile associated with the ratio of salivary miRNA associated with concussion?

Chapter and Title	Chapter Content - Question / Rationale / Findings
2 **Differences in injury profiles between female and male athletes across the participant classification framework: A systematic review and meta-analysis**	**Question:** • Where are sex and gender differences more apparent in sports related injuries? **Rationale:** • There has been a significant increase in the participation of women and girls in sport. In parallel with this increased participation a disproportionate increase in the incidence of ACL injuries was observed in women and girls. It has become widely acknowledged that female athletes are at increased risk of ACL injury due to a complex interaction of factors relating to sex and gender. It remains unclear if there are other sex and gender specific injury patterns in sport. This is important to understand so that effective primary and secondary prevention strategies can be developed and applied. **Approach:** • A systematic review of literature and meta-analysis. **Findings:** • Female athletes are at increased risk of bone stress injuries (BSI), knee, foot and ankle injuries and sports related concussion (SRC). • Female athletes also have an increased symptom severity and duration following SRC (note: this was an incidental finding of the literature review and was not included in the meta-analysis). • Male athletes have a higher incidence of hamstring injury, hip/groin injury and acute fractures. **Novel contributions:** • This meta-analysis confirmed that there are differences in injury profiles between male and female athletes beyond ACL injuries. • The findings highlight where the differences are more apparent • The findings can inform areas where development and implementation of primary and secondary prevention strategies are needed.

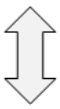


Link between Chapters 2 and 3:

Having identified that there are differences between the sexes in the incidence of and recovery from sport related concussion, the next step was to understand why this may occur. The literature suggests that it may be due to a reporting bias, fluctuating sex hormones, weaker neck musculature and smaller neuroanatomy in females.

Chapter and Title	Chapter Content - Question / Rationale / Findings

3 **Differences between the sexes in concussion knowledge and attitudes in community football players in New Zealand.**	**Question:** • What is the level of concussion knowledge and attitudes in community football players in New Zealand and does this differ between male and female players? **Rationale:** • To understand if there is a difference in the level of concussion knowledge between male and female football players. • To understand if there is a difference in the attitudes towards concussion (including intention to report) between male and female football players. **Approach:** • N = 97 • Online survey shared via New Zealand Football's club database. • Players 16 years and above. • Rosebaum Concussion Knowledge and Attitudes Survey (RoCKAS) **Findings:** • Male players had a statistically significantly higher CKI than female players. • Female players with a history of concussion had a statistically significantly higher CKI than female players without prior concussion. • Over 30% of all players indicated that they would continue playing with symptoms of concussion. Despite 95% of female players knowing that concussion affects performance and can have long term consequences if not managed early. **Novel contributions:** • This study sets an important baseline for *community* football players in New Zealand. • Female players indicated that they would continue playing with symptoms of concussion. This is the first study to investigate this aspect in female athletes.



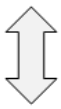
Link between Chapters 3 and 4:

Having understood how concussion knowledge and attitudes (including an indication of reporting intention) in male and female football players may contribute to the observed gender differences in incidence and outcome of the injury, the remainder of the thesis focused on how hormone profile and hormone related symptomology may interact with concussion in females. A feasibility study was used to test a data collection protocol and to understand baseline symptomology in the absence of concussion in physically active females with a regular menstrual cycle.

Chapters 4 and 5 are based on data from the same group of participants but aim to report on independent aspects of the feasibility study.

Chapter and Title	Chapter Content - Question / Rationale / Findings
4 **Menstrual cycle related symptoms in healthy naturally cycling physically active females overlap with symptoms associated with concussion.**	**Question:** • How do hormone related symptoms change across the menstrual cycle in healthy physically active females in the absence of injury? • Is the data collection protocol feasible for participants to complete? • Does the data collection protocol provide the information required? **Rationale:** • To test the data collection protocol for use in female athletes with concussion. • Symptoms associated with concussion are non-specific and are congruent with hormone related symptoms. • Symptoms are a key determinant in managing concussion, the magnitude of change in baseline symptoms across the menstrual cycle in females in the absence of injury has not yet been reported. • Menstrual cycle related symptoms may interact with concussion symptoms and may need to be considered as a confounder in the overall clinical picture in females with concussion. **Approach:** • N = 8 • Healthy physically active females aged 25-47 years with regular menstrual cycle (21-35 days). • Prospective data collection protocol including daily tracking of symptoms and sleep characteristics in WILD AI menstrual cycle tracking app, weekly online survey (Low Energy Availability in Females Questionnaire; LEAF-Q). • Sleep and LEAF_Q data were collected to use as covariates in the analysis. **Findings:** • There was a statistically significant association between day of the menstrual and symptom score. • Sleep quality changed across the menstrual cycle and showed a statistically significant association with symptom score. • The magnitude of change in symptom score across the menstrual cycle was similar to that which meets one of two criteria for concussion diagnosis. **Novel contributions:** • This was the first study to investigate and quantify the magnitude of day-to-day change in symptoms across the menstrual cycle and to identify the high degree of congruence between menstrual cycle symptoms and other clinical concerns including concussion.
 *Link between Chapters 4 and 5:*	Having understood the magnitude of change in baseline symptoms across the menstrual cycle this information can be considered in the context of concussion assessment. Chapter 4 also confirms that app based daily symptom monitoring is a feasible method of data collection for future studies. The second part of this study compared salivary hormone concentrations against blood-based measures to test the potential for salivary measures to be used as a reliable part of the suite of data collection tools that support monitoring of hormone levels over multiple timepoints as a more accessible method of data collection.

Chapter and Title	Chapter Content - Question / Rationale / Findings
5 **Correlation between salivary and blood hormone concentrations using an automated ECLIA technique - challenges and pitfalls.**	**Question:** • How do salivary measures of sex hormones estradiol and progesterone compare to those measured in blood? • Is daily saliva sampling a feasible protocol for participants to adhere to? **Rationale:** • To test the data collection protocol for use in future research. • Saliva is a non-invasive method of measuring hormone concentrations and is more convenient to the participant when a high volume of data collection is required. It is necessary to test the use of an automated ECLIA technique for analysing salivary measures of sex hormones against those measured in blood to ensure the method will be reliable. **Approach:** • N = 8 • Prospective data collection protocol for three-to-five menstrual cycles including daily saliva samples and two blood samples in the last month of data collection. • Healthy females with a regular menstrual cycle aged 25-47 years. **Findings:** • Progesterone and estradiol both showed a positive correlation between blood plasma and salivary measures; (r_m=0.996, $p<0.001$ and r_m=0.0705, p=0.0517 respectively). • There was a weak non-significant correlation for measures of cortisol (r_m=0.245, p=0.526). • Further work to validate this technique in saliva is required before it can be reliably applied to quantify levels of estradiol and progesterone. **Novel contributions:** • This was the first study to compare salivary estradiol and progesterone with blood-based measures analysed using an automated ECLIA technique. • This study used a repeated measures correlation (rmcorr) data analysis technique.



Link between Chapters 5 and 6:

The findings from chapters 4 and 5 were used to inform the design of the studies reported in chapters 6-8.

There is currently no objective test to diagnose and monitor concussion, however there are several candidate salivary biomarkers that show potential. Research related to concussion biomarkers shows a similar gender bias to other fields of research. It is important to understand from the outset whether sex may be a confounder of salivary concussion biomarkers to ensure accurate interpretation. Having confirmed the feasibility of a daily data collection protocol that can be done by the participant from home this approach was built into a single-case time series observational study protocol to monitor a female football (soccer) player for three consecutive menstrual cycles during the season.

Chapter and Title	Chapter Content - Question / Rationale / Findings
6 **Concussion biomarkers across the menstrual cycle in an un-injured football player: A single-case observational time series study.**	**Question:** • Does the ratio of salivary miR-27a-5p/miR-30a-3p (miR ratio) change across the menstrual cycle? • Does miR ratio differ between the menses and pre-menses phase of the menstrual cycle? • Does salivary morning cortisol change across the menstrual cycle? • Does salivary morning cortisol differ between the menses and pre-menses phase of the menstrual cycle? **Rationale:** • Sex differences are evident in the expression of miRNA in other disease states • Changes in hormone concentrations may have an impact on the expression of miRNAs. • This is important to understand in the absence of injury before investigating in females with concussion to ensure accurate interpretation of the data. • It is also important to understand before confirming the use of miRNAs in the diagnosis and management of concussion based on male data. • Salivary morning cortisol has also been identified as a prognostic concussion biomarker; some evidence suggests this can vary by menstrual cycle phase. **Approach:** • Single-case design – female football player. • Prospective data collection protocol for three consecutive menstrual cycles including daily saliva sample, daily log in WILD AI menstrual cycle tracking app, weekly online survey (Low Energy Availability in Females Questionnaire; LEAF-Q). **Findings:** • miRNA-27a-5p/miR-30a-3p shows some cyclical variation across the menstrual cycle, within a narrow range of 0.73-1.1. • The third menstrual cycle was shorter and showed a statistically significantly different miR between the menses and pre-menses phase of the menstrual cycle. • Salivary morning cortisol stayed within a normal reference range and showed a statistically significant difference between the menses and pre-menses phase of the menstrual cycle. **Novel contribution:** • This is the first study to capture salivary biomarker data across consecutive menstrual cycles in the absence of injury (baseline). • Sub-clinical menstrual disturbances may alter concussion specific salivary miR ratio in the absence of injury.
Link between Chapters 6 and 7:	Having understood baseline symptomology and concussion biomarkers over multiple timepoints in females in the absence of concussion, it is important to understand how these metrics interact with concussion. The findings from the feasibility study (chapter 5) informed the decision not to pursue the use of salivary measures of sex hormones and to prioritise saliva sampling and analysis for concussion biomarkers.

Chapter and Title	Chapter Content - Question / Rationale / Findings
7 **Female RNA Concussion (FeRNAC) study protocol: Assessing hormone profiles and salivary RNA in females with concussion by emergency departments in New Zealand: A prospective cohort study.**	**Questions:** • Does recovery time following concussion differ by hormone profile? • Does miR-27a-5p/miR-30a-3p expression differ by hormone profile? • Does symptom burden differ by hormone profile? • Does LEAF_Q score change during the recovery period? **Rationale:** • Changes in hormone concentrations may have an impact on how females experience concussion both in terms of symptom burden and recovery time. It was not clear from previous literature whether hormone profile may impact concussion specific biomarkers. **Approach:** • Protocol methodology description. • Prospective cohort study. • All females presenting to ED in Wellington, New Zealand with suspected concussion within three days of injury. • Data collection includes a single saliva sample and two weekly surveys: the LEAF-Q and the Brain Injury Screening Tool (BIST) symptom report. **Findings:** • This is a protocol paper. **Novel contribution:** • This is the first study to investigate concussion specific salivary biomarkers in relation female hormone profiles. • This is the first study to investigate time to recover from concussion in relation to hormone profiles.
Link between Chapters 7 and 8:	The protocol was accepted and implemented in the ED environment in the next chapter.
8 **The Female RNA in Concussion (FeRNAC) study.**	**Questions:** • Does recovery time following concussion differ by hormone profile? • Does miR-27a-5p/miR-30a-3p expression differ by hormone profile? • Does symptom burden differ by hormone profile? **Rationale:** • Changes in hormone concentrations may have an impact on how females experience concussion both in terms of symptom burden and recovery time. In addition, it was not clear from previous literature whether hormone profile may impact concussion specific salivary miR-27a-5p/miR-30a-3p. **Approach:** • N = 36 • Prospective cohort study. • Females presenting to ED in Wellington, New Zealand with suspected concussion within three days of injury. • Data collection included a single saliva sample and two weekly surveys: the LEAF-Q and the Brain Injury Screening Tool (BIST) symptom report. **Findings:** • Hormone profile but not initial symptom score was a statistically significant predictor of time to return to learn/work. • There was no statistically significant mean difference between hormone profile groups for initial symptom score or miR-27a-5p/miR-30a-3p. **Novel contribution:** • This is the first study to investigate concussion specific salivary biomarkers in relation female hormone profiles. • This is the first study to investigate time to recover from concussion in relation to hormone profiles.

	• This is the first study to use qPCR to measure miR-27a-5p/miR-30a-3p.

Chapter and Title	Chapter Content - Conclusions
9 **Summary and conclusions.**	**Question:** • What is the impact of the female sex hormones on concussion? **Findings:** • The sex difference observed in sport related concussion was unlikely to be due only to a reporting bias in females. • Menstrual cycle symptoms were congruent with concussion symptoms and changed across the menstrual cycle in the absence of injury. • Salivary concussion biomarkers miR-27a-5p/miR-30a-3p showed some cyclical variation across the menstrual cycle in the absence of injury. • Sub-clinical menstrual disturbance may alter miR ratio in the absence of concussion. • Hormone profile but not initial symptom score was a predictor of time to return to learn/work after concussion. • Salivary miR-27a-5p/miR-30a-3p was below 1.0 in females with concussion. • There was no meaningful difference in salivary miR-27a-5p/miR-30a-3p between different hormone profiles. **Practical application:** • There is a need for more targeted concussion education in community sport that also addresses the underlying motivation to disclose a suspected concussion, or not. • Health practitioners should consider hormone profile and related symptoms when managing females with concussion. • Salivary biomarkers may offer an objective tool for use in research and clinical settings. • Salivary measures of estrogen and progesterone may have utility to monitor within-person changes overtime multiple time points but are not yet valid for hormone profile verification. **Future research opportunities:** Studies in female athletes to understand the: • Motivations of female athletes to report concussion, or not. • Threshold ratio of miR-27a-5p/miR-30a-3p for concussion diagnosis. • Neuroendocrine response to concussion. • A consensus on the methodological approach to concussion research that includes females.

Chapter 2: Differences in injury profiles between female and male athletes across the participant classification framework: A systematic review and meta-analysis.

This chapter has been published as:

Hardaker, N.J., Hume, P.A., Sims, S.T. Differences in injury profiles between female and male athletes across the participant classification framework: A systematic review and meta-analysis. *Sports Medicine* 2024, 54(6): 1595-1665. http://doi.org/10.1007/s40279-024-02010-7

2.1 Overview

Background: Female sex is a significant determinant for ACL injury. It is not understood if sex is a key determinant for other sports related injuries. **Objective:** To identify where differences in injury profiles are most apparent between the sexes in all sports across the 6-tiered participant classification framework. **Methods:** This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the 'implementing Prisma in Exercise, Rehabilitation, Sport medicine and SporTs science (PERSiST) guidance. The databases, PubMed, CINAHL, Web of Science SPORTDiscus, Medline, Scopus, Cochrane Library and EBSCO were searched from inception to 24 April 2023. Longitudinal, prospective, and retrospective cohort studies, cross sectional and descriptive epidemiology studies that used standard injury data collection were included. Studies were excluded if injuries were not medically diagnosed and if injuries were not reported and/or analysed by sex. Two reviewers independently extracted data and assessed study quality using the Downs and Black checklist. **Results:** Overall, 180 studies were included (8 tier-5, 40 tier-4, 98 tier-3, 30 tier-2, 5 tier-1 studies; one study included data in two tiers). Of those, 174 studies were of moderate quality, and six studies were of limited quality. In sex comparable sports, there was moderate evidence that female athletes had greater risk of knee injury (RR 2.7; 95% CI: 1.4-5.5), foot/ankle injuries (RR 1.25; 95% CI: 1.17-1.34), bone stress injury (RR 3.4; 95% CI: 2.1-5.4) and concussion (RR 8.46; 95%CI 1.04-68.77) than male athletes. Male athletes were at increased risk of hip/groin injuries (RR 2.26; 95% CI: 1.31-3.88) and hamstring injuries (RR 2.4; 95% CI: 1.8-3.2) compared to females, particularly in dynamic sports. Male athletes were 1.8 (1.37-2.7) to 2.8 (2.45-3.24) times more likely to sustain acute fractures than female athletes, with the highest risk in competition. **Discussion:** Most studies in all cohorts were of moderate quality (mean/range of scores tier-5 17±2.2[14-20], tier-4 16.9±1.9[11-21], tier-3 16.9±1.5[11-20], tier-2 16.3±2.2[11-20], tier-1studies 15.6±1.3[14-17] out of 28 on the Downs and Black checklist), with only six studies of limited quality. Female athletes' propensity towards bone stress injuries highlights opportunities to reinforce development of optimal bone health during adolescence and to outline the effects of energy availability. Earlier strength

development and exposure to neuromuscular training programmes and modification of skill development in female athletes may be effective strategies in reducing lower limb injury risk. Key components of neuromuscular training programmes could be beneficial in reducing hip/groin and hamstring injury risk in male athletes. There may be a need for sex specific prevention and return to sport protocols for sports related concussion in female athletes. **Conclusions:** Female sex was a key determinant for sports related injuries beyond ACL injury and included foot/ankle injury, bone stress injury and sports related concussion. Male sex was a key determinant in hip/groin, hamstring injury and upper limb injury.

2.2 Introduction

Title IX is the federal civil rights law in the United States that was enacted as part of the Education Amendments of 1972. It prohibits sex-based discrimination of any education program that receives funding from the federal government and was pivotal in increasing the opportunities for females to participate in sport and exercise [9]. Sport is now a viable career option for female athletes and Tokyo 2021 was the first gender equal Olympic Games with females comprising 49% of participants and parity of medals available for male and female athletes [56]. In parallel with increasing participation of females in sport, a disproportionate increase in ACL injury has been observed, particularly in sports that involve rapid deceleration, change of direction or jump-landing movements [25-27, 57]. Female athletes have 4-10 times increased incidence of non-contact ACL injury [27, 57, 58].

There is a significant body of evidence supporting the understanding of ACL risk profiles in female athletes [25, 26, 57, 59-61] and the development of effective primary prevention programmes. Alterations in biomechanics, muscle strength, and neuromuscular control that females experience at the onset of puberty are different to those observed in males, these divergent changes may contribute to an increased susceptibility to ACL injury [25, 60, 61]. It is plausible that these and other factors create sex specific injury profiles across sport, but this has not been thoroughly investigated. Injury is a barrier to participating in training and/or competition, therefore understanding injury patterns across sport will contribute to the development of effective, targeted interventions to minimise the risk of injuries and support athletes in maintaining consistent participation in sport and exercise. Bittencourt's [62] complex systems approach for sports injuries moved risk factor identification to injury pattern recognition. The identified features of complex systems include relationships (interactions) among determinants, and regularities (profiles) that characterise emerging injury patterns. Sex is considered a contributing unit in the web of determinants. Throughout this review the term 'sex' is used to refer biological sex. That is sex assigned at birth, which is self-reported, and therefore it is assumed that athletes included in the studies of this review are unlikely to have undergone sex verification.

The aim of this review was to investigate original studies and identify where differences in injury profiles are most apparent between the sexes in all sports across the 6-tiered (0-5) athlete classification framework

[63]. For greater accuracy and to avoid recall bias, this review focused on medically diagnosed injuries (i.e., self-report injuries were excluded). Due to the volume of research in ACL injury mechanisms, risk and prevention [25-27, 57-61, 64-67], ACL injury was excluded from the current review.

2.3 Methods

The protocol for this review was pre-registered on Prospero; CRD42017058806 (last updated on 7th June 2023).

2.3.1 Literature search methodology

The PRISMA guideline [68] review methodology (literature search; assessment of study quality; data collection of study characteristics; analysis and interpretation of results; recommendations for practice and further research) and the 'implementing Prisma in Exercise, Rehabilitation, Sport medicine and SporTs science (PERSiST) guidance was used to evaluate the effects of sex on injury profiles in sport.

2.3.2 Search strategy

PubMed, CINAHL, Web of Science SPORTDiscus, Medline, Scopus, Cochrane Library and EBSCO databases were searched for terms linked with Boolean operators ('AND', 'OR'): 1. Gender OR Sex OR Male OR Female; 2. Sport* OR athlet* OR player; 3. Injur* OR "overuse injur*" OR "injury prevention" OR intervention; 1 AND 2 AND 3 in full text and English. Searching was from inception to 24 April 2023. Manual searching of reference lists and the 'Cited by' tool on Google Scholar were used to identify additional articles.

2.3.3 Study selection

Titles and abstracts of potentially eligible studies were screened by lead author (NH) with a 10% check by co-authors (PH and SS). Full text of all studies that were not excluded after initial title and abstract screening were retrieved and assessed for eligibility by lead author (NH) and independently checked by a co-author (PH). Disagreements between researchers during full-text screening were resolved through discussion with a third co-author (SS).

A study was considered eligible if it met the inclusion criteria provided in Table 2.1. Studies were included if:

(i) Injury rate and/or injury incidence was reported or analysed by sex;

(ii) Injury reporting was standardized (i.e., there was a consistent system in place for recording/documenting injuries;

(iii) Injury rate per player or injury incidence per athletic exposures and/or injury rate ratios were reported.

Papers were excluded if:

(i) They focused exclusively on ACL injuries;

(ii) In multi-injury studies, ACL injury data was not reported independently and therefore could not be removed;

(iii) Content was unavailable in English;

(iv) Content was unavailable in full text format;

(v) Data collection was through self-report and/or injury was not medically diagnosed;

(vi) The study did not provide additional information for any of the identified sections and subsections of this review.

Figure 2.1 shows the flow of information through the systematic review.

2.3.4 Injury definition

Acute and overuse/chronic injuries were included if sustained due to sports participation and if they were defined as requiring attention from a healthcare professional that resulted in diagnosis. Injuries were reported as per original data which included incidence rates (i.e., injuries per training and competition exposure hours or per total athlete exposures [AEs]) or prevalence (i.e., number of injuries per registered athlete or, percentage of registered athletes).

Table 2.1: Study inclusion and exclusion criteria.

Population	Inclusion: Healthy active participants, all levels of sport, up to 65 yrs.
	Exclusion: General population – i.e., not sport or activity related injuries reported, over 65 yrs.
Intervention	Inclusion: Standardized injury data collection intervention.
	Exclusion: Self report measure, not medically diagnosed injury.
Comparisons	Inclusion: Analysed and/or reported by sex.
	Exclusion: No sex analysis.
Outcomes	Inclusion: Injury rate, injury incidence, injury rate ratio, odds ratio, injury proportion ratios, reported by sex.
	Exclusion: Illness clearly from aetiology other than transfer of kinetic energy (as per definition in IOC consensus statement).
Study design	Inclusion: Longitudinal cohort studies, prospective and retrospective cohort studies, cross sectional, descriptive epidemiology.
	Exclusion: Systematic or narrative reviews, case reports and case series, commentaries and opinion pieces.
Other limiters	Inclusion: Full publications in the English language.
	Exclusion: Unavailable in English or in full text format, did not provide additional information for any of the identified sections and subsections of this review.

2.3.5 Athlete classification

All participants in eligible studies were considered 'athletes' and were classified according to the tiered system presented by McKay et al. [63]. Tier-0 is defined as 'sedentary' with criteria "do not meet minimum

activity guidelines and undertake occasional or incidental physical activity" (46% of the global population). Tier-5 is defined as 'World class' with criteria "Olympic and/or world medallists, world-record holders and athletes achieving within 2% of world-record performance and/or world leading performance, top 3-20 in world ranking, maximal or near maximal training within the given sport norms and exceptional skill level" (0.00006% of global population). Within this classification system, data relating to adolescent athletes has been sub-categorised due to the unique aspects of the developing musculoskeletal system.

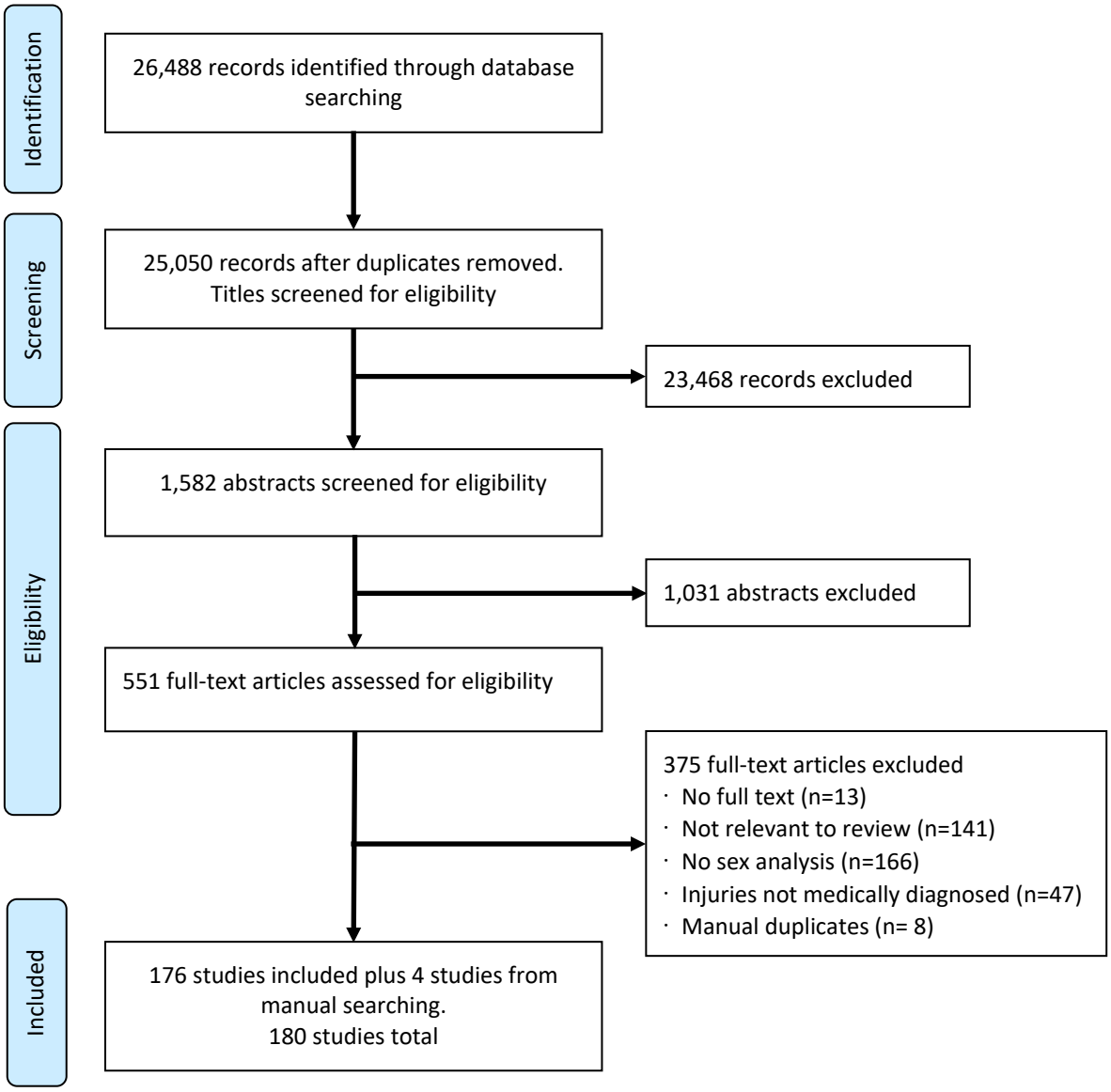


Figure 2.1: Flow diagram of identification, screening, eligibility and study inclusion of previously published studies.

2.3.6 Data extraction

Lead author (NH) extracted data from included studies; data were checked by a co-author (PH) and conflicts resolved after discussion with the third co-author (SS). Data extraction included study design, participant numbers and characteristics (age, sex, BMI), and study characteristics (data collection methods, type of injury, sex differences, type of sport, injury incidence rate [IIR], injury rate [IR], relative risk [RR],

incidence rate ratio [IRR] or injury rate ratio with confidence intervals [CI]). As a result of extracting data, it was clear that studies reported analysis for fractures and overuse injuries by sex, therefore analyses for fractures and overuse injuries are provided as types of injuries in addition to body site analyses.

2.3.7 Assessment of study quality

Studies identified for final inclusion were observational in nature and were therefore analysed using the modified Downs and Black checklist [18] that has good validity, test-retest and interrater reliability for methodological quality of randomised control trials [RCT] and non-RCTs. The Downs and Black checklist considered methodological quality of each study irrespective of the number and type of different out-comes assessed in each study. The modified Downs and Black checklist of 27 questions (Appendix V, Supplementary Table 2.1) related to quality of reporting (ten questions, with one question carrying 2 points), external validity (three questions), internal validity–bias (seven questions) and internal validity–confounding (six questions), and statistical power (one question). In the modified version the scoring of item 27 that rates whether the study performed a power calculation or not was changed from a score out of five to a score out of one to ensure consistent scoring across studies (zero points awarded if no power calculation was attempted, and one point awarded if a power calculation was described). On the reporting item the 'intervention' in this context was the data collection method described in studies. Similarly, in item four addressing 'compliance with the intervention', this was applied to the data collection method. The maximum possible score for the modified Downs and Black was 28.

Two authors (NH, PH) reviewed all included studies and SS also independently reviewed a randomly selected 10% of studies. With comparison of scoring between co-authors, any scores that differed were discussed at the conclusion of the review process and a final designation of the score agreed by consensus. There were disagreements be-tween researchers (NH and PH) on two papers, which were resolved through discussion with the third co-author (SS). As there were only two cases of ratings being different, kappa analysis was not reported. Each study was given a methodological quality index categorization [18] using the modified Downs and Black questions: poor (<7/28 with <25%), limited (8-13; 25-49%), moderate (14-21; 50-74%), or strong (21+; 75%+). If a study was of 'poor' or 'limited' quality; consideration was given to the direction of the effect reported. Greater weight was placed on moderate quality studies when drawing conclusions, if a single 'poor' or 'limited' quality study ran contrary to most of the moderate quality studies, those data were excluded from the key findings.

Meta-analyses by body site and injury type were conducted to calculate pooled fixed effects (Mantel-Haenszel method for dichotomous data) using RevMan 5.3 software. Analyses were completed where data collection and reporting methods were similar and allowed RR to be calculated. A pooled estimate for the IRR was calculated for each outcome with two or more studies and summarized in a forest plot. Heterogeneity was assessed using the I^2 statistic (>60% considered to represent substantial heterogeneity; >75% considerable heterogeneity). Where an outcome had an I^2 value between 30-60%, a random effects

model was used to cope with the moderate heterogeneity. For any outcomes that had an I^2 value greater than 75%, the meta-analysis was excluded due to substantial heterogeneity as per the Cochrane guideline [69].

2.4 Results

2.4.1 Studies extracted

A total of 26,448 studies were identified, 1,438 were duplicates and were removed. A further 23,468 studies were removed after screening by title and 1,031 removed after screening by abstract. After eligibility assessment of 551 full text articles, 375 additional studies were excluded due to: 1) full text not available (n=13); 2) not relevant/did not answer research question (n=141); 3) did not include sex analysis or injury rates (n=166); or 4) injuries were not medically diagnosed (n=47). A further eight studies were duplicates identified manually and excluded. Data from 180 studies were included in this review for analysis (see Figure 2.5).

Table 2.2 outlines studies that showed significant, or not, sex differences for overall (all injury types combined) injury rates across the classification framework. It is to be noted that the total number of studies reported in some tables adds up to 181 as one study [70] included data for two different tiers. Table 2.3 reports studies that showed significant, or not, sex differences by injury location.

Table 2.2: Studies that showed significant, or not, sex differences for acute and overuse combined injury rates for Tier 1-5 participants.

Tier	Significant sex difference	No significant sex difference	Not reported on
Tier-5: 8 studies	Team sports: 1 study [71] male athletes higher IIR. 1 study [72] female athletes higher IIR.	Team sports: 0 studies.	2 studies [73, 74].
	Individual sports: 0 studies male athletes higher IIR. 1 study [75] female athletes higher IIR.	Individual sports: 3 studies [76-78].	
Tier-4: 40 studies	Team sports studies: 5 studies [79-83] male athletes higher IIR. 1 study [84] female athletes higher IIR.	Team sports studies: 6 studies [85-90].	6 studies: [91-96].
	Individual sports: 9 studies [97-105] male athlete higher IIR. 5 studies [106-110] female athletes higher IIR.	Individual sports: 8 studies [70, 111-117].	
Tier-3: 98 studies (*3 studies in 2 categories)	Team sports: 9 studies [118-125]* [126] male athletes higher IIR. 8 studies [127-134] female athletes higher IIR.	Team sports: 10 studies [135-142] [143]* [144]*.	51 studies: [70, 145-194].
	Individual sports: 4 studies: [155, 183, 195] [125]* male athletes higher IIR. 7 studies [196-202] female athletes higher IIR.	Individual sports: 12 studies [203-212] [143]* [144]*.	
Tier-2: 30 studies (*2 studies in 2 categories)		Team sports: 7 studies [213-217] [218]* [219]*.	18 studies [220-237].
	Individual sports: 1 study [238] female athletes higher IIR.	Individual sports: 6 studies [218, 239-242]* [219]*.	
Tier-1: 5 studies	Team sports: 3 studies [243]* [244]* [245]* male athletes higher IIR. 0 studies female athletes higher IIR.	Team sports: 0 studies.	2 studies [246, 247].
	Individual sports: 3 studies [243]* [244]* [245]* male athletes higher IIR. 0 studies female athletes higher IIR.	Individual sports: 0 studies.	

*study includes data from both team and individual sports combined

Table 2.3: Studies that showed significant, or not, sex differences by injury location for Tier 1-5 participants.

Tier	Lower extremity	Upper extremity	Trunk	Head/Neck/Concussion
Tier-5	ADULTS: Willauschus, 2021 [78]; Engebretsen, 2010 [75]; Edouard, 2018 [76]	Trease, 2020 [74]; Engebretsen, 2010 [75]	Harris, 2020 [72]; Kim, 2020 [73]	Engebretsen, 2010 [75]; Junge, 2006 [71]
Tier-4	ADULTS: Abadi, 2021 [97]; Kim, 2021 [107]; Mattiussi, 2021 [117]; Edouard, 2014 [101]; Edouard 2015 [105]; Edouard, 2016 [99]; Gescheit, 2017 [108]; Hagglund, 2009 [79]; Hunt, 2017 [89]; Johansen, 2015 [109]; Larruskain, 2018 [82]; Moreno-Perez, 2019 [102]; Toohey, 2019 [81]; Traneus, 2016 [84]; Westermann [115]	Abadi, 2021 [97]; Chimera, 2022 [106]; Gescheit, 2017 [108]; Johansen, 2015 [109]; Traneus, 2016 [84]; Westermann, 2015 [115]	Abadi, 2021 [97]; Fröhlich, 2021 [98]; Moreno-Perez, 2019 [102]	Mertz, 2022 [95]; Vedung, 2020 [87]; Hill, 2019 [91]; Toohey, 2019 [81]
Tier-3	ADULTS: Chan, 2021 [161]; Comeau, 2023 [139]; Cross, 2010 [123]; Cross, 2013 [124]; Dalton, 2015 [146]; Eckard, 2017 [131]; Eckard, 2017 [143]; Fares, 2019 [210]; Fraser, 2021 [202]; Fulstone, 2016 [147]; Harmer, 2008 [208]; Kerbel, 2018 [151]Kerr, 2016 [70]; Lievers, 2020 [141]; Mauntel, 2017 [153]; Post, 2022 [133]; Roos, 2017 [138]; Sallis, 2000 [248]; Trojan, 2019 [158]; Tummala, 2018 [159]; Beynnon, 2005 [166]; Opar, 2014 [183]; Vaandering, 2022 [142]; Rizzone, 2017 [156]; Trojan, 2019 [133, 158]	Comeau, 2023 [139]; Deckey, 2020 [162]; Fares, 2019 [210]; Hibberd, 2016 [149]; Kerr, 2015 [207]; Lanzi, 2017 [175]; Melvin, 2018 [83]; Owens, 2009 [154]; Sallis, 2000 [248]; Schroeder, 2022 [140]	Hassebrock, 2019 [125]; Sallis, 2000 [248]; Schroeder, 2022 [140]	Covassin, 2003 [145]; Covassin, 2016 [169]; Deckey, 2020 [148]; Gessel, 2007 [148]; Hurtubise, 2015 [150]; Roos, 2017 [138]; Schroeder, 2022 [140]; Simmons, 2017 [157]; Zuckerman, 2015 [160]; Zupon, 2018 [90]; Vaandering, 2022 [142]; Zupon, 2018 [90]
	ADOLESCENTS: Belilos, 2023 [201]; Borowski, 2008 [129]; Cierna, 2017 [196]; Collins, 2008 [118]; Darrow, 2009 [127]; Fernandez, 2007 [170]; Hinton, 2005 [119]; Kerr, 2011 [172]; Kerr, 2018 [174]; Kolstrup, 2016 [130]; Marshall, 2020 [209]; Messina, 1999 [121]; Mitchell, 2015 [179, 180]; Nelson, 2007 [181]; O'Connor, 2020 [212]; Pierpoint, 2016 [198]; Powell, 2000 [128]; Rauh, 2000 [200]; Stracciolini, 2014 [187]; Stracciolini, 2015 [188]; Swenson, 2013 [190]; Swenson, 2013 [189]; Yard, 2008 [135]	Belilos, 2023 [201]; Collins, 2008 [118]; Hinton, 2005 [119]; Kerr, 2011 [173]; Kerr, 2018 [174]; Maciejewski, 2016 [205]; O' Connor, 2020 [212]; Pytiak, 2018 [184]; Stracciolini, 2014 [187]; Stracciolini, 2015 [188]	Cierna, 2017 [196]; Hinton, 2005 [119]; Kerr, 2018 [174]; Stracciolini, 2014 [187]; Stracciolini, 2015 [188]	Belilos, 2023 [201]; Borowski, 2008 [129]; Bretzin, 2021 [134]; Bretzin 2018 [167]; Cierna, 2017 [196]; Collins, 2008 [118]; Hinton, 2005 [119]; Huffman, 2008 [171]; Kerr, 2011 [172]; Kerr, 2017 [120]; Kerr, 2018 [174]; Lincoln, 2011 [176]; Marar, 2012 [177]; O' Connor, 2017 [182]; Tirabassi, 2016 [194]; Yard, 2008 [135]
Tier-2	ADULTS: Gill, 2021 [232]; Hollander, 2021 [233]; Mintz, 2021 [242]; Sugimoto, 2020 [237]	Gill, 2021 [232]; Mintz, 2021 [242]; Sugimoto, 2020 [237]; Rugg, 2021 [231]	Gill, 2021 [232]; Rugg, 2021 [231]	Gill, 2021 [232]; Morrissey, 2020 [236]

	ADOLESCENTS: Hosea, 2000 [223]; Honrado, 2021 [234]; Khodee, 2020 [235]; Owoeye, 2020 [215]; Leppanen, 2017 [224]; Pasanen, 2018 [225]; Quatman, 2009 [239]; Reid, 2012 [240]; Sokka, 2020 [216]; Willick, 2021 [238]; Zynda, 2022 [217]	Khodaee, 2020 [235]; Quatman, 2009 [239]; Reid, 2021 [240]; Robinson, 2014 [227]; Sokka, 2020 [216]; Forward, 2014 [222]; Hoge, 2020 [230]	Sugimoto, 2020 [237]; Polites, 2014 [226]; Quatman, 2009 [239]; Reid, 2012 [240]	Chatha, 2020 [214]; Chun, 2021 [218]; Delaney, 2014 [220]; Khodaee, 2020 [235]; Polites, 2014 [226]; Schallmo, 2017 [228]; Tsushima, 2019 [229]; Zynda, 2022 [217]; Hoge, 2020 [230]
Tier-1	ADULTS: Aman, 2019 [246]	Stogner, 2020 [245]		Aman, 2019 [246]; Xiao, 2022 [247]

2.4.2 Study methodological quality

Final quality rating (mean modified Downs and Black) scores are reported for studies in Tables 2.4 to 2.8 (individual criteria scores are in Supplementary Table S2). Quality rating scores (mean ±SD; range) were highest for tier-5 (17 ±2.2; 14-20), followed by tier-4 (16.9 ±1.9; 11-21), tier-2 (16.3 ±2.2; 11-20), tier-3 (16.9 ±1.4; 11-20) and tier-1 (15.6 ±1.3; 14-17). All studies clearly described their objectives and outcomes. Studies of limited quality were included in the results if they reported similar trends to the moderate quality studies. No studies were excluded on this basis. Risk of bias was initially assessed using the Risk of Bias In Non-randomized Studies - of Exposure (ROBINS-E) tool. The preliminary analysis questions in the ROBINS-E suggested that all studies could be considered at “high risk of bias” therefore no further detailed domain based RoB assessment was carried out. Internal validity scores in the Downs and Black checklist ranged from two to five out of a possible seven (Appendix V, Supplementary table 2.1).

Table 2.4: Participant characteristics and main findings for the Tier-5 athlete studies

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Willauschus 2021 [78]; 18, 64% Moderate	Foot & ankle injuries in taekwondo.	107 athletes (47% female) from a national Olympic taekwondo training centre were investigated prospectively for training & competition injuries over 5 yrs (January 1, 2014–December 31, 2019). Age 19.3±3.2 (15-27), male 20.9±3.4 (15-26), female 19.5±3.0 (15-27). yrs of experience 9.6±3.3 (6-20), male 10.3±3.8 (6-20), female 9.1±3.5 (6-20).	Overall IIR ankle joint 13.14/1000h (95%CI 1.5-25.9). No sig. sex diff.; male 14.8 (95%CI 2.7-17.9), female 12.7 (95%CI 3.6-21.1). For the foot 4.9 (95%CI 2.0-7.9). No sig. sex diff. male 5.3 (95%CI 2.0-7.8), female 3.9 (95%CI 2.6-7.0). The IIR during competition was higher overall for both ankle joint (training 2.9 [95%CI 2.0-3.7] competition 20.9 [95%CI 15.4-26.5] p<0.01) and foot injuries (training 2.7 [95%CI 2.0-7.9] competition 6.8 [95%CI 5.4-8.2]; p<0.01). This pattern was evident in males & females.
Harris 2020 [72]; 16, 57% Moderate	Rib stress injuries in 2012–2016 (Rio) Olympiad.	151 Australian rowing team athletes for 88773 athlete days. 85 qualified athletes (2013-15) plus 66 'shadow squad' athletes seeking qualification in 2016.	Overall IIR 0.2714/1000 athlete days (2013-15), shadow squad (2016) 0.1344/1000 athlete days. Rib injury prevalence 9.15%, (4%-15.4% over 2013-2015). Shadow squad prevalence 4.27%. Prevalence highest among lightweight athletes 13.04% in first 3 yrs of Olympiad. Overall, 4- year period prevalence for at least one rib stress injury 9.9% (incl. all 151 athletes monitored from 2013 to 2016) with 2.7% having more than one (n=4). Sex diff. in prevalence (p=0.05). Prevalence highest in females 11.94%, lightweight (13.04%) & sweep (11.49%) athletes in 2013–2015 Australian rowing team & in females (8.51%), lightweight (5.88%) & scull (8.11%) athletes in 2016 shadow squad.
Kim 2020 [73]; 20, 71% Moderate	Acute & overuse injuries in aquatic sports.	241 athletes, trained average of 4.5 hrs/day, 5 days/wk. For 1 yr, athletes were present at training centre for 9.5 months (41.25 wks). Swimmers & divers trained 4 hrs/day, 5 days/wk, for 9 months (39 wks); water polo players & artistic swimmers trained 5 hrs/day, 5 days/wk, for 10 months (43.5 wks). Swimming; 30 males 24.4±4.4 yrs, 1.84±0.03 cm, weight (kg) 78.9 (5.6), BMI 23.3 (1.0), 31 females 23.7±2.6 yrs, 1.71±0.02 cm, 63.4±1.3 kg, BMI 21.6±0.6. Diving; 32 males 23.6±0.4 yrs, 1.67±0.05 cm, 62.0±4.1 kg, BMI 22.3±0.2, 24 females 22.8±1.7 yrs, 1.56±0.04 cm, 52.3±0.9 kg, BMI 21.4±1.1. Water Polo; 73 males 24.4±3.4 yrs, 1.82±0.05	Overall IR 2.74 (95%CI 2.55–2.93)/1000 hrs of training. Swimming IR 4.60 (95%CI 4.04-5.17) (male 4.10, female 5.13 injuries/1000 hrs of training; RR = 1.25, [95%CI 0.34-4.57]). Diving IR 3.15 (95%CI 2.69-3.62), (male 2.61, female 3.72; RR = 1.43, [95%CI 0.29-6.92]). Overall IIR 13.41 (95%CI 12.48-14.34)/1000 AEs. Swimming IIR 19.37 (95%CI 16.99-21.75)/1000 AEs, (male 17.43, female 21.35/1000 AEs; RR 1.22 [95%CI 0.65-2.29]). Diving IIR 13.32 (95%CI 11.35-15.28)/1000 AEs, (male 11.18, female 15.51; RR 1.39 [95%CI 0.65-2.98]). Injury patterns by site & type - no sig. sex diff. (most notable diff. in diving, male = lower extremity, trunk & female = trunk, upper extremity).

		cm, 86.5±9.2 kg, BMI 26.0±2.8. Artistic swimming; 51 females 19.1±1.9 yrs, 1.63±0.03 cm, 53.13±4.0 kg, BMI 19.83±1.1.	
Trease 2020 [74]; 18, 64% Moderate	Acute & overuse injuries in rowing.	153 athletes over London 2012 & Rio 2016 Olympic cycles (8 yrs). Demographic information available as supplementary information online.	No sig diff. in height or weight of athletes in any year studied. Overall IIR for 2 Olympic cycles 4.1–6.4 injuries/Athlete Days (AD's). Chest wall injuries female RR 1.4 (95%CI 1.2-1.7). Low back pain female RR 0.5 (95%CI 0.4-0.6). Forearm injury female RR 1.06 (95%CI 0.8-1.5). Knee injury female RR 1.10 (95%CI 0.8-1.6).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Edouard 2018 [76]; 19, 68% Moderate	Acute & overuse injuries in gymnastics.	Not included.	Overall IR 84.1±17.5/1000 registered gymnasts (54 female (66%, 27 male (33%)). No sig. sex diff. (female 86.4±22.0 vs male 79.9±28.9/1000 gymnasts). Time loss IR 32.2±11.1/1000 gymnasts. No sig. sex diff. (female 27.2±12.8 vs male 41.4±21.2/1000 gymnasts). Sig. sex diff. for ankle injuries; female 27.4±18.7 vs male 3.4±6.7/1000 gymnasts & training injuries; female 51.4±25.3 vs male 17.2±15.0/1000 gymnasts. Overall female IR RR=1.08; (95%CI 0.69 to 1.68). Female time-loss RR=0.66; (95%CI 0.33 to 1.32). Female ankle injury RR=7.95; (95%CI 1.00 to 63.1). Female training injury RR=2.98; (95%CI 1.10-8.09).
Engebretsen 2010 [75]; 17, 61% Moderate	Acute & overuse injuries in winter sports; skiing, snowboarding, skating, hockey, luge, bobsled.	2567 registered athletes (1045 females, 1522 males) No further detail reported.	Overall IR; 111.8 injuries/1000 registered athletes. Sig. (p=0.003) sex diff. in overall IR; female 131.1/1000 athletes, male athletes 93.3. Face, head & cervical spine (female 19.7%, male 21.4%) & knee (female 16.1%, male 10.7%) most prominent injury locations. followed for females by wrist (8.0%) & for male athletes by thigh (10.0%). Contusions (female 31.6%, male 25.5%, ligament sprains (female 19.8%, male 10.6%) & muscular strains (female 8.1%, male 16.3%) most common injury types. Female overall injury RR=1.4 (95%CI 1.1 to 1.8).

Junge 2006 [71]; 14, 50% Moderate	Acute & overuse injuries in football, handball, basketball, field hockey, baseball, softball, water polo, volleyball, beach volleyball.	Specifics not included.	Sex diff. overall IIR of time-loss injuries male 24.7/1000 player matches female 15.9 ($p<0.05$). Handball IR higher in female 145/1,000 player hrs, male 89/1,000 player hrs. sig. ($p< 0.05$) more injuries in male players than in female players were expected to result in time loss male 40/1,000player hrs, female 36/1,000 player hrs. No diff. in incidence of time-loss injuries was observed between male & female players. Sig. sex diff. ($p<0.05$) in injury proportions; concussions female 4% vs male, 0.5% sprains female 19% vs male 9%, fractures female 3% vs male 6% & lacerations female 5% vs male 11%. Football: type of injuries differed sig. ($p<0.01$), with fewer contusions & lacerations & more sprains & concussion in females. Basketball; location differed sig. ($p<0.05$), with twice as many head injuries (female 29%, Male 15%).
Kim 2015 [77]; 14, 50% Moderate	Acute & overuse injuries in judo.	24 male, 24 female athletes training at facilities.	Total IR 4.1 injuries/year for each athlete; 4.0 injuries/male athlete (4.5 for lightweights & 3.5 for heavyweights, 4.2 injuries/female athlete (4.5 for lightweights & 4.0 for heavyweights). Annual injury incidence 4.2 injuries/1000 h of training/athlete; male 4.1 injuries/1000 h of training (4.6 for lightweights vs. 3.6 heavyweights ($z=4.7893$, $p<0.001$)). Female 4.3 injuries /1000 h of training (4.6 for lightweights vs. 4.2 for heavyweights ($z=1.9111$, $p>0.05$)). Sig. sex diff. ($p=0.0228$) for grade III injuries. However, this was sig. only in a comparison between male heavyweights vs. female heavyweights ($p=0.0041$) NOT between male lightweights & female lightweights 42 ($p=0.6347$). Weight category female OR-1.04 (95%CI; 0.78 to 1.37, & the distribution of injury rate in the two categories was analogous to each other. Sex & weight diff. in IR not statistically sig. ($p=0.8060$).

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; HR: hazard ratio; IRR: incidence rate ratio; RR: relative risk; IQR: interquartile range

Table 2.5: Participant characteristics and main findings for the Tier-4 athlete studies.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Abadia 2021 [97]; 19, 61% Moderate	MSK injuries in professional tennis players during tournament play.	161 tennis players (44%) female. Athlete rank; singles 742 (27–2668), doubles 595 (13–1886). Most players (63.4%) competed both in singles & doubles matches. Age(yrs) 22.0±4.1, Height (cm) 1.74±0.09, weight (kg) 68.6±10.4, playing experience (yrs) 15 (6–31). Data collected over 5 ITF tournaments in 2019.	Across all tournaments 1167.7 hrs of play caused 36 musculoskeletal injuries. Overall IIR 30.8 injuries/1000 player hrs (95%CI 28.2–33.5). Male IIR 35.7 injuries/1000 player hrs (95%CI 32.1–39.2), female IIR 23.6 injuries/1000 player hrs (95%CI 19.7–27.4), male RR 1.4 (95%CI 0.7-2.8; p=0.280). 15% female players sustained an injury, 22% males sustained an injury. Overall acute IIR 18.8 (95%CI 16.6–21.1), overuse IIR 2.6 (95%CI 1.7–3.5), recurrent IIR 9.4 (95%CI 7.7–11.1). 68% male injuries were acute, 4% overuse & 25% recurrent. 45% female injuries were acute, 18% overuse & 36% recurrent. 32% male injuries were to the upper limb, 48% to the trunk & 20% to the lower limb. 9% female injuries were to the upper limb, 18% to the trunk & 64% to the lower limb.
Chimera 2022 [106]; 16, 57% Moderate	MSK injury & illness during Canada Games competitions from 2009-2019.	Retrospective data analysis. 8710 male & 8391 female athletes. Summer games 10169 (5457 male; 4712 female), winter games 6932 (3253 male; 3679 female). 3160 injuries in male athletes, 3272 injuries in female athletes.	Male IIR 362.8/1,000 athletes, female IIR 389.9 (p=0.004). Shoulder injuries accounted for highest proportion of injuries in male (11.3%) & female (11.8%) athletes. Muscle strains accounted for the highest proportion of injury type in both male (36.4%) & female (37.8%) athletes. Acute injuries accounted for 54.3% injuries in male & 46.9% in female athletes. Chronic injuries accounted for 45.7% injuries in male & 53.1% in female athletes. Overall injury & illness incidences were sig. higher in Winter, cf. to Summer, Canada Games. No sig diff. in overall IIR in females competing in Winter cf. to Summer Games. In female athletes, highest IIR freestyle skiing. Female OR in softball 3.8 [95%CI 2.9-5.1], canoe-kayak 2.4 [95%CI 1.5-3.8], rowing 2.2 [95%CI 1.53.2], sailing 5.6 [95%CI 2.8-11.1], swimming 1.7 [95%CI 1.2-2.6], tennis 2.3 [95%CI 1.4-3.8], triathlon 3.8 [95%CI 2.0-7.1], fencing 22.0 [95%CI 6.7-71.9], alpine skiing 2.3 [95%CI 1.5-3.5], biathlon 3.1 [95%CI 1.8-5.2], judo 1.8 [95%CI 1.2-2.8], snowboarding 2.6 [95%CI 1.5-4.5].
Fröhlich 2021 [98]; 19, 68% Moderate	Acute & overuse injuries in elite competitive alpine skiers throughout an entire season (i.e., an off-season preparation period & competition period).	44 members of Swiss National Ski Team, 25 females & 19 males, competing at different international levels (i.e., WC, EC, FIS races). Overall sample 21.4±2.8 yrs, 172.3±8.7 cm, 72.9±10.7 kg, BMI 24.4±2.0 kg/m2. 25 females 21.0±2.7 yrs, 166.6±5.4 cm, 65.6±6.6 kg, 23.6±1.9 kg/m2. 19 males 21.8±3.0 yrs, 180.0±5.7 cm, 82.5±6.8 kg, 25.5±1.67 kg/m2. Male height, weight & BMI sig. [p<0.01], higher than females.	Across entire season; traumatic IR 120.5/100 athletes, overuse IR 63.6 & overall IR 184.1. Competition season; traumatic IR 77.3/100 athletes, overuse IR 18.2, & overall IR 95.5. Across entire season; traumatic injury female RR 1.031 (95%CI 0.630-1.690), overuse injury female RR 0.988 (95%CI 0.549-1.779), overall injury female RR 0.971 (95%CI 0.626-1.507). Sig. sex diff. for overuse injuries in competition season; female IR 24.0 (95%CI 20.7-27.3), male IR 10.5 (95%CI 7.4-13.7). No sex diff. for traumatic injuries (female 52.0 [95%CI 48.1-55.9], male 57.9 [95%CI 52.8-63.0]). Preparation period; traumatic IR 36.4 (95%CI 34.2-38.5), overuse IR 43.2 (95%CI 41.1-45.4), overall IR 70.5 (95%CI 68.4-72.5). Sig. sex diff. for traumatic injuries in preparation period; female 44.0 (95%CI 40.1-47.9), male 26.3 (95%CI 21.8-30.9). Sig. sex diff. for overuse injuries in the preparation period male 52.6 (95%CI 47.5-57.8), female 36.0 (95%CI 32.2-39.8). Type & body site of injury not reported by sex.

Kim 2021 [107]; 19, 68% Moderate	Acute & overuse injuries in adolescent judo. 10 months of data collection in 2019 prospective cohort study.	120 male athletes: 40 lightweight 18.82±2.31 yrs, 169.73±3.39 cm, 69.74±5.33, BMI 23.41±1.47, 40 middleweight 18.77±2.24 yrs, 177.48±4.21 cm, 85.11±6.45 kg, 25.11±1.68 kg/m2; 40 heavyweight 18.39±2.89 yrs, 181.24±6.86 cm, 108.82±12.49 kg, 33.98±4.95 kg/m2; 120 female athletes: 40 lightweight 18.13±2.90 yrs, 159.58±5.93 cm, 53.24±5.74 kg, 21.44±1.30 kg/m2; 40 middleweight 18.61±2.22 yrs, 166.19±3.55 cm, 67.45±4.57 kg, 23.94±1.99 kg/m2; 40 heavyweight 18.38±2.97 yrs, 172.09±4.32 cm, 90.11±11.87 kg, 31.33±6.63 kg/m2.	Overall IIR 12.60/1000 AEs, & the injury rate was lower in male athletes than in female athletes (female 15.66, male 9.49/1000 AEs; p<0.001). Sex diff. in IIR for all categories; Lightweight (male 10.24 [95%CI 8.15–12.71], female 16.71 [95%CI 14.02–19.77]), Middleweight (male 9.32 [95%CI 7.34–11.66]), female 15.46 [95%CI 12.91–18.37]), heavyweight (male 8.92 [95%CI 6.99–11.22], female 14.84 [95%CI 12.31–17.73]). Location of injuries was most frequent for lower extremity (39.71%), upper extremity (36.63%), trunk (16.69%), & head & neck (6.97%), female higher injury rate across all areas (p=0.023). Females higher rate of bone stress injury & tendinosis.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Mattiusi [117]; 19, 68% Moderate	Acute & overuse injuries in ballet. Prospective cohort study 2015-20.	123 elite professional dancers (66 female, 28.0±8.3 yrs; 57 male, 27.9±8.5 yrs across ranks of apprentice, artist, first artist, soloist, first soloist, principal & principal character artist. Dancers who joined or left company during study period were included for duration of their time in company.	283,453 individual dancer events (class: 99,733; rehearsal: 152,588; performance: 31,132). Scheduled dance events represented 417,693 hrs of individual dance exposure (class: 115,772; rehearsal: 209, 529; performance: 92, 392) over 5 seasons. Sig. main effect of company rank observed on medical attention injury incidence rate ($p<0.001$), medical attention IR lower in apprentices (2.5/1000 hrs 95%CI 1.9-3.2) than first soloists (4.5/1000 hrs; 95%CI 3.7-5.5; $p=0.003$) & principals (4.7/1000 hrs 95%CI 3.9-5.8; $p=0.002$). No sig. main effects of sex ($p=0.031$) or sex by rank ($p=0.659$) for medical attention IR or time loss IR (sex; $p=0.496$), sex by rank; $p=0.205$). Overall, injuries to distal lower extremity & joint/ligament tissue had greatest burden across all dancers. Ankle injuries (synovitis, impingement & bursitis) were greatest burden in female dancers. In male dancers, stress fractures to foot & lower leg had greatest burden. Nineteen of 21 stress fractures in male dancers were attributed to jumping & landing activities & 18 were non-traumatic.
Mertz 2022 [95]; 14, 50% Moderate	Maxillofacial injuries from NCAA Division I American Football, baseball, men's & women's basketball, men's & women's water polo, men's & women's tennis, men's & women's indoor volleyball, women's beach volleyball, women's lacrosse, & women's football.	Collegiate age (17-24 yrs) NCAA database 2015-19. No further demographic info included.	193 athletes across 13 sports sustained at least 1 maxillofacial injury over 4 athletic seasons. In males, American Football accounted for 26.4%, basketball 16.1% & water polo 14.0%. In females, basketball accounted for 9.8%, lacrosse 5.7%, & football 5.2%. Across all sports, male athletes experienced 70.5% maxillofacial injuries, while female athletes experienced 29.5% injuries. Overall incidence of maxillofacial injuries was 2.06/1000 AE hrs. Male IIR 1.92/1000AE, female 2.4/1000 AE hrs. Men's basketball (8.30 injuries/1000 AE hrs) & men's water polo (8.15 injuries/1000 AE hrs) highest IIR of all sports. Women's basketball highest IIR of women's sports (5.79 injuries/1000 AE hrs). Men's & women's tennis & baseball had lowest rates, all with fewer than 1/1000 AE hrs. Basketball male RR 1.43. Water polo male RR 3.79. Volleyball male RR 1.6.
Vedung 2020 [87]; 18, 64% Moderate	Concussion in football.	51 Swedish elite football teams. 959 players, 389 female 23±4.2 yrs, & 570 male 25±4.6 yrs.	Overall concussion game IIR 1.19/1000 hrs of game play, male 1.18, female 1.22 (NS). 1st league; concussion game IIR 1.27/1000 hrs of game play, male 1.33, female 1.23 (NS). Semi-pro; concussion game IIR 1.20/1000 hrs of game play, male 1.11, female 1.33 (NS). 83% concussions occurred during games. Two players sustained two concussions, & 36 concussions were distributed on 34 players. To compare the SRC IIR between male & female

			on the same level of play; female 1st league cf. to male 2nd league (females 0.8/1000 hrs of game play; males 0.9/1000 hrs of game play; p=0.79).
Allen 2012 [100]; 20, 71% Moderate	Acute & overuse injuries in professional ballet.	52. 27 female; 25±6 yrs, 162.2±3.7 cm, 49.2±4.0 kg & BMI 18.9±1.6 kg/m2. 27 male; 23±5 yrs, 179.6±4.3 cm, 71.7±4.7 kg & 22.2±1.4 kg/m2.	Overall IR 6.8 injuries/dancer (females, 6.3; males, 7.3; p>0.05). Severity of injury higher (p<0.05) in male dancers (9 days) than in female dancers (4 days). No sig. sex diff. for injury incidence due to extrinsic factors; females 1.49/1000 hrs, male 1.64/1000 hrs greater injury incidence related to intrinsic factors for males (3.12/1000 hrs) cf. to females (2.65/1000 h). Female had sig. (p<0.05) higher incidence of first-episode injuries (49%) than males & reported fewer recurrences (40%) & exacerbations (11%) (p<0.05). No correlation between age & incidence (r=-0.20, p=0.16) & age & number of injuries (r=-0.19, p=0.18).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Augusticova 2019 [111]; 16, 57% Moderate	Acute & overuse injuries in karate.	5 weight divisions per sex (<60 kg, <67 kg, <75 kg, <84 kg, & >84 kg for male athletes & <50 kg, <55 kg, <61 kg, <68 kg, & >68 kg for female athletes). All female bouts are scheduled for 2 minutes, all male bouts are scheduled for 3 minutes (real time).	Sig. sex diff. for IIRAE female 2.46, male 6.98. No sig. sex diff. for IIRME female 1.23, male 2.33. (Overall IIRAE & IIRME were 5.13 (95%CI, 3.82-6.74) & 1.98 (95%CI, 1.47-2.60, respectively. Female IIRAE RR, 0.35 (95%CI, 0.17-0.70). Female IIRME RR, 0.53 (95%CI, 0.27-1.05).
Bere 2015 [85]; 16, 57% Moderate	Acute & overuse injuries in volleyball.	No demographic info included. Competitions for under-18, under-19, under-20, under-21 & under-23 age categories were classified as junior events.	Match IIR 10.7/1000 player hrs (95%CI 9.5 to 12.0); male 10.5 (95%CI 7.6-13.4, female 7.8 (95%CI 5.5-10.1). No sig. sex diff. Time-loss IIR during match play 3.8/ 1000 player hrs (95%CI 3.0 to 4.5); male 2.1 (95%CI 0.8-3.3, female 2.6 (95%CI 1.3-3.9). No sig. sex diff. Overall injury male RR: 1.09, (95%CI 0.86 to 1.38). Incidence of time-loss injuries during match play was 3.8/ 1000 player hrs (95%CI 3.0 to 4.5). Time loss male RR 1.04, (95%CI 0.70 to 1.55).
Cierna 2017 [196]; 17, 61% Moderate	Acute & overuse injuries in karate.	295 karate athletes (119 female, 176 male)	Overall IIRAE, 35.6 (95%CI, 22.8-53.0, & overall IIRME 10.9 (95%CI, 7.0-16.2). No sig. sex diff.: females had a slightly higher IIRME & IIRAE. Female RRME 1.33 (95%CI, 0.61-2.90). Female RRAE 1.19 (95%CI, 0.54-2.60). Overall injury proportion 8.1 (95%CI, 5.2-12.1)
Edouard 2014 [101]; 18, 64% Moderate	Acute & overuse injuries in athletics.	Not included.	Sig. (p=0.001) sex difference in overall IR; Male 122.0±23.6, female 69.5±15.9/1000 registered athletes. Sig. (p=0.03) sex difference in time-loss IR; Male 56.9±16.5, female 31.5±9.4/1000 registered athletes. Male overall injury RR=1.8 (95%CI, 1.2-2.5, males time-loss RR=1.7 (95%CI, 1.0-2.9). Anatomical location differed sig. for location (p=0.03); specifically, groin (8.9% vs. 0%, thigh (27.8% vs. 28.6%, knee (17.8% vs. 4.8%, & ankle (8.9% vs. 7.1%) injuries were more frequent in males.
Edouard 2015 [105]; 17, 61% Moderate	Acute & overuse injuries in Athletics	Not included.	Sig. sex diff. for overall IR; male 110.3±6.8/1,000 registered athletes, female 88.5±6.7. Sig. sex diff. for overall outdoor IR; male 116.3±7.6/1,000 registered athletes, female 93.7±7.4. No sex diff. was found for cause & severity of injury. Sig. sex diff. time loss injury; male, 54.0±5.3, female 43.2±5.2. Sig. sex diff.; hip/groin IR male 6.9±1.9, female 3.0±1.4, thigh IR male 32.4±4.1, female 19.7±3.5, lower leg IR male 22.04±3.4, female 16.2±3.2. Sig. sex diff. stress fracture IR male 0.9±0.7, female 2.7±1.3, strain IR male 34.1±4.3, female 20.7±3.6. Overall injury male RR=1.25 (95%CI 1.13-1.37). Overall outdoor injury male RR=1.24 (95%CI 1.12 to 2.51). Male thigh injury RR=1.64 (95%CI 1.32-2.05, male lower leg RR=1.36 (95%CI 1.05-1.75) male hip/groin injuries RR=2.26 (95%CI 1.31-3.88), male muscle strains RR 1.64 (95%CI 1.33-2.04, male thigh strains RR 1.66 (95%CI 1.25-2.19, male stress fractures RR 0.32 (95%CI 0.12-0.81) than female athletes. Male sprint injury RR 1.32 (95%CI 1.06-1.66), middle distance injury RR 1.48 (95%CI 1.06-2.06), male race walk injury RR 2.55 (95%CI 1.27-5.10). Male time loss RR 1.25 (95%CI 1.07-1.46).

Edouard, 2016 [99]; 16, 57% Moderate	Acute & overuse muscle injuries in athletics.	Mean age not reported. Age groups: <20, 20-22, 23-25, 26-29, 30-34, >35. 3289 injuries in total.	1351 hamstring injuries. Overall incidence of muscle injuries sig. sex diff. Male 51.9±6.0 vs female 30.3±5.0 injuries/1000 registered athletes. Overall incidence of hamstring injuries sig. sex diff.; male 22.4±3.4 vs female 11.5±2.6 injuries/1000 registered athletes. Hamstring injury incidence in indoor training similar for both sexes; Male-0.8±0.3 vs female 0.9±0.3 injuries/1000 registered athletes. The proportion of muscle injuries sig. higher among male (45.6%) than female athletes (34.7%) ($\chi2$=18.1; p<0.001). For male athletes, the proportion of muscle injuries was sig. higher among time-loss & in-competition time-loss injuries (about 57%) than among all, training & in-competition injuries (about 46%; p<0.05). Of 1082 injuries during 9 international athletics championships, there were 185 hamstring injuries, constituting 17.1% total injuries. Hamstring injury frequencies were sig. higher in male (19.3%) than female (13.5%) athletes ($\chi2$=6.1; p=0.01); 61.1% (113). Muscle injuries; Male RR=1.71; (95%CI 1.45-2.01). Hamstring injuries; Male RR=1.94; (95%CI 1.42-2.66). Male hip/groin RR 3.01 (95%CI 1.54 to 5.87).

Reference & Quality score	**Focus**	**Participants characteristics, age (mean ±SD)**	**Findings/Comment**
Gescheit, 2017 [108]	All injuries musculoskeletal injuries (omitting lacerations/abrasions & bruising/ haematomas) in Tennis.	Male & female professional singles ranking of singles cohorts were 84±71 & 81±76, respectively. Junior & wheelchair tennis player injuries were excluded.	Female players overall IR: 201.7/10 000 GE, male 148.6 (Sig diff not reported). Female players IR 33.6±1.6/Australian Open tournament/year, male players 24.8±1.2. Most common male injury region; knee 3.5±1.6, ankle 2.3±1.3 & thigh 2.3±1.5 injuries/year. Most common in females; shoulder 5.1±1.1 injuries/year foot 3.2±1.1, wrist 3.1±1.5 & knee 3.1±1.1. No sex diff.
Hagglund 2009 [79]; 20, 71% Moderate	Acute & overuse injuries in football.	239 males (51%), 25±5 yrs (16-37), 79±6 kg. 228 females, 23±4 yrs (15-41), 63±6 kg.	Sig. sex diff. for overall training IR; Male 4.7 vs female 3.8/1000 hrs, (p=0.018) & match play male 28.1 vs female 16.1, p< 0.001). No sex diff. in severe IR (absence > 4 wks) (0.7/1000 hrs in both groups). Male players sig. great IR for 'muscle strain'; 2.6 vs 1.6/1,000 hrs & 1.0 vs 0.2/1000h. Greater proportion of hip/groin injuries in male players (18% vs 11%; p<0.02). Greater proportion of knee injuries (22% vs 16%; p<0.02) in female players (18% vs 11%; p<0.02). Greater proportion of meniscal/cartilage injury in female players (4% vs 1%; p<0.019). Greater proportion of tendon (11% vs 3%; p=<0.001) & contusion injuries (18% vs 11%; p<0.007) in male players. Greater proportion of overuse injuries (18% vs 11%; p<0.006) in female players.

Hame 2004 [88]; 18, 64% Moderate	Acute & overuse-fractures in baseball/softball, basketball, cross-country/track, golf, gymnastics, football, swimming/diving, tennis, volleyball, water polo, American Football.	No demographic info included. Collegiate sports in the USA	Overall IR of all fractures 0.0439/athlete (95%CI 0.039-0.049). No sig. sex diff. (females 0.04038 & males 0.0461). Overall IRR=1.041 (CI, 0.917-1.422, p=0.236). Female stress fracture RR; 3.37 (95%CI, 2.11-5.38; p=0.001). Male fracture RR, 1.803 (95%CI, 1.37-2.37; p=0.001). Of 349 total primary fractures, 269 (77.1%) were fractures & 80 (22.9%) were stress fractures.
Hamid 2016 [113]; 18, 64% Moderate	Acute & overuse injuries & illness at Asian games.	276; 167 (60.5%) male, 109 (39.5%) female. Median 23±5 yrs.	Overall IR 30.1/100 athletes. (Athletes in individual events had higher proportion of more severe injury (37.8% vs. 12.8%)). No sig. sex diff. (p=0.28) in overall IR; male 32.9/100 athletes (95%CI 24.8 to 42.9, female 25.7 (95%CI 17.1 to 37.1). Only sig. sex diff. in IR for hockey players (p<0.001). Male hockey RR; 15 (95%CI 2.3-631.5. No sex analysis on injury type.
Harringe 2007 [86]17, 61% Moderate	All injuries resulting in >1wk absence.	42. 16 males; 21.8±1.9 yrs, 178.9±4.1 cm, 77.1±9.6 kg, gymnastics experience 15.1±3.0 yrs, training 11.0±2.0 hrs/wk. 26 females; 18.0±2.4 yrs, 161.4±7.0 cm, 54.4±6.4 kg, gymnastics experience 12.8±3.3 yrs, training 11.0±2.0 hrs/wk.	Overall IR; 2.2 injuries/1000 gymnastics hrs for males (95%CI 1.1-3.4) & females 2.2 (95%CI 1.4-3.0). No sig. sex diff. No sex analysis for anatomical location.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Hill 2019 [91]13, 46% Limited	Concussion in cricket.	555. In 2015-2016 season 278; 172 male & 106 female domestic & international players. In 2016-2017 season 277; 179 male & 98 females.	Match IR; Male 7.2 head impacts/per 1000 player days 2.3 concussions/1000 player days. Female 3.7 head impacts/1000 player days, 2.0 concussions/1000 player days. No sex analysis. Male: 32% head impacts resulted in concussion. Female: 54% head impacts resulted in concussion. 'Inclusion criteria match-only head impacts & concussions of elite-level male & female players competing in Cricket Australia-sanctioned international or domestic matches. Per 1,000 player days. Batters (male & female) sustained 75% head (helmet) impacts. Most common batting mechanism was being struck by ball being bowled by fast bowler (67% all impacts, although this mechanism did not occur in women's cricket, likely as result of comparatively slower bowling.
Hunt 2017 [89]; 16, 57% Moderate	Acute & overuse foot/ankle injuries in NCAA Div I sports.	1076 (580 males, 496 females).	Sig. sex diff. Female 4.07 vs male 3.94 injuries/1000 AE's. Female athletes sustained foot/ankle injuries at a sig. higher rate (53%) cf. with male athletes (47%) ($p<0.05$). Female athletes sustained a sig. higher rate of MTIs than male athletes (0.99 vs. 0.72/1,000 AE's; $p<0.05$). Among total injuries, female athletes accounted for a sig. higher number of bone stress injuries (23%) than males (10%) ($p<0.05$). Bone stress injuries that resulted in MTI 69% females vs. 31% males. Tendinopathies; 65% females vs. 35% males.
Johansen 2015 [109]17, 61% Moderate	Acute & overuse injuries in skiing.	Not reported.	Overall absolute IR; 26.4 injuries/100 athletes/season. Sig. ($p<0.05$) sex diff. for overall absolute IR; male 23.2 injuries/100 athletes/season, female 34.6. Sig. sex diff. for; absolute knee IR female 10.1 injuries/100 athletes/season, male 3.7 injuries, & shoulder/clavicle injuries females 6.3 injuries/100 athletes/season, males 2.5 injuries. No sex diff. for other injuries. No sig. sex diff. for relative IR male 7.5 injuries/1,000 runs, female 9.6. Absolute female knee RR 1.49 (95%CI 1.07-2.08). Relative female knee RR 2.72, (95%CI 1.35-5.51). Female shoulder/clavicle RR 2.55, (95%CI 1.06-6.14).
Kerr 2016 [70]17, 61% Moderate*	Acute & overuse injuries in cross country running.	Age 17-24 yrs. Div I - Male: 127 injuries (46%); Female: 146 injuries (54%)	Overall practice IR 5.70/1,000 AE (4.69-6.72) Female 6.52 (5.40-7.64). Overall competition IR 3.41/1,000 AE (0.68-6.13) Female 9.50 (4.85-14.16). Overall total IR 5.53/1,000 AE (4.56-6.49) Female 6.75 (5.65-7.84). Sex diff. not sig.
Kirialanis 2002 [114]17, 61% Moderate	Acute & overuse injuries in Artistic gymnastics	100 male, 13±3.2 yrs, 38.8±12.8 kg, 144.8±17 cm; 87 female, 11.5±2.3 yrs, 32.4±9.6 kg, 138.2±13.1 cm.	Overall IR 1.3/gymnast/year. Annual incidence 1.4 injuries/1000 hrs of training. No sig. sex diff. in IR ($p=0.770$) male 139%, female 125.2%.

Larruskain 2018 [82]; 21, 75% Moderate	Acute & overuse injuries in football.	Male 50 Age 25±4yrs, Player seasons 127, Height 182±6cm, Body Mass 76±6kg. Female 35 Age 25±5yrs, Player seasons 99, Height 167±6cm, Body Mas 59±7kg.	Sig. sex diff. for Total injury incidence; male 8.31/1000 player hrs, female 6.3 (p<0.01, training; male 4.78, female 3.43 (p<0.02) & match; male 29.86, female 22.57 (<.04). Sig. sex diff. for overuse injury; male 4.19, female 2.84 (p<0.01). Lower limb injury; male 7.33, female 5.79 (p<0.02). Hip & Groin; male 1.62, female 1.02 (p<0.05). Pubalgia; male 0.44, female 0.04 (p<0.02). Hamstring strain; male 1.52, female 0.79 (p<0.01). Quadriceps strain; male 0.44, female 0.98 (p<0.01). Muscle contusion; male 1.52, female 0.32 (p<0.01). Total injury male RR; 1.32 (95%CI, 1.09-1.59). Training injury male RR; 1.39 (95%CI, 1.06-1.83). Match injury male RR; 1.32 (95%CI, 1.01-1.73). Overuse injury male RR; 1.48 (95%CI, 1.12 1.95). Lower limb male RR; 1.27 (95%CI, 1.04-1.55). Hip & Groin male RR; 1.58 (95%CI, 1.00-2.50). Pubalgia male RR; 11.10 (95%CI, 1.48-83.44). Hamstring male RR; 1.93 (95%CI,1.16-3.20). Quads male RR; 0.44 (95%CI, 0.24-0.82). Muscle contusion male RR; 4.82 (95%CI, 2.30-10.08). IPR quads strain; male 5%, female 16% (p<0.05). Knee ligament injury; male 3%, female 9% (p<0.05). Pubalgia male; male 5%, female 1% (p<0.05). Contusion; male 18%, female 5%. Total, training, & match exposure hrs per player-season were 17%-20% higher for men cf. to women (p<0.05). Furthermore, the male team had 35% more training sessions (p<0.01) & 30% more matches (p=0.02) per wk, but the match hrs/total hrs of exposure ratio similar (p=.94). IPR ACL injury; male 1%, female 4%.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Melvin 2018 [83]; 16, 57% Moderate	Acute & overuse upper extremity injuries in ice hockey.	No demographic information reported. Div I, 620 male injuries (86%) & 104 female injuries (14%).	Div I, male overall 231.51/100,000AE (95%CI 213.29-249.74), competition IR 735.40 (95%CI 668.84-801.95), practice IR 74.01 (95%CI 62.20-85.81); female overall IR 115.22 (95%CI 93.08-137.36), competition IR 307.76 (95%CI 236.17-379.35), practice IR 49.11 (95%CI 32.36-65.87). Div I overall male RR 2.01 (95%CI 1.63-2.47).
Major 2014 [92]; 16, 57% Moderate	Acute injuries in Snowboarders.	1432; 927 male, 505 female.	Overall IR 6.4/1000 runs. No sig. sex diff.; male 6.7 female 5.9. No sex analysis by discipline or injury type. Data not reported by sex for injury type. Overall injury male RR; 1.3 (95%CI 0.9-1.7).
McCurdie, 2017 [110]	Acute & overuse injuries in tennis.	Elite players. Age not reported. 700 injuries.	Overall injury rate 20.7 per 1000 sets played; Male players (17.7 injuries per 1000 sets played) had a lower injury rate than female players (23.4 injuries per 1000 sets played).
Moreno-Perez 2019 [102]; 16, 57% Moderate	Acute & overuse injuries in tennis.	162; 119 (73%) male age 20.7±5.2 yrs, body mass 73.9±7.3 kg, average playing experience 13.3±6 yrs. 43 female (27%) age 17.9±2.7 yrs, body mass 62.9±6.8 kg, average playing experience 10.6±3.5 yrs. One season.	Overall IR 1.03/1,000 games played. Overall IR for male 1.47±3.26 & female 1.18±1.43/1,000 games. Significance diff. not reported. Lower limb most common in males (38%), Trunk most common in females (41%). Muscle strain most common diagnosis in both sexes. Sex analysis not done on IR.
Neidel 2019 [93]; 16, 57% Moderate	Stress fractures in triathlon.	86 (47 male (55%) 39 female (45%)). Age 17.5±3.3) yrs. BMI 19.9±1.4).	OR Female 2.2 0.8-6.0 (p=0.1330).
Owoeye 2017 [80]; 15, 54% Moderate	Acute & overuse injuries in football.	18-32 yr, 756 male: 356 (54%), 116 injuries. 300 female (46%), 50 injuries.	Male players overall incidence 113.4 injuries/1000 hrs (95%CI 93.7-136.0) equivalent to 3.7 injuries/match & time-loss incidence of 15.6 injuries/1000 hrs. Female players had overall injury incidence of 65.9 injuries/1000 hrs (95%CI 48.9-86.8) equivalent to 2.2 injuries/match & time-loss incidence of 7.9 injuries/1000 hrs. Male players sig. higher risk of injuries [IRR=1.72 (95%CI 1.23-2.45)].
Park 2017 [104]17, 61% Moderate	Acute & overuse injuries in fencing.	Annually, 15 elite fencers (7 male, 8 female) for each weapon category (sabre, epee foil); total 45, male 21, female 24. Athletes trained on average 4.5 hrs/day, 5 days a wk. Over the year athletes were present at training centre for 11 months (47.7 wks, totalling 1073 hrs of training).	Total IR per athlete was 3.3 injuries/year. For sabre; 3.9 injuries/year (male 4.4; female 3.5, epee 3.2 (male 3.0; female 3.4) & foil 2.7 (male 2.9; female 2.4). Overall IR across fencing; 3 injuries/1,000 hrs. Sig. sex diff. for Sabre, overall IR 3.7 injuries/1000 hrs; male 4.1 vs female 3.3 (p<0.001). Epee, overall IR 3.0 injuries/1000 hrs of training; male 2.8 vs female 3.2 (p<0.02). Foil; overall IR 2.5 injuries/1000 hrs of training; male 2.7 vs female 2.2 (p<0.004). Injury by exposure-sig. sex diff.; overall IR 13.7 injuries/1000 AE; male 14.4 vs female 13.1 (p<0.0001). Sabre, overall IR 16.5 injuries/1000 AE, male 18.4 vs female 14.8 (p<0.0001). Epee, overall IR 13.5 injuries/1000 AE, male 12.5 vs female 14.3 (p<0.002). Foil, overall IR 11.1 injuries/1000 AE, male 12.4 vs 10.1 (p<0.001).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Park 2019 [103]; 18, 64% Moderate	Acute & overuse injuries in taekwondo	283 athletes. Age>18 yrs. Athletes trained an average of 4 hrs/day, 5 days a wk. Over the course of a year, Athletes were present at the training centre for 9 months (39 wks, totalling 780 hrs of training per year. Athletes participated in nine training sessions for each period of 2 wks (4.5 training sessions/wk).	Total IR per athlete was 4.6 injuries/year, sig. sex diff. stated but numbers not reported. Sig sex diff. for featherweight & welterweight categories; female higher IR per athlete-but numbers not reported. No sig. sex diff. for flyweight or heavyweight categories. IR per 1,000 hrs 5.9 injuries/1000 hrs of training. No sig. sex diff. across any weight categories. Exposure; overall 26.1 injuries/1000 AE, with lower rates in male athletes (male 20.5 vs female 31.7 injuries/1000 AE). No sig. sex diff. in the flyweight or heavyweight categories. Sig. sex diff. in featherweight & welterweight athletes. Per 1,000 hrs of training female overall RR 1.54(0.48 to 4.97). Overall IR AE; female RR=1.55, 95%CI 0.89 to 2.68). By AE-Featherweight; female RR=1.82, 95%CI 1.05-3.14; welterweight; female RR=2.27, 95%CI 1.41-3.68. Similar distributions of affected body regions in male & female athletes (p=0.98)
Steenstrup 2014 [94]; 16, 57% Moderate	Acute head injuries in alpine & freestyle skiing, snowboarding.	5247 snowboard, freestyle & alpine skiing athletes during seven seasons (2006-2013, (3203 male & 2044 female).	Sig. sex diff. (p<0.05) for overall absolute IR male 3.9 injuries/100 athletes, female 5.8. & in Freestyle male 4.5, female 7.4 & snowboard male 3.8, female 7.3. women had a higher injury incidence cf. with men, while no sig. sex diff. in alpine skiing; male 3.4, female 3.6. 1.05 (0.65 to 1.70). No sig. sex diff. overall or within disciplines for head/face injuries/1000 competition runs. Overall absolute female RR 1.48 (1.15 to 1.90). Snowboard female RR 1.93 (1.27 to 2.91). Freestyle female RR 1.63 (1.07 to 2.47). Alpine female RR 1.05 (0.65 to 1.70).
Toohey 2019 [81]; 19, 68% Moderate	Acute & overuse injuries in rugby sevens.	90; (55 male & 35 female) were under surveillance during 2-year period, over which 8457 on-field player participation hrs were completed. From the male squad 46 (83.6%) players (age 24.0±3.8 yrs) sustained 213 injuries; female squad 27 (77.1%) players (age, 22.9 6 3.1 yrs) sustained 152 injuries over 2 seasons.	Overall IR 43.2/1000 player-hrs. Sig. (p<0.05) sex diff. overall IR male 45.0/1000 player-hrs, female IR 40.8. Overall IRR 1.36; (95%CI, 1.35-1.37). No sig. sex diff. overall-male IRR 1.39; (95%CI, 0.38-1.40, female IRR 1.32; (95%CI, 1.31-1.34). Female IRR, 0.91; (95%CI, 0.90 0.91). Time-loss injuries female IRR, 0.90; (95%CI, 0.89-0.91) & non-time loss injuries Female IRR, 0.92; (95%CI, 0.91-0.94). Trunk region injury female RR, 1.75; (95%CI, 1.28-2.40; p=0.010). Head/neck region injury female RR, 0.58; (95%CI, 0.37-0.93; p=0.011). Hip/groin injury Female RR, 1.67;(95%CI, 1.22-2.28; p=0.017). Lumbar spine injury female RR, 1.91; (95%CI, 1.38-2.64; p=0.018). Head injury male RR, 0.54; (95%CI, 0.32-0.92; p=0.010). Burden of injury-male: non-anterior cruciate ligament (non-ACL) knee injuries (120-day absence/1000 player-hrs, ACL knee injuries (117-day absence, & ankle sprains (106-day absence). Female: foot sprains (157-day absence per 1000 player-hrs, ACL injuries (127-day absence-*note longer than male) & ankle sprains (118-day absence). Male hamstring strains 103 (vs female 31-day absence per 1000 player-hrs) & concussion 53 (vs female 9-day absence per 1000 player-hrs).

Traneus 2016 [84]17, 61% Moderate	Acute & overuse injuries in floorball.	122 male, 25±4.6 yrs, floorball experience 12.7±4.6 yrs, exposure floorball preseason 5.3±0.9 hrs/wk, exposure floorball game season 6.3±0.9 hrs/wk. 116 female 22±4.2 yrs, floorball experience 10.9±3.2 yrs exposure floorball preseason 5.0±0.6 hrs/wk, exposure floorball game season 6.0±0.6.	Injury incidence; male 2.6/1000 exp hrs & 3.9 female. Of 238 floorball players, 34 players (14%) sustained 38 injuries during preseason & 101 players (42%) sustained 148 injuries during game season. In total; 0.8 injuries/player. Higher proportion in female 57% than male 43% (p=0.02). Male ACL 2 injuries (1%, 11 female (6%) (p=0.02). No sig. sex diff. in injury location in pre-season. No sig. sex diff. for overuse injuries. During season sig. sex diff. in the % ankle (male 10, female 15.5; p=0.0004) knee (male 4, female 11; p=0.008) & shoulder (4 male, 0.5 female; p=0.04) injuries. Across full season, sig. sex diff. in % ankle (7.5 male, 20 female; p=0.0003) & knee injuries (male 7, female 15.5; p=0.007). Sig. sex diff. for traumatic injuries in % ankle (5.5 male, 15 female; p=0.001) & knee (3 male, 11 female; p=0.001).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Westerman 2015 [115]; 16, 57% Moderate	Acute & overuse injuries in gymnastics.	119, male 64, female=55. No further demographic info included.	Overall IIR female 9.37/1,000 AE male 8.78. No sig. sex diff. Sig sex diff. for; Neck clavicle-male 0.59, female 0.14, Wrist/hand/finger/thumb-male 2.12, female 0.61, Hip/groin/thigh-male 0.07, female 0.65, Ankle/Foot/heel/toe-male 1.54, female 3.64. Neck clavicle-female RR 0.24. Wrist/hand/finger/thumb-female RR 0.29. Hip/groin/thigh-female RR 8.92. Ankle/Foot/heel/toe-female RR 2.37. Symptom resolution for all symptoms was categorized as 1 hour, 0.1 hour to 1 day, 0.1 day to 3 days, 0.3 days to 1 wk, 0.1 wk to 2 wks, 0.2 wks to 4 wks, & 0.4 wks. We classified SRCs that had a symptom resolution time over 4 wks as having a long symptom resolution time.
Wolf 2009 [116]17, 61% Moderate	Acute & overuse injuries in swimming.	94 swimmers; 44 male, 50 female. Divs. I college swimmers.	Overall IIR male-4.00 injuries/1000 AEs vs. female 3.78 injuries/1000 exposures not sig. No sig. sex diff. by injury location or discipline (Sprint vs. distance) or stroke (freestyle, fly, breaststroke, backstroke). Overall male RR, 1.33 (0.81-1.37).
Yang 2012 [249]; 18, 64% Moderate	Acute & overuse injuries in baseball, basketball, cross-country & track & field, football, gymnastics, swimming & diving, wrestling, rowing, football, softball, volleyball.	573 injured athletes; 319 male & 254 female.	Sex comparable sports sig. sex diff. overall; acute IR male 30.4 vs female 27.3/10 000 AE's (p<0.0001). Overuse IR female 16.8 vs male 14.9 per 10 000 AE's (p<0.0001). The highest overuse IR was in female field hockey 70.5/10 000 AEs. The highest acute IR across sports was in female football, with 190.0/10 000 AEs. No sex diff. in types of overuse injuries in 10 sex-comparable sports. RR for acute vs overuse injury higher among male athletes; 3.67 (95%CI=3.02, 4.46) than female athletes; 1.53 (95%CI=1.28, 1.83). Male athletes had a lower proportion of sprains & strains (60.1% vs. 66.6%, p=0.0465) & a higher proportion of open wounds (8.1% vs. 3.5%, p=0.0044). Male athletes sustained more major injuries (34.0% vs 26.8%) & fewer no-time-loss injuries (27.2% vs 45.9%) (p=0.0021). Sex diff. in injury severity for overuse injuries; proportion of major injuries incurred by male athletes almost twice that of females (45.9% vs 23.1%, p<0.0001) in 16 study sports & 10 sex-comparable sports (49.0% vs 29.4%, respectively, p=0.0002).
Zupon 2018 [90]; 15, 54% Moderate	Back, neck & spine injuries in ice hockey.	College age 17-22 yrs. Demographic data not included. Men's ice hockey data: 39 Division I programme provided 87 team-seasons. Women's ice hockey data: 13 Division I programme provided 31 team-seasons.	Male competition IR 1.22 (95%CI 0.95–1.50), practice IR 0.23 (95%CI 0.16–0.30), overall IR 0.47 (95%CI 0.39–0.55). Female competition IR 0.84 (95%CI 0.44–1.24), practice IR 0.24 (95%CI 0.11–0.37), overall IR 0.40 (95%CI 0.26–0.54). No sig. sex diff.

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; HR: hazard ratio; IRR: incidence rate ratio; RR: relative risk; IQR: interquartile range.

Table 2.6: Participant characteristics and main findings for the Tier-3 athlete studies.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
ADULTS			
Chan 2021 [161]; 17, 61% Moderate	Severe foot injuries in 25 NCAA sports.	NCAA data: 2004-2005 to 2008-2009 & 2009-2010 to 2013-2014. 3,607 injuries included. College age 17-22yrs. Demographic data not included.	Overall IIR 30.62/100,000 AEs. Among all foot injuries, the operative rate was 4.6%, the season ending rate 6.9%, & the career-ending rate 1.0%. Male sports with the highest foot IIR football 56.20, cross-country 38.91, basketball 38.77, & American Football IIR 34.98. Female sports with the highest foot IIR gymnastics 88.58, cross-country 52.88, football 46.48, & outdoor track 46.28. Overall severe IIR; female (5.89), male (5.64). Male sports with highest severe foot IIR basketball 10.71, indoor track 7.16, & American Football 7.08. Female sports with highest severe foot IIR cross-country 17.15, gymnastics 14.76, & outdoor track 14.65. Sex diff. in operation rate for severe foot injury (male RR 2.43; 95%CI 1.71-3.44). Sex diff. in season ending injuries (male RR 1.89; 95%CI 1.48-2.41). Severe injuries sustained by male athletes were more likely to be due to contact than in female athletes (RR 2.23; 95%CI 1.76-2.83). Male sports contact (v non-contact) injuries RR 1.56 (95%CI 1.39-1.75). Female sports contact (v non-contact) injuries 0.43 (95%CI 0.34-0.55).
Chandran 2016 [122]; 15, 54% Moderate	Acute & overuse injuries in football.	18,239 injuries included. College 17-22 yrs. Demographic data not included.	Over 2004-09; Overall IR female 7.3, male 7.6/1,000 AE's ($p<0.05$). New injuries IR female 6.3, male 6.7/1,000 AE's ($p<0.05$). Contact injuries (no significant difference). Non-contact IR female 2.7, male 2.9/1,000 AE's ($p<0.05$). Game IR female 14.3, male 17.1/1,000 AE's ($p<0.05$). Practice IR female 5.0, male 5.0/1,000 AE's (no significant difference). Short term IR female 4.1, male 4.5/1,000 AE's ($p<0.05$). Long term injuries (no significant difference). Injuries requiring surgery IR female 0.19, male 0.12/1,000 AE's ($p<0.05$). Overall female RR 0.95. New injuries female RR 0.94. Non-contact injuries female RR 0.91. Game injuries female RR 0.84. Short term injuries female RR 0.91. Injuries requiring surgery female RR 1.63.
Comeau 2023 [139]; 20, 71% Moderate	Acute & overuse injuries in ice hockey, basketball, football, wrestling, & volleyball, American Football (male only) & rugby (female only).	295, 182 male & 113 female varsity athletes attending a single Canadian University. Males 21.0±2.3 yrs, 184.2±8.2 cm, 90.5±17.6 kg, BMI 26.6±4.6 kg/m2. Female 20.1±2.2 yrs, 169.6±7.3 cm, 69.9±10.6 kg, 24.3±3.1 kg/m2.	Total proportion of athletes reporting one or more injuries, male 68.7%, females 68.1% (NS). Greater proportion female wrestlers (100%) with at least one acute injury cf. to male wrestlers (40.0 %) ($p<0.05$). No sex diff. for chronic injuries across sports. Greater proportion of athletes sustained an acute injury cf. to chronic injury within each sex overall. Overall IR no sex diff.; female 1.4 injuries/athlete, male 1.4/athlete. Hockey; male 2.1, female 1.5 ($p<.05$), basketball; female 2.4, male1.5 ($p<.05$) & football; male 1.6, female 0.6 ($p<.05$). Most common injury locations were hip, groin, or thigh & lower leg, ankle or foot for both male & female athletes. Sig. higher proportion of female wrestlers experienced shoulder injury (62.5%) cf. to male wrestlers (0.0%). Sig. higher proportion of female basketball players experienced an injury to hip, thigh, or groin (50.0%) cf. to male players (6.7%). Male football players (40.0%) reported a higher proportion of hip, thigh, or groin injuries cf. to female football players (4.5%). Sig. higher proportion of male football players (50.0%) sustained a muscle/tendon injury cf. to female football players (18.2%). Overall, male &

	female athletes did not differ sig. in mean number of events a player missed due to injury (male=4.8, female=5.4). When cf. on sport-by-sport basis, female athletes missed more events cf. to respective male team in hockey (female=11.1, male=2.1, $p<0.05$), basketball (female=6.4, male=3.1, $p>0.05$) & volleyball (female=9.3, male=4.3, $p>0.05$). Mean time to injury as percentage of competitive season overall male 32.3%, female 28.9 % (NS), basketball male 42.6 % or 67 days, female 17.9 % or 28 days ($p<.05$), & volleyball male 41.2% or 65 days, female 8.8% or 14 days ($p<.05$). Time to concussion or head trauma differed between males & females (collapsed across sport) where females had sig. shorter time to concussion cf. to males ($p<0.5$).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Covassin 2003 [145]; 17, 61% Moderate	Concussion in football, lacrosse, basketball, softball/baseball & gymnastics.	College age 17-22yrs. Demographic data not included.	Concussions accounted for 6.2% all reported injuries. Highest percentage of concussion: female lacrosse players (13.9%) in game followed by female football (11.4%), male ice hockey (10.3%), male lacrosse (10.1%), football (8.8%), female basketball (8.5%). Male incidence of concussion higher in practice, female concussion incidence higher in games. Football game injuries - Female 2.04/1,000 AE vs. Male 1.10/1000AE. Lacrosse game injuries - Female 1.37/1000AE vs. Male 1.41/1000AE. Basketball game injuries - Female 0.54/1000AE vs. Male 0.29/1000AE. Softball/baseball game injuries - Female 0.34/1000AE vs. Male 0.43/1000AE. Gymnastics competition injurie - Female 0.11/1000AE vs. Male 0/1000AE. Female concussion higher ($p<0.01$) in games than practice in; lacrosse (IDR 3.26), football (IDR 14.57), basketball (IDR 2.35), field hockey (IDR 1.59). Male concussion higher ($p<0.01$) in games than practice in; hockey (IDR 17.35), lacrosse (IDR 10.07), football (IDR 13.75), basketball (IDR 2.07), baseball (IDR 5.38).
Covassin 2016 [169]; 18, 64% Moderate	Concussion in football, basketball, ice hockey, lacrosse, softball, baseball.	Male: 779 concussions (46%) Female: 903 concussions (54%). Some athletes and multiple concussions. College age 17-24 yrs. Actual number of athletes not reported.	In sex comparable sports, sex diff. ($p<0.05$) in; softball/baseball female IR 2.34/10,000AE's, male IR 1.20, basketball female IR 4.67, male IR 3.34, football female IR 6.45, male IR 4.19. No sig. diff. in ice hockey female IR 6.57 male IR 5.94 or lacrosse female IR 4.99, male IR 5.44. Baseball/softball female RR=1.95, (95%CI=1.45-2.65), Basketball female RR=1.4 (95%CI=1.17-1.67), Football female RR=1.54 (95%CI=1.31-1.83), Ice hockey female RR=1.1 (95%CI=0.84-1.30), Lacrosse male RR 1.1 (95%CI=0.832-1.43). Females typically had greater time loss from sport following concussion than male athletes in both training & competition related injuries. In this study only by 1-3 days.
Cross 2010 [123]; 16, 57% Moderate	Acute & overuse hamstring injuries in Football, field hockey, American Football, tennis, cross country	NCAA athletes Aged 17-24 yrs. Specific age not reported. Male: 1277 injuries (81% injured) Female: 299 injuries (19%)	Overall hamstring strain male IRR = 1.62 (95%CI 1.28-2.05). Highest IR in male sports; football (6.91 injuries/10,000 athlete-exposures [AEs]) & American Football (6.04/10,000 AEs). Highest IR in female sports; football 3.81 injuries/10,000 AEs & field hockey 3.79/10,000 AEs.
Cross 2013 [124]; 17, 61% Moderate	Acute & overuse hamstring injuries in football.	519 injured athletes. 309 male injuries (59%), 210 female injuries.	Sex diff. ($p<0.05$) in; overall IR male 0.58/1,000 AE's, female 0.37, game IR, male 1.2, female 0.5, practice IR, male 0.41, female 0.31 & during in-season males 0.49, female 0.25. No sig. sex diff. in injury rates during preseason. Male athletes had higher proportion of recurrent hamstring strains than female athletes (Male, 22%, female 12%; $p=0.003$). Overall Male IRR 1.64 (95%CI 1.37-1.96), game male IRR 2.42 (95%CI 1.82-3.23), practice male IRR 1.34 (95%CI 1.06-1.68), in-season male IRR 1.98 (95%CI 1.56-2.52). Preseason male IRR 1.04 (95%CI 0.79-1.37).

Dalton 2015 [146]; 17, 61% Moderate	Acute & overuse hamstring injuries in American Football, wrestling, volleyball, field hockey, gymnastics, ice, hockey, football, basketball, lacrosse, baseball/softball, indoor & outdoor track, cross country, tennis, swimming & diving.	No demographic info. reported. 1142 injuries reported	Overall IR 3.05/10,000AE's. Overall competition IR 5.24/10,000AE's, overall practice IR 2.56/10,000AEs. Overall competition RR = 2.05 (95%CI, 1.81-2.32). American Football, men's football & women's football contributed greatest number of hamstring strains (35.3%, 9.9% & 8.3% respectively). Sex analyses: football overall male RR=1.60 (95%CI 1.22-2.11), male competition RR=1.96 (95%CI 1.29-2.98), male practice RR=1.46 (95%CI 1.02-2.10). In women's football, preseason rate higher than in-season/postseason (RR=2.01 [95%CI 1.33-3.04]). No sig. diff. found in males across the season. Basketball overall female RR=2.05 (95%CI 1.19-3.53) practice female RR=2.56 (95%CI 1.30-5.05). In women's basketball, preseason rate higher than in-season/postseason (RR=3.07 95%CI 1.61-5.86). No sig. diff. found in males across the season. Baseball/softball overall male RR = 1.66 (95%CI 1.07-2.59), competition male RR=4.51 (95%CI, 2.90-6.12). Indoor track overall male RR=1.88 (95%CI, 1.26-2.78) practice male RR=2.37 (95%CI, 1.46-3.85).
Deckey 2020 [162]; 18, 64% Moderate	Hand & wrist injuries in basketball.	NCAA database 2009-14. College age 17-22yrs. Demographic data not included.	Hand & wrist injuries IIR female 4.20/10,000 AEs, male 7.76. Female competition (cf. to practice) IRR 2.40 (95%CI 1.54-3.72) male competition (cf. to practice) IRR 3.31 (95%CI 2.45-4.47). Female preseason IPR (cf. with male) 0.47 (95%CI 0.26-0.85); female in-season IPR (cf. to male) 0.59 (95%CI 0.44-0.80). Guards had the highest IIR. Female guards IRR 1.19 (95%CI 0.83-1.72). Female centres IRR 0.89 (95%CI 0.41-1.92) female forwards IRR 0.61 (95%CI 0.36-1.06). Contact injuries were the most common injuries in females (96.0%) & males (90.1%) (NS).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Deckey 2020 [126]; 15, 54% Moderate	Neck & cervical spine injuries.	NCAA data from 2009-14. Across 22 sports.	Overall IIR 7.05/100,000 AEs. In sex-comparable sports, male IIR 2.66/100,000 AEs, female IIR 1.95/100,000 AEs. In male sports, American Football had the highest IIR 29.09/100,000 AEs. In female sports, ice hockey had the highest IIR 11.51/100,000 AEs. Overall IIR was higher during competition (17.90) than practice (4.54) per 100,000 AEs). Competition RR 3.94 (95%CI 3.27-4.75). In sex comparable sports, male competition (v practice) RR 3.34 (95%CI 2.04-5.46), female competition (v practice) RR 5.57 (95%CI 2.96-10.49). Gymnasts' female competition (v practice) RR 29.81 (95%CI 2.70-328.75). Contact comprised the largest proportion of neck & cervical spine injuries (n = 9576, 83.2%) in all sports. Among sex-comparable sports, contact mechanism was evident in male sports 63.1%, & female sports 84.6%. In sex-comparable sports, female contact RR 1.34 (95%CI 0.88-2.05), male non-contact RR 4.25 (95%CI 1.06-16.98). In sex comparable sports, 73.4% male athletes (n=1,147) returned to play within 24 hrs, & 53.2% female athletes (n=613) returned to play within the same time frame. Male athletes 1.38 times more likely than female athletes to return to play within 24 hrs of injury (IPR 95%CI 0.79-2.41). In sex comparable sports, strains/contusions were most common in both male & female athletes (71.7% & 96.1%, respectively).
Eckard 2017 [131]; 16, 57% Moderate	Quadriceps strain in American Football, wrestling, field hockey, gymnastics, volleyball, baseball/softball, basketball, cross country, ice hockey, lacrosse, football, swimming & diving, tennis, track.	414, Collegiate age athletes. 116 male (28%), 298 female.	Overall IIR 1.07/10,000 AE's. In sex-comparable sports; overall IIR female 1.97/10,000 AEs vs male 0.65 ($p<0.05$); RR 3.03 (95%CI 2.45-3.76). Sig. diff. ($p<0.05$) in sex comparable sports; pre-season female 4.46/10,000AE vs male 1.08/10,000AEs; female RR 4.32 (95%CI 3.12-5.99), the regular season female 1.08/10,000AE vs male 0.53/10,000AEs, in competition female 1.79/10,000AE vs male 1.11/10 000 AEs; RR 1.61 (95%CI 1.08-2.41) & in practice female 2.02/10,000AE vs male 0.53/10 000 AEs; Practice female IRR 3.81 (95%CI 2.94-4.94). Sport specific; regular season IIR baseball/softball female 1,19/10,000AE vs male 0.35/10,000AE's & indoor track female 2.19/10,000AE vs male 0.69/10,000AE's. Sport specific; regular season baseball/softball female RR 3.41 (95%CI 1.25-9.30) & indoor track female RR 3.18 (95%CI 1.17-8.62).
Eckard 2017 [143]; 16, 57% Moderate	Acute & overuse hip/groin injuries in American Football, wrestling, volleyball, field hockey, gymnastics, ice, hockey, football, basketball, lacrosse, baseball/softball, indoor & outdoor track, cross	No demographic info. reported.	Overall hip flexor IR 1.60/10,000 AEs. The sports with the highest rates of hip flexor strains were men's (3.77/10,000 AEs) & men's ice hockey (2.47/10,000 AEs). Overall adductor IR 1.29/10,000 AEs. The sports with the highest rates were men's (3.15/10,000 AEs) & men's ice hockey (2.47/10,000 AEs). Sex-comparable sports; no sig. sex diff. for hip flexor IR; male 1.81, female 1.59/10,000 AEs. Hip adductor IR sig. higher in male (1.71) than female (1.15/10,000 AEs). Hip flexor; overall male IRR 1.14 (95%CI 0.96-1.36); male IRR 1.68 (95%CI 1.19-2.37); baseball/softball male IRR 0.44 (95%CI 0.20-0.98). Hip adductor; overall male IRR 1.49 (95%CI 1.22-1.81); ice hockey male IRR 1.83 (95%CI 1.13-2.96) & football male IRR 1.83 (95%CI 1.25-2.69). Noncontact mechanism hip flexor strain male IPR 1.03 (95%CI 0.88-1.21, hip adductor strain male IPR 0.99 (95%CI 0.85-1.16). Overuse represented the second-largest proportion of

	country, tennis, swimming & diving.		injuries among hip flexor strains (23.4%, 180) & hip adductor strains (18.4%, 114). Overuse hip flexor strain female IPR 1.45 (95%CI 1.10-1.92). Overuse hip adductor strain male IPR 1.07 (95%CI 0.73-1.56).
Fares 2019 [210]; 18, 64% Moderate	Acute & overuse injuries (sustained in fight but not resulting KO or TKO were excluded) in mixed martial arts & ultimate fight championship.	285 UFC matches included 291 injuries documented from all nine weight divisions. Of 285 matches, 249 (87%) involved male fighters, 36 (13%) involved female fighters. No further info. reported.	Decision was the most common way a match ended in both male (48%) & female (50%) fights. KO/TKOs were sig. (P = 0.0007) higher in matches involving male fighters (36%) (female fighters 14%). Submissions were sig. (p=0.001) higher in matches involving females (36%) (males 16%). Overall injury rate 51/100 AE. Head injuries were the most common; overall head IR rate 34/100 AE (male 28/100AE, female 29/100AE), lower limb overall IR 9/100 AE (male 8/100AE, female 2/100AE; p<0.001), upper limb 8/100 AE (male 6/100AE, female 19/100AE; p<0.0003). Male IR 54/100 AE, female IR 30/100 AE (sig not reported).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Fraser 2021 [202]; 18, 64% Moderate	Lateral ankle sprains in military athletes.	Injury data for 272,970 enlisted male tactical athletes, 56,732 enlisted female tactical athletes, 24,534 male officers, & 6020 female officers included from 2006-15.	Overall IR male tactical athletes 27.9/1000 person-yrs, female tactical athletes 34.5/1000 person-yrs, male officers 12.6/1000person-yrs, & female officers 16.4/1000 person-yrs. Female enlisted RR 1.24 (95%CI 1.23-1.25; p < 0.01). Female officers RR 1.30 (95%CI 1.27-1.34; p < 0.01).
Fulstone 2016 [147]; 15, 54% Moderate	Acute knee Injury in football.	8116 athletic injuries; 1,083,752 AE's. 556,558 female AE's, 527,194 male AE's. No demographic data reported.	11% Division I teams, 5% Division II & 8% Division III teams participated. To preserve statistical power, Division II & III teams pooled for analyses. Division I athletes contributed 467360 AEs & Division II/III athletes contributed 616, 393 AEs. Overall IR 7.5/1000 AEs (all injuries), overall knee injuries 1.06/1000 AEs. Sex diff. (p<0.05) overall; female 1.19/1,000 AE's & male 0.91/1,000 AE's. Overall female RR 1.31, (95%CI 1.16-1.47). Overall female OR 1.61 (95%CI 1.00-2.58). Time lost from participation sig. higher in females (p<0.05). Female OR 1.44 (95%CI 1.27-1.63). Rate of knee injuries in Division I players higher than in Division II/Division III players (RR 1.20 95%CI 1.07-1.35). After adjusting for contact & level of competition, female OR 1.44 (95%CI 1.27-1.63). Contact (v non-contact) also a sig. predictor of knee injury OR 1.20 (95%CI 1.05-1.37), event type & level of competition did not.
Gessel 2007 [148]; 18, 64% Moderate	Concussion in American Football, football, volleyball (excl. from discussion, basketball, wrestling, baseball/softball).	14–24-year-olds. 396 high school concussions; 482 collegiate concussions	Overall concussion IIR 0.23/1000AE's. Practice IIR 0.11/1000AE's, competition IIR 0.53/1000AE's. Highest IIR-Collegiate American Football 0.61/1000AE & collegiate girls' football 0.63/1000AE. Football; female IIR 0.36/1000 AEs, male 0.22/1000 AEs (p=0.03), RR 1.68 (95%CI 1.08-2.60). Basketball; females IIR 0.21/1000 A-Es vs males 0.07/1000 A-Es (p=0.01), RR 2.93 (95%CI 1.64-5.24). Football concussion as proportion of total injuries; females 15.1% vs. male 9.4%; IPR 1.61 (95%CI 1.59-1.64). Basketball concussion as a proportion of total injuries female 11.7% vs. male 3.8%; IPR 3.09 (95%CI 2.98-3.20). Male symptom resolution & return to play quicker than female in high school basketball. Greater proportion baseball players males returned within 6 days than softball players females.
Haines 2022 [165]; 14, 50% Moderate	Acute & overuse injuries in equestrian.	327,912 (91.51%) female, 30,407 (8.48%) male athletes. 148,530 (33.47%) junior equestrian athletes, 295,200 (66.53%) senior athletes. Data from 2015-19. No other	Overall IIR across all equestrian disciplines 780 injuries/100 000 AE. IR per discipline was less than 1.2%; 3-day eventing 1.18%, hunter–jumper 1.11%, western 0.06%. Most common injury types in 3-day eventing & hunter jumper were 'possible head injury' & 'fractures & bone injuries,' followed by 'muscle & tendon' injuries. These were also the most common injury types over all disciplines. 779 injury reports (23%) that had no injury type filled out on the form that were filed into the 'uncategorized' injury type. Male IR 0.84% & Female IR 0.97% (p<0.001). 23.8% participants did not have a sex specified, so these results are only based on 76.2% participants. Senior IR 0.70%, junior IR 0.91% (p<0.001).

		demographic info included.	
Harmer 2008 [208]; 16, 57% Moderate	Thigh, knee & ankle acute & overuse injuries in fencing.	78,223 competitors (male=60.7%; female=39.3%). Age 8-70+, participated in various events in the targeted competitions. Over 5 seasons; 184 time-loss injuries were recorded.	Overall IIR 0.3/1,000 AE's. No sig. sex diff. in overall IIR-male 0.27 (95%CI=0.22-0.32) vs female 0.36 (95%CI=0.29-0.44). Female time loss injury overall RR=1.35; (95%CI=1.01-1.81). Female fracture RR 2.6 not sig. No sig. sex diff. for any injury by anatomic location. Similar profiles for most common type-by location injuries: thigh strains (Female 14%, male 14.3%) & ankle sprains (Female 11.6%; male 13.3%). Acute & overuse not reported separately.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Hassebrock 2019 [125]; 17, 61% Moderate	Acute & overuse lower back & lumbar spine injuries in American Football, wrestling, baseball; women's volleyball, field hockey, gymnastics, & softball; & men's & women's ice hockey, football, basketball, lacrosse, indoor track & field, outdoor track & field, cross-country, swimming & diving, & tennis.	No demographic info included. NCAA athletes; age 17-23	Overall IR 6.01/1000AE's (95%CI 5.62-6.39). In sex-comparable sports, males had sig. higher IR overall than females (4.94 vs 3.94/1000 AEs). In specific sports; males sig. higher IR than females in lacrosse (8.02 vs 1.86) & basketball (4.58 vs 2.64). In sex-comparable sports, male IRR 1.25 (95%CI, 1.05-1.48). Within specific sports; male IRR in Lacrosse 4.31 (95%CI 2.07-8.96) & basketball, 1.73 (95%CI 1.17-2.54).
Hibberd 2016 [149]; 17, 61% Moderate	Shoulder-AC joint injury in American Football, wrestling, baseball; women's volleyball, field hockey, gymnastics, & softball; & men's & women's ice hockey, football, basketball, lacrosse, indoor track & field, outdoor track & field, cross-country, swimming & diving, & tennis.	No demographic info included. College age athletes 17-23 yrs	AC joint sprain overall IIR 1.72/10,000 AEs. Sig diff in sex-comparable sports; overall IIR male 1.85 vs. female 0.40. In soccer; overall IIR male 1.19 vs. female 0.52 & competition male 3.86 vs. female 1.83. Ice Hockey IIR male 6.40 vs female 2.12. Lacrosse overall IIR male 1.57 vs female 0.14, competition IIR male 4.3 vs. female 0.36, practice IIR male 1.03 vs. female 0.08. In sex-comparable sports; male RR 4.67 (95%CI 3.56-6.14). In football overall male, RR 2.30; (95%CI 1.18-4.46) & male competitions RR 3.14; (95%CI 1.34-7.34) & No sig. male practice RR 1.58 (95%CI 0.53-4.69). Ice Hockey-overall male RR 3.01 (95%CI 2.08-4.37), competition male RR, 3.11 (95%CI 2.09-4.64) & male practice RR 4.08 (95%CI 1.47-11.32). Lacrosse-overall male RR 11.61 (95%CI 2.79-48.37) male competition RR 12.08 (95%CI 1.60-91.42) male practice RR 12.30 (95%CI 1.64-92.12). In sex-comparable sports; Injuries due to player contact; male IPR 1.50 (95%CI 1.06-2.13), surface contact; female IPR, 1.55 (95%CI 1.01-2.38).
Hurtubise 2015 [150]; 16, 57% Moderate	Acute severe injury in American Football (m) rugby (f) basketball, volleyball, ice hockey, cross country/track & field.	NCAA athletes-typically aged 17-24 yrs. 1,155 male injuries (70% injured), 502 female injuries	Overall female OR; 1.40 (CI 1.05-1.86). Female concussion OR 1.85 (CI 1.28-2.67). Sig. greater proportion of all injuries are concussion in female athletes 11% vs. male 6.2% ($p<0.001$). Female had sig. higher proportion of severe injuries (17.7%) than males (13.3%) ($p=0.02$).

Hopkins 2022 [163]; 18, 64% Moderate	Recurrent injuries in track & field.	NCAA database 2009-14. College age 17-22yrs. Demographic data not included.	Overall, 474 injuries, 13.1% classified as recurrent injuries. T&F athletes missed average 15.6 days following initial injury & 24.2 days following recurrent injury. T&F athletes missed 55% (95%CI 17-107; p<0.01) more time following recurrent injury than initial injury. When stratified by sex, diff. was greater in women's T&F; recurrent injuries 75% (95%CI 16-167; p<0.01) more time loss than initial injuries cf. to men's T&F; recurrent injuries 34% (95%CI 9-96; p=0.14) more time loss than initial injuries. No sex diff. in proportion of injuries classified as recurrent (IPR 0.96 [95%CI 0.7-1.3]), however, women's T&F athletes experienced 27% (95%CI 5-55; p=0.02) more time loss across all injuries than men's T&F athletes. Recurrent injuries (cf. to new injury) time loss RR 1.50 (95%CI 1.13-1.98; p<0.01), female (cf. to male) time loss RR 1.15 (95%CI 0.96-1.41; p=0.18). (i.e., time loss is due to recurrence not sex).
Kerbel 2018 [151]; 16, 57% Moderate	Acute & overuse Hip/groin Injuries in football, ice hockey, cross country, basketball, tennis, track, gymnastics, lacrosse.	No demographic info included.	Overall hip injury rate of 53.06/100,000 AE's. Sports with highest hip IR/100,000 AE's; male football (110.84, male ice hockey (104.90, female ice hockey (76.88). Sig. sex diff.; overall male 59.53/100,000AE's female 42.27. Proportion of injuries due to player contact male 17.0% vs female 3.6% (p<0.05); Overuse/gradual female 29.1% vs male 16.7% (p<0.05). NTL injuries; female 62.5% vs male 54.2% (p<0.05). Overall Game RR, 2.18 (95%CI, 1.99-2.39). Overall male RR, 1.41 (95%CI, 1.28-1.55). Female practice RR, 1.00 (95%CI, 0.82-1.21) conversely male competition RR 3.14 (95%CI, 2.82-3.50). Player contact male IPR, 4.80 (95%CI, 3.10-7.42). Overuse injury female IPR, 1.74 (95%CI, 1.46-2.06). NTl female IPR, 1.15 (95%CI, 1.07-1.25).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Kerr 2015 [207]; 16, 57% Moderate	Acute & overuse injuries in swimming & diving. RIO database 2008-19.	9 male & 13 female swimming/diving programmes included. No other demographic info included.	No sig. sex diff. for Overall IIR-training, competition, swimming or diving injuries. Sig. sex diff. for overuse injuries in swimming; female 1.04/1000 AEs, male 0.66/1000 AEs. Male divers sustained a greater proportion of shoulder injuries (32.0%) than female divers (5.4%). Swimming overuse female RR 1.58; (95%CI 1.14 to 2.19). Diving shoulder injury male IPR 5.92 (95%CI 1.37-25.59).
Kerr 2016 [70]; 17, 61% Moderate*	Acute & overuse injuries in cross country running.	Age 17-24 yrs. 89 male injuries (44%), 114 female injuries (56%) (Div II & III)	Div II - overall practice IR male 1.62/1,000AAE (0.77-2.47), female IR 1.96 (0.85-3.07). Overall competition IR male 2.82/1,000 AE (0.00-6.74), female IR 6.41 (0.00-13.66). Total overall IR male 1.71/1,000 AE (0.87-2.55), female 2.28 (1.13-3.43). Div III - overall practice IR male 5.14 (3.88-6.40), female 6.13 (4.85-7.40). Competition IR male 5.77/1,000 AE (2.00-9.53), female 5.77 (2.19-9.34). Total overall IR male 5.21/1,000 AE (4.01-6.41), female 6.09 (4.89-7.29). Sig. sex diff. for hip/groin IR female 0.65/1000AE, male 0.15/1000 AE; RR 4.32 (95%CI 1.89-9.85). Ankle IR male 0.60/1000AE, female IR 0.29; RR 2.07 (95%CI 1.07-3.99). Overall female RR 1.25 (95%CI 1.05-1.50). Largest proportion of severe injuries male: trunk (16.7%), hip/groin (14.3%), & lower leg (13.2%); Female: trunk (22.7%), foot (22.5%) & thigh (18.4%). Largest proportion of severe injuries; male stress fractures (30.0%), strains (11.6%) & spasms (11.1%); female fractures (60.0%), stress fractures (58.3%) spasms (11.1%).
Kronisch 2002 [152]; 20, 71% Moderate	Acute fractures in mountain biking.	93; (71 male, 22 female). Male mean age 28.4 yrs (range 15-59), height 181 cm (range 156-195), & weight 72.9 kg (range 54-100). Females mean age 30.8 yrs (range 22-52), height 167 cm (range 151-182), &weight 58.3 kg (range 51-66). Females with fractures had a mean age of 30.8 yrs, & those without fractures had a mean age of 30.8 yrs. Males with fractures had a mean age of 31.2 yrs, & those without fractures	For all events combined, female IR; 0.77% vs. male 0.40% (p=0.01). Female downhill IR; 0.76% vs. male 0.40% not sig. Female DS IR; 1.61% vs. male 0.40% (p=0.02). No sig. sex diff. in CC race over the 8-year study period. Female fracture IR downhill; 0.51% vs. male 0.11%, (p<0.02, Female dual slalom 0.96% vs. male 0.10%, (p<0.02, female overall fracture IR 0.35% vs. male 0.08%, (p<0.001). The diff. between the female fracture IR CC, downhill, dual slalom (0.17% vs 0.51% vs 0.96%, p 0.04). Overall Injury female OR; 1.94 overall female fracture OR; 4.17. Female fracture downhill OR; 4.78. Female fracture dual slalom OR; 9.64. Fractures sustained by higher percentage of females than males (45.5% vs 21.1%, p<0.03).

		had a mean age of 27.6 yrs.	
Lacasse 2021 [132]; 11, 339% Limited	All injuries in college football.	One season (2019) College age (17-24 yrs). No other demographic info included. N=27 male players, n=34 female players.	69.35% injuries female players, 30.65% male players (p=0.031).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Lanzi 2017 [175]; 15, 54% Moderate	Shoulder-posterior glenohumeral instability injuries in rugby, wrestling, track, judo, swimming, gymnastics, American Football, ski, baseball, handball, lacrosse, fencing, paintball, cheerleading.	No specific demographic info reported. Retrospective cohort study (2006-2012) within the population at the US Military Academy using Cadet Illness & Injury Tracking System (CIITS). Data from 113 posterior instability injuries analysed (105 male, 8 female).	Overall IR 4.28/1000 person-yrs or 0.08/1000AE's. Sig. sex diff. overall; male IR of 4.67/1000 person-yrs, female IR 2.04/1000-person yrs (p<0.05). Intercollegiate athletes overall IR; male 9.01/1,00py, female 4.18 (p<0.04) IR; male 0.08/1,000 AE's, female 0.04 (p<0.06). Overall male IRR 2.29 (95%CI 1.11-4.69). Intercollegiate; population based male IRR of 2.3 (95%CI 1.04-5.08; p=0.04), exposure-based IRR of 2.14 (95%CI 0.97-4.74; p=0.06).
Lievers 2020 [141]; 18, 64% Moderate	Foot injuries in multiple NCAA sports.	College age 17-22yrs. Demographic data not included. 2009-15.	Overall highest foot IIR in gymnastics (10.62/10,000AE), lowest IIR were in female (0.70) & male (0.61) swimming & diving. In sex comparable sports, sex diff. in Track & field female 4.92/10,000AE, male 3.29; male foot injury RR 0.67 (95%CI 0.50-0.89; p=.0039). No sex diff. in injury types across or within sports (foot/toe contusion, midfoot injury, plantar fascia, turf toe, metatarsal fracture, forefoot extensor/flexor tear).
Marchena-Rodriguez 2020 [211]; 18, 64% Moderate	Acute & overuse injuries in badminton.	150 players, 69.33% male. Overall 50.18±10.72 yrs, male 50.18±10.72, female 51.58±12.95, BMI 25.56±3.36 kg/m2, male 25.56±3.36, female 23.78±3.87. Mean duration playing experience 27.2 yrs.	Injuries suffered by male & female players in ratio 2.07:1.99 (p=0.130: F=2.31). Overall IR 0.134±0.1/1000 hrs training, male 0.138±0.1, female 0.131±0.08 (p=0.981).
Mauntel 2017 [153]; 16, 57% Moderate	High ankle sprain in American Football, wrestling, baseball; women's volleyball, field hockey, gymnastics, & softball; & men's &	No information reported.	Overall high ankle sprain IIR 1.00/10,000 AEs. Higher IIR during competitions than practice (p<0.05). Similar trends were observed between competitions & practices for males (comp 1.78, practice 0.35) & females (comp 1.02, practice 0.19) in sex comparable sports. Sig. sex diff. for; overall IIR male 0.64/10,000Aes vs female 0.36, competition IIR male 1.78 vs female 1.02, practice IIR male 0.35 vs female 0.19. Sport-specific sex diff.; ice hockey competition IIR male 3.36/10,000AE vs female 1.27; basketball practice IIR male 0.68/10,000AE vs female 0.11. In sex-comparable sports; overall male RR, 1.77; (95%CI,

	women's ice hockey, football, basketball, lacrosse, indoor track & field, outdoor track & field, cross-country, swimming & diving, tennis, cross country.		1.28-2.44) competition male RR, 1.75 (95%CI, 1.15-2.68) practice male RR, 1.87 (95%CI, 1.13-3.09). Sport-specific sex diff. male ice hockey competitions (RR, 2.64; 95%CI, 1.03-6.78, male basketball practice RR, 6.20 (95%CI, 1.42-27.12).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Melvin 2018 [83]; 16, 57% Moderate	Acute & overuse upper extremity injuries in Ice hockey.	No demographic information reported. Div II&III, 684 male injuries (79%) & 185 female injuries (21%).	Sig. sex diff. for overall IR; male 235.97/10,000AE's, female 124.61 (p<0.05), competition IR; male 733.23, female 303.33 (p<0.05). Preseason IR; male 149.09, female 52.67 (p<05), In season IR; male 256.94, female 142.96 (p<0.05), Postseason IR; male 142.73, female 49.01 (p<0.05). Overall male RR; 1.89 (CI 1.67-2.15). Preseason male RR; 2.83 (CI 1.69-4.74), In season male RR; 1.80 (CI 1.57-2.05), Postseason male RR; 2.91 (CI 1.33-6.38). No sig. diff. by player position, upper extremity region injured or injury type. Mechanism of injury: contact with player, male IPR; 1.54 (CI 1.24-1.90), contact with playing surface, male IPR; 1.54 (CI 1.24-1.90). Div II male overall IR 167.69/100,000AE (95%CI 135.31-200.08), competition IR 540.85 (95%CI 420.82-660.88) practice IR 53.19 (95%CI 32.34-74.04); female overall IR 148.23/100,000AE (95%CI 100.47-195.99), competition IR 316.80 (95%CI 181.30-452.30), practice IR 87.28 (95%CI 44.51-130.04). Div III male overall IR 260.07/100,000AE (95%CI 238.93-281.22), competition IR 784.38 (95%CI 707.99-860.78), practice IR 102.47 (95%CI 87.33 to 117.60); female overall IR 126.82/100,000AE (95%CI 106.38-147.25), competition IR 296.74 (95%CI 234.38-359.09), practice IR 69.81 (95%CI 52.29-87.32). Div II overall male RR 1.13 (95%CI 0.78-1.65). Div III overall male RR 2.05 (95%CI 1.71-2.46).
Owens 2009 [154]; 16, 57% Moderate	Glenohumeral instability in American Football, wrestling, volleyball, field hockey, gymnastics, ice, hockey, football, basketball, lacrosse, baseball/softball, indoor & outdoor track, cross country, tennis.	17,799 shoulder injuries, 4080 (23%) of which were glenohumeral instability.	Overall GH IIR 0.12/1000AE's. Highest Male sport-Football 0.4/1,000 AE's. Highest female sport 0.18/1,000 AE's. 60% occurred during practice (0.9/1,000 AE's) (comp IR 0.31/1000AE's). Overall Male IIR 0.15/1,000 AE's (95%CI 0.14-0.15, Female IIR 0.06/1000AE's (95%CI 0.05-0.06). No sig. diff in sex comparable sports. Sig. sex diff. in competition football, male-0.17, female 0.09/1,000 AE's. Practice RR 3.5(95%CI 3.29-3.73). Overall male RR 2.67 (95%CI 2.43-2.93). In football competition, male RR 1.99 (95%CI 1.28-3.12).
Post 2022 [133]; 14, 50% Moderate	Overuse injuries in multiple high school sports (2014-19). (12 sports; baseball/softball, basketball, cross-country, American Football, ice hockey/field hockey, lacrosse, football, swimming, tennis, track &	RIO high school injury database. 2014-19. No demographic info reported.	Overall IIR 5.3/10 000AEs (95%CI 5.1-5.7). NTL overuse IIR 3.4/10 000 AEs (95%CI 3.1-3.6), TL overuse IIR 2.0/10 000 AEs (95%CI 1.8-2.2). Overall overuse female IRR 1.9 (95%CI 1.7-2.1). Highest IIR, female cross-country 19.2/10 000 AEs (95%CI 15.0-24.2), female track & field 16.0/10 000 AEs (95%CI 13.5-18.8), female field hockey 15.1/10 000 AEs (95%CI 10.2-21.6). Overuse injury rates were higher for the lower extremity than the upper extremity (IRR 5.7 [95%CI 4.9-6.7]). In male & female sports, most injured anatomical locations - lower leg (male 24.9%, female 31.7%), knee (male 20.5%, female 20.1%); most common diagnoses - medial tibial stress syndrome (male 10.1%, female 18.0%), patellar tendinitis (male 9.1%, female 8.3%).

	field, volleyball, wrestling, gymnastics).		
Rizzone 2017 [156]; 16, 57% Moderate	Stress fractures (lumbar spine, pelvis, lower limb) in all NCAA sports	College age 17-22yrs. Demographic data not included.	Overall IIR5.70/100 000 AEs. Highest IIR; female cross-country 28.59/100 000 AEs, female gymnastics 25.58/100 000 AEs, & female outdoor track 22.26/100 000 AEs. Highest male stress fracture IIR cross-country-16.14/ 100 000 AEs. Sex-comparable sports; female higher overall IIR-9.13/100 000 AEs vs. male 4.44/100 000 AEs; female RR=2.06 (95%CI=1.71, 2.47). Specifically, female basketball 14.04 (vs. 8.29); RR 1.69 (95%CI 1.26, 2.28, cross-country 28.59 (vs. 16.14); RR 1.77 (95%CI 1.05-2.98, football 7.38 (vs. 4.37); RR 1.69 (95%CI 1.09-2.63, indoor track 11.63 (vs. 4.6); RR 2.53 (95%CI 1.18, 5.42), & outdoor track 22.26 (vs. 7.2); RR 3.09 (95%CI 1.765 5.80). Among sex-comparable sports; proportion of stress fractures occurring in the lower back/lumbar spine/pelvis higher in male (23, 12.8%) than female (22, 6.9%). The proportion of stress fractures at the femur higher in female (39, 12.2%) than male (8, 4.4%).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Roos 2017 [138]; 17, 61% Moderate	Acute & overuse injuries in football.	No specific demographic data included. 44 male & 64 female football programmes (104 & 167 team seasons of data, respectively). 1554 male & 2271 female injuries.	No sig. sex difference in overall IIR; male 8.07/1,000 AE's, Female 8.44, Competition IIR; male 17.53 vs female 17.04/1000AE, Practice IIR male 5.47 vs female 5.69/1000AE. 47.2% (733) male injuries & 47.5% (1079) female injuries were non-time loss. Concussion IIR; female 0.59 vs male 0.34/1000AE. Sig. sex diff. IIR; preseason practices, female 10.10 vs male 8.57/1000AE, severe injuries female 9.2% vs male 5.1%. Hip/groin competition male 1.86 vs female 0.84/1000AE, Upper leg male 3.01 vs female 1.98/ 1000AE, hip/groin practice male 0.80 vs female 0.57/1000AE. Head/face in competition female 3.06 vs male 2.19/1000AE. Knee in competition female 3.44 vs male 2.15/ 1000AE. Knee practice female 0.89 vs male 0.70/1000AE. IIR strains in competition male 3.88 vs female 2.58/1000AE. Lacerations in competition male 0.60 vs female 0.28/1000AE. Contusions in practice male 0.68 vs female 0.45/1000AE. Concussion in competition female 1.83 vs male 0.94/1000AE. Overuse IIR practice female 1.31 vs male 1.01/1000AE. cf. with women, men had a higher hip/groin strain rate than women (0.78 vs 0.46/1000AE; RR 1.69 (95%CI 1.33 to 2.14). Overall concussion female 0.59 vs male 0.34/1000AE & knee sprain female 0.66 vs male 0.42/1000AE. Male competition RR 1.03 (95%CI 0.94 to 1.13). Male practice RR 0.96 (95%CI 0.88-1.05). Concussion female RR 1.76 (95%CI 1.32-2.35). Preseason practice female RR 1.18 (95%CI 1.04-1.34). Severe injury female IPR 1.78 (95%CI 1.39-2.28). Hip/groin male RR 2.20 (95%CI 1.56-3.11). Upper leg male RR 1.52 (95%CI 1.19-1.95). Hip/groin practice male RR 1.41 (95%CI 1.09-1.82). Head/face female RR 1.39 (95%CI 1.09-1.78). Knee competition female RR 1.60 (95%CI 1.25-2.05). Knee practice female RR 1.28 (95%CI 1.01 to 1.63). Strain male RR 1.51 (95%CI 1.21 to 1.87). Comp laceration male RR 2.18 (95%CI 1.19-4.00). Practice contusion male RR 1.51; (95%CI 1.14 to 2.00). Concussion comp female RR 1.94; (95%CI 1.35 to 2.79). Overuse practice female RR 1.30 (95%CI 1.07 to 1.59). Overall concussion female RR 1.76 (95%CI 1.32 to 2.35). Overall knee sprain female RR 1.59 (95%CI 1.22 to 2.07). Concussions caused by ball contact female RR 2.43 (95%CI 1.37 to 4.33).
Sallis 2000 [248]; 18, 64% Moderate	Acute & overuse injuries.	3767 participants; 18–22-year-old male & female college athletes. Divs. III.	Male athletes; 1018 injuries (54.3%) female athletes; 856 (45.7%). Overall IR, female athletes, 52.5/100 participant-yrs male athletes, 47.7/100 participant-yrs-not statistically sig. Sig. sex diff. in swimming-female 47.08 vs. male 12.37 & water polo-female 18.38 vs. male 7.10 ($p<0.001$) in pattern of injury. Swimming; back/neck-female 8.19/100partcipant yrs vs. male 1.45, shoulder-female 21.05 vs. male 6.55, hip-female 2.34 vs. male 0.00, knee-female 5.85 vs. male 1.45. Water polo; shoulder-female 8.09 vs. male 3.40. Football; lower leg-female 10.48 vs. male 4.51. Sig. sex diff. overall for hip-female 4.17 vs. male 2.25, lower leg-female 5.39 vs. male 3.61, Shoulder-female 6.92 vs. male 3.09, thigh-female 4.53 vs. male 6.79.
Schroeder, 2022 [140]; 15, 54% Moderate	All injuries in water polo.	NCAA database. 3 male, 4 female teams were observed for 2016-21. 1	No sex diff. in overall IIR, male RR 1.0 (95%CI 0.9-1.2). Sex diff.; weight room injuries male RR 2.6 (95%CI 1.3-5.2), spine/neck injury male RR 2.1 (95%CI 1.2-3.5), muscle spasm/cramp/soreness male RR, 2.3 (95%CI 1.4-4.0), fracture male RR 3.1 (95%CI 1.3-7.7), visceral damage/trauma male RR (3.8; 95%CI 1.3-

		male, 1 female team observed 2018-21. 729 injuries included.	11.3). Shoulder tendinopathy male RR 3.0 (95%CI 1.3-7.0), lumbar spine muscle spasm/cramps/soreness male RR 3.7 (95%CI 1.4-9.9). Concussion - injury mechanism in males; contact with another player (57.8%), contact with a playing device (28.9%), in females; contact with a playing device (42.1%), contact with another player (23.7%). Offseason practice concussion male RR 3.25 (95%CI 1.2-8.8), concussion via contact with another player male RR 2.9 (95%CI 1.3-6.4).
Simmons 2017 [157]; 17, 61% Moderate	Acute injuries (head, neck, facial) in ice hockey.	No demographic info. included.	Overall IIR sig sex diff.; male 1.75/1,000 AE's vs female 1.16/1000 AE's. Competition IIR; male 5.86 vs female 2.95. Time loss IIR competition male 2.56/1000 AEs vs female 2.21. Time loss IIR practice male 0.28/1000 AEs vs female 0.32. Proportion cervical spine/neck female 25.6% male 11.5%. Most common HFN injury was concussion in both sexes. The proportion of competition HFN injuries that were concussion female 68.2% male 42.5%. Concussion IIR; practice-male 0.25/1,000 AE's vs female 0.30. Competition-male 2.49/1,000 AE's vs female 2.01. No sex analysis done. Overall male RR 1.51 (95%CI 1.25-1.84). Competition male RR; 1.99 (95%CI 1.58-2.50). Non time-loss injury male IPR 2.37 (95%CI 1.59-3.51). Cervical spine/neck female IPR 2.23 (95%CI 1.05-4.75). Competition female concussion IPR 1.60 (95%CI 1.34-1.93).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Trojan 2019 [158]; 16, 57% Moderate	Knee-patellofemoral pain in all NCAA sports	College age 17-22yrs. Demographic data not included.	Overall IIR; male 14.15/100,000 AEs vs female 19.35/100,000 AEs. Lacrosse practice male RR 0.32 (95%CI 0.13-0.79), football practice male RR 0.53 (95%CI 0.29-0.96). Basketball competition male RR 2.41 (95%CI 1.11-5.24). Football overall male RR 0.51 (95%CI 0.31-0.84). Sex comparable sports; practice male RR 0.70 (95%CI 0.55-0.89), overall male RR 0.77 (95%CI 0.63-0.95). Patellar tendinitis accounted for 100% patellofemoral injuries in women's outdoor track, 85.7% patellofemoral injuries in men's swimming, & 83.3% patellofemoral injuries in men's outdoor track. PFPS was the most prevalent injury in female field hockey, lacrosse & softball, accounting for 50.0%, 29.4% & 29.4% injuries, respectively.
Tummala 2018 [159]; 16, 57% Moderate	Acute & overuse ankle injuries in basketball.	College age 17-22yrs. Demographic data not included. 1,388; (1298 male, 90 female) ankle injuries.	Male IR 1.49 injuries/1000 AEs, female IR 1.21/1000AEs. Male competition IR; 2.51/1000 AEs, Male practice IR; 1.23/ 1000 AEs. Male preseason IR 2.00/ 1000 AEs vs. in-season IR; 1.37/1000 AEs & postseason IR; 0.84/1000 AEs. Female IR 1.21 injuries/1000 AEs. Female competition IR; 2.00/1000 AEs vs. practice IR; 0.98/1000 AEs. Female preseason IR; 1.45/1000 AEs vs. in-season IR; 1.18/1000 AEs & postseason IR; 0.45/1000 AEs. Females have a sig. lower rate of ankle injuries overall; IPR 0.81 (95%CI, 0.75-0.88). Female were sig. more likely to be injured during season; IPR 1.08 (95%CI 1.02-1.14) & less likely to be injured during preseason; IPR, 0.87 (95%CI 0.77-0.99). Female sig. greater acute noncontact mechanisms; IPR 1.20 (95%CI 1.06-1.35), less often because of contact; IPR 0.88 (95%CI 0.81-0.95). Female sig. more likely to have a recurrent injury; IPR 1.77 (95%CI 1.22-2.57). Male injuries 37.3% resulted in a time loss of less than 7 days & 56.7% lasted longer than 7 days. Female injuries 67.2% resulted in a time loss of less than 7 days & 29.0% lasted longer than 7 days. Female IIR lower but a greater proportion of female injuries are at the ankle.
Zuckerman 2015 [160]; 18, 64% Moderate	Concussion in American Football, wrestling, baseball; women's volleyball, field hockey, gymnastics, & softball; & men's & women's ice hockey, football, basketball, lacrosse, indoor track & field, outdoor track & field, cross-country, swimming & diving, tennis, cross country.	No Demographic info included. NCAA athletes. 2009-14	Overall concussion IIR 4.47/10,000 AE. Overall competition IIR 12.81/10,000 AEs & practice IIR 2.57/10,000 AEs. Highest IIR wrestling 10.92/10,000 AEs. Sig. sex diff. in; Basketball overall IIR f 5.95/10,000AE vs. m 3.89 & competition IIR f 10.92 vs. m 5.6; Female basketball overall RR=1.53; (95%CI, 1.16-2.03) & competition RR=1.95; (95%CI, 1.21-3.13). Football overall IIR f 6.31 vs. m 3.44 & competitions IIR f 19.38 vs. m 9.69; Football overall female RR=1.83; (95%CI, 1.34-2.51) & competition female RR=2.00; (95%CI, 1.35-2.96). Lacrosse overall IIR f 5.21 vs. m 3.18; Lacrosse overall female RR 1.64 (95%CI 1.12 2.40). Softball overall IIR f 3.28 vs. m 0.90, & competition IIR f 5.61 vs. m 1.20 & practice IIR f 1.75 vs. m 0.72; Softball overall female RR=3.65; (95%CI, 2.09-6.39, competitions female RR 4.66 (95%CI 2.17-10.03), & practice female RR 2.44 (95%CI 1.05-5.65).

Zupon 2018 [90]; 15, 54% Moderate	Back, neck & spine injuries in ice hockey.	College age 17-22yrs. Demographic data not included. Men's ice hockey data: 27 Division III programmes provided 60 team-seasons. Women's ice hockey data: 16 Division III programmes provided 36 team seasons. No division II institutions included due to playing in a different league.	Male competition IR 1.49 (95%CI 1.10–1.89), practice IR 0.46 (95%CI 0.33–0.58), overall IR 0.47 0.71 (95%CI 0.58–0.85). Female competition IR 1.50 (95%CI 0.96–2.05), practice IR 0.71 (95%CI 0.48–0.93), overall IR 0.92 (95%CI 0.70–1.14). No sig. sex diff. in proportion of NTL injuries, severe, recurrent, or required surgery injuries. Overall male IRR 0.85 (95%CI 0.67-1.08), competition male IRR 1.13 (95%CI 0.81-1.59), practice male IRR 0.67 (95%CI 0.48-0.94). No sig. sex diff. in proportion of NTL injuries (IPR 1.08 95%CI 0.90-1.30), severe (IPR 1.93 95%CI 0.43-8.77), recurrent injuries (IPR 1.02 95%CI 0.60-1.74) or injuries that required surgery (IPR 0.86 95%CI 0.08-9.36).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
ADOLESCENT			
Baugh 2016 [197]; 15, 54% Moderate	Acute & overuse injuries in Rowing	Demographic info not included. High school age. The number of programs providing data varied by sport & year. 2011-2012 through 2013-2014 academic yrs. Eight boys' rowing programs provided 13 team-seasons of data. Eleven girls' rowing programs provided 17 team-seasons of data.	Boys' & girls' rowing IR of 2.39/1000 AEs (95%CI 1.78-3.00) & 8.60/1000 AEs (95%CI 7.38-9.82). No diff. existed in injury distributions by body part or diagnosis in boys' & girls' rowing. Overall rate in girls was 3.60 times that of boys (95%CI 2.69-4.82). Competition rates were higher than practice rates in boys (RR 2.01 95%CI 1.12-3.62) but not in girls (RR 1.06 95%CI 0.72-1.56).
Belilos 2023 [201]; 17, 61% Moderate	Acute & overuse injuries in swimming & diving. RIO database 2008-19	High school athletes. No further demographic information included.	Overall IIR 2008-19 across swimming & diving a 0.26/1000 AEs, (95%CI 0.24- 0.28), male 0.20/1000 AE, female 0.31/1000 AE. IIR higher in practice than competition (P=0.051). Overall female RR 1.57 (95%CI 1.32-1.86; p<0.05), practice female RR 1.53 (95%CI 1.27- 1.85; p<0.05), competition female RR 1.81 (95%CI 1.15- 2.84; p<0.05). There was no sig. diff. in the proportion of injuries among classes (freshmen 23.1%, sophomores 27.2%, juniors 26.6%, & seniors 23.1%). The shoulder was the most commonly injured anatomic location in males (43.0%) & females (39.9%). There was no sig. diff. between overuse injuries in males (48.5%) & females (48.2%). In diving, injury to the head accounted for 47% injuries in males & 29% in females. A greater proportion of injuries in females were to the knee (16.7%) than in males (5.9%).
Beis 2007 [204]; 20, 71% Moderate	Acute & overuse injuries in Taekwondo	2739 young & adult males & females participating in the national championships of Hellenic Federation of Taekwondo. The adult men & women were age >18yrs, junior boys & girls were 14-17yrs, boys & girls, 11-13 yrs.	No sig. sex diff. Overall IR female 11.36/1,000 A-E, male 7.40/1,000 AE's or for age groups; Adult (age> 18yrs) male 6.85/1,000 AE's, female 2.43, junior (age 14-17yrs) male 8.97/1,000 AE's, female 17.01, child (age 11-13 yrs) male 6.16/1,000 AE's, female 9.37. Across age groups no sig. sex diff. adult male OR 2.861 (95%CI 0.350-23.395, Junior boys OR 0.532 (95%CI 0.207-1.362). Child boys OR 0.668 (95%CI 0.256-1.745). Overall male OR 0.703 (95%CI 0.383-1.293). No sig. sex diff. across mechanism of injury or body part.

Borowski 2008 [129]; 17, 61% Moderate	Acute & overuse injuries in Basketball	No demographic info included. High school sports in the USA	Overall IR; 1.94/1,000 AE's. IR greater ($p<0.05$) during competition (3.27) practice (1.40). IR was sig. ($p<0.05$) greater among girls (2.08) than among boys (1.83). IR greater during competition than practice RR 2.33 (95%CI 2.10-2.57). IR was sig. greater among girls RR 1.14 (95%CI1.03-1.26). Female basketball players sustained a greater proportion of concussions (IPR 2.41 95%CI 1.49-3.91) & knee injuries (IPR 1.71 95%CI 1.27-2.30). Boys more frequently sustained fractures (IPR 1.87 95%CI 1.27-2.77) & contusions (IPR 1.52 95%CI 1.00-2.31). The ankle/foot (39.7%), knee (14.7%), head/face/neck (13.6%), arm/hand (9.6%), & hip/thigh/upper leg (8.4%) were most injured. Most frequent injury diagnoses were ligament sprains (44.0%) muscle/tendon strains (17.7%), contusions (8.6%), fractures (8.5%), & concussions (7.0%).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Bretzin 2018 [167]; 18, 64% Moderate	Concussion in Male sports included basketball, baseball, American Football, ice hockey, lacrosse, football, swimming & diving & wrestling. Female sports included basketball, competitive cheer, lacrosse, football, softball, swimming/diving & volleyball.	No specific demographic info. In the 2015-2016 school year, there were a total of 755 Michigan high schools (i.e., 9th-12th grades) that participated in this study.	Overall clinical incidence for all sports was 1.7/100 player-seasons (95%CI=1.6-1.8); 1.9 (95%CI=1.8-2.0) for male sports & 1.5 (95%CI=1.4-1.6) for female sports. Female athletes were at a 1.9 (95%CI 1.8-2.2) times greater risk for enduring SRCs than male athletes in sex-comparable sports, with a greater risk in baseball/softball (RR 2.7 [95%CI=1.9-3.8]), basketball (RR 2.5 [95%CI 2.1-2.9]) & football (RR 1.6 [95%CI 1.4-1.9]). Female student-athletes had sig. (p<0.001) longer time loss (days) than male student-athletes (13.8±12.9 vs. 12.0±12.1). Sig. sex diff. in RTP (days) in Basketball (p=0.024); Male 12.7±10.5 Female 15.1±13.5. Mean number of missed school days did not differ between sexes (p=0.70).
Bretzin 2021 [134]; 18, 64% Moderate	Concussion in football	n= 43,741 male & 39,637 female soccer players (2016-19).	1507 football related SRCs (37.0% male). A greater proportion of female athletes than male athletes were in lower grades in school 9th grade; female 308 (32.4%), male 117 (21.0%) (p<0.001), junior varsity soccer; female 337 (35.5%) male 141 (27.1%) (P =0.004) or had a prior history of concussion; female 199 (21.0%), male 78 (14.0%) (p<0.001). Most documented SRC occurred during competition in both male (813 [85.5%]) & female (476 [85.6%]) athletes (p=0.55). Overall IIR 1.8 (95%CI 1.72-1.90)/100 athletes per season. Female RR 1.88 (95%CI 1.69-2.09; p <0.001).
Changstrom 2015 [168]; 19, 68% Moderate	Overuse Injuries-stress fractures in American Football, boys' & girls' football, volleyball, basketball, wrestling, baseball & softball, lacrosse, track & field, swimming & diving, cross country, girls' field hockey, girls' gymnastics, boys' ice hockey, girls cheerleading.	Age 15.8 (±13-19) yrs (median age 16 yrs), BMI, 22.7(±15.6-41.5) (female-21.3 & male-24.0).	Overall IR 1.54/100,000 AE's. Sig. sex diff. (p<0.05) between overall in sex comparable sports; female 2.22/100,000 AE's, male 1.27/100,000 AE's. Track & field; female, 4.76, male 1.79 (p<0.05) & basketball female, 2.71, male 1.05 (p<0.05). No sig. diff. for lacrosse female 2.8 male 1.5, cross country female 10.62, male 5.42, football female 2.3, male 1.69 or baseball/softball female 0.23, male 0.75. Sex comparable sports; Female RR, 1.75 (95%CI 1.38-2.23). Track & field Female RR 2.66 (95%CI, 1.63-4.36), Basketball Female; RR 2.58 (95%CI 1.59-4.20). No sig. diff. for Lacrosse Female RR; 2.54 (95%CI 0.94-6.86), Cross country female RR 1.96 (95%CI 0.77-4.98), football female RR 1.36 (95%CI 0.84-2.19) or baseball/softball female RR 0.31 (95%CI 0.09-1.10). Rate ratios for volleyball & swimming & diving could not be calculated because no stress fractures were reported for boys' volleyball & girls' swimming & diving.
Cierna 2017 [196]; 19, 68% Moderate	Acute & overuse injuries (competition only) in Karate	246; 160 male, 86 female. Age 12-17 yrs.	Overall IIR-AE 45.2/1000AE (95%CI 38.78-52.58, IIR-ME 35.88/1,000ME (95%CI 30.70-41.68). Injury Proportion/100athletes (IP) 9.87 (95%CI 8.44-11.46). No sig. sex diff. in overall IP, IIR-AE or IIR-ME. Overall girls RR-AE 1.71 (95%CI 1.01-2.91), Overall girls RR-ME 1.69 (95%CI 1.00-2.86). Most frequently

			injured site; Male Head/neck (66%) & lower limb (21%), female Head/neck (56%) & trunk & lower limb (12% each). Most common injury type; male-contusion (79%) & fracture (4%), female-contusion (84%) & epistaxis (15%).
Cierna 2018 [195]; 18, 64% Moderate	Acute & overuse injuries in Karate	Junior (age 16-18yrs) 948 male, 604 female. U21, 738 male, 522 female. Total, 1,686 male, 1,126 female.	Sig. sex diff. for overall IR-male=10.74/100 athletes, female 6.75, IIR male 48.69/1,000 AE's, female 30.28 & male 20.06/1,000ME's, female 14.12. Overall IIR female RR 0.6 (95%CI 0.5-0.8) (AE, female RR 0.7 (95%CI 0.5-0.9) (ME).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Collins 2008 [118]; 18, 64% Moderate	Acute & overuse injuries in Rugby Union	n=594, age 16.5±1.2yrs, height 175.26±8.64cm, weight 76.77±14.36kg. 517 males age 16.5±1.2yrs, height 176.53±8.13cm, weight 78.62±13.59. 77 females, age 16.3±1.2yrs, height 165.61±6.86cm, weight 62.19±11.88kg.	Total 594 injuries (103 practice, 488 competition, 3 unspecified). Overall IIR 5.2/1000 total AE. Sig. sex diff.; male 5.5/1000 total AE, female 4.1/1000AE. Practice IIR 1.3/1000AE (male 1.3 vs. female 1.0; not sig., match IIR 15.2/1000AE, (sig sex diff; male 14.8 vs. female 19.5). Overall injury male RR1.3 (95%CI 1.1-1.7; p=0.02). Practice (vs. match) RR 12.1 (95%CI 9.8-14.9; P.001). Match injury female RR 1.3; (95%CI 1.0-1.7; p=0.048). Male time loss RR1.5 (95%CI 1.1-2.0; p=0.005). Overall, most injured body sites were head (21.7%, ankle (13.3%, shoulder (12.8%) & knee (11.1%); most common diagnoses-fractures (16.0%, concussions (15.8%) & ligament sprains (incomplete tears) (15.7%). 87.0% injuries were in male athletes. Patterns of injury; head (male 22.1%, female 19.5%, ankle (male 13.2%, female 14.3%, shoulder (male 13.2%, female 10.4%) & knee (male 10.6%, female 14.3%). Male time-loss injuries (>10 days) 52.3% (vs. female 35.1%). All career ending injuries were in male athletes (11) 4 fractures, 2 complete dislocations, 2 nerve injuries, 1 concussion, 1 torn cartilage & 1 unknown shoulder injury. Injuries were most commonly a new injury (86.4%) rather than a recurrence or complication of a previous injury.
Darrow 2009 [127]; 18, 64% Moderate	Acute & chronic injuries (severe time loss injuries) in Football, Basketball, Volleyball, Wrestling, Baseball/softball	1378 severe injuries. No demographic info. High school age in USA	Overall, 0.39 severe injuries/1000AE's. Football had highest IR (0.69, wrestling (0.52, girls' basketball (0.34) & girls' football (0.33). Sig. sex diff. overall (0.45 male, female (0.26). Sex comparable sports sig. sex diff.; female 0.29/1,000 AE's vs. male 0.23. More specifically, female basketball (0.34) male basketball (0.24) (RR,1.43 95%CI 1.10-1.86; p=0.009). The severe injury rate sig. greater in competition (0.79) than practice (0.24). Overall Male RR 1.74 (95%CI 1.54-1.98; p<0.001). Sex comparable sports, female RR 1.28 (95%CI 1.08-1.52; p=0.006). Female Basketball RR 1.43 (95%CI 1.10-1.86; p=0.009). Overall competition RR 3.30 (95%CI 2.97-3.67; p<0.001). Female football knee IPR 2.14 95%CI 1.30-3.53; p=0.001). Male football fracture IPR 1.90 95%CI 1.11 3.26; p=0.016). Female football ligament sprains (complete tear) IPR 2.88 95%CI 1.26-6.57; p=0.005). Female basketball IPR 2.17 (95%CI 1.38-3.41; p<0.001). Male basketball foot/toe injuries; IPR 5.33 (95%CI 1.22-23.35 p=0.012). Male basketball fracture IPR 2.49 (95%CI 1.59-3.91; p<0.001). Female basketball IPR 1.95 (95%CI, 1.88-2.02; p<0.001).
Fernandez 2007 [170]; 17, 61% Moderate	Acute lower extremity injury in Baseball, American Football & wrestling; Softball & volleyball; boys' & girls' basketball & football	No demographic info included. High school sports in the USA	LESRI rate 1.33/1,000 AEs across all sports. Highest male IR; 2.01/1,000 in football & Highest female IR; 1.59/1,000 in football. Female with ligamentous knee injuries were 2 times more likely to require surgery than males (67% vs. 35%; p<0.01). Female had 1.5 times proportion of season-ending LESRIs of males (12.5% vs. 8%; p<0.01). Among high school athletes in 2005, 2,298 of 4,350 injuries (52.8%) were LESRIs. IPR for surgery in football 3.57 (95%CI=1.20 to 10.61) times higher in Female players (7.3% vs. 2.0%); p=0.014. IPR of female surgeries for ligamentous tears of knee (e.g., anterior cruciate ligament [ACL] tears) were 1.90 (95%CI 1.24 to 2.93) higher (67.4% vs. 35.4%) p=0.005. Overall, season ending LESRI 1.55 (95%CI 1.12 to 2.15) more likely in females. (12.5% vs. 8%) p=0.008.

Hinton 2005 [119]; 16, 57% Moderate	Acute & overuse injuries in Lacrosse	2259 male & 1141 female participants. Most participants were upper-level high school players aged 15 to 18 yrs who aspired to play collegiate lacrosse. No specific demographic info reported	Sig. sex diff. in overall IR; male 2.89/1,000 AEs, female 2.54/1,000 AE's, Ankle IR; male 0.47/1,000 AE's, female 0.64, neck IR; male 0.08, female 0.02, shoulder IR; male 0.24, female 0.10, upper leg IR; male 0.22, female 0.10, back/trunk IR; male 0.22, female 0.10, concussion IR; male 0.29, female 0.10. Overuse IR male 0.24, female 0.36 *Not sig. Overall male IRR 1.14 (95%CI 1.00-1.30). Game male IRR 3.00 (95%CI 2.26-4.05). Ankle injury male IRR 0.73 (95%CI 0.55-0.98), neck injury male IRR 3.89 (95%CI 1.13-20.73), shoulder injury male IRR 2.38 (95%CI 1.32-4.55), upper leg injury IRR 2.15 (95%CI 1.18-4.14), back/trunk injury male IRR 2.11 (95%CI 1.15-4.06), concussion male IRR 2.99 95%CI (1.65-5.79), overuse injuries male IRR 0.69 (95%CI 0.46-1.03).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Huffman 2008 [171]; 15, 54% Moderate	Rare injuries & conditions in American Football, Football, Volleyball, Baseball/softball, Basketball, Wrestling	Demographic info not included. High school age. 2005-07 data analysed	Overall, RIC IRs were higher among boys (12.4/100,000AE's) than girls (2.51/100,000AE's) & higher in competition (15.9) than in practice (6.32). Eye injuries IR of 1.10/100 000 AEs. IRs higher in boys (1.27) than girls (0.75) not sig. Neck & cervical IRs were higher in boys (7.99) than girls (0.92). Dehydration & heat illness IRs were higher in boys (2.34) than girls (0.42). Overall, RIC-RR 4.93 (95%CI 3.39-7.18) & higher in competition than in practice (6.32) RR 2.52 (95%CI 2.02-3.14). Eye injuries RR 1.69 (95%CI 0.80-3.57) not sig. Neck & cervical-RR 8.69 (95%CI 4.73-15.96). Dehydration & heat illness RR 5.59 (95%CI 2.24-13.97).
Jayanthi 2020 [164]; 19, 68% Moderate	Injuries associated with sport specialisation - multiple sports	n=1208 participants at baseline. Final study sample n=579 participants. By 18-month follow-up survey n=308 (53%) participants remained in the study. By 3-year follow up study n=61 (11%) completed the sports participation. Mean age at baseline 14.05±2.26 yrs, injured participants 14.41±2.08 yrs, uninjured participants 13.03±2.46 yrs (p<.001). (more detailed characteristics included in the paper).	Univariate analysis; female OR 1.28 (95%CI 1.01-1.62; P=.04). (increasing chronological age OR, 1.11 (95%CI 1.05-1.17); p<.001, higher BMI OR 1.04 [95%CI 1.12-1.27]; P=.05, increased wkly hrs of physical activity OR 1.19 per 5 h/wk (95%CI 1.02-1.05; p <.001) & organized sports OR 1.39 per 5 h/wk (95%CI 1.27-1.52; p<.001). Highly specialized (cf. to low specialised) athletes OR 1.72 (95%CI 1.35-2.20) & 1.52 times greater odds of an injury than moderately specialized athletes (95%CI 1.18-1.96). Highly specialized athletes RR 1.41 (95%CI 1.06-1.87; p=.02) (after adjusting for sex, age, time from baseline, BMI, & wkly hrs in organized sports). Overuse injuries Female OR 1.50 (95%CI, 1.14-1.99; P =.004). Multivariate analysis for overuse injuries consisted of the following variables: sex, time, BMI, total wkly hrs of physical activity, wkly sports hrs, exceeding the wkly hrs of organized sports by the age recommendation, & degree of sport specialization. Female OR 1.43 (95%CI 1.05-1.96; p=.02) cf. to males. After controlling for all variables, athletes categorized as highly specialized had 1.46 (95%CI 1.04-2.04) times greater odds of an overuse injury than low specialized athletes (P=.03).
Kerr 2011 [172]; 16, 57% Moderate	Acute Injuries (contact) in American Football, , Volleyball, Basketball, Wrestling, Baseball/Softball	9010 player-player contact injuries. (46% all injuries).	Overall IIR-11.6/10 000 AEs (practice rate=6.2/10 000 AEs, competition rate=26.1/10 000 AEs). In football; A larger proportion of females had knee injuries (25.1%) than males (16.9%; p=0.003). In Basketball; player-player contact injuries represented a greater portion of total injuries among males (45.7%) than females (35.3%; p<0.001). A larger proportion of females had knee injuries (14.1%) than males (7.5%; p=0.002), a larger proportion of females had concussions (17.5%) than males (6.9%; p<0.001). In football; female knee IPR 1.48 (95%CI 1.14-1.93). In Basketball; male player-player contact

			IPR 1.29 (95%CI 1.16-1.44), female knee IPR 1.87 (95%CI 1.24-2.83, female concussion IPR 2.53 (95%CI 1.71-3.74).
Kerr 2011 [173]; 19, 68% Moderate	Acute injury-Dislocation/separation injuries in American Football, football, wrestling, baseball/softball, basketball, volleyball	No demographic info included. High school age 14-18yrs. 2005-09	Overall males sig. higher IR (1.30/10,000AE's) vs. female (0.33). In gender-comparable sports, no sig. sex diff. (m 0.39 vs. f 0.34; p=0.406). Football, no sig. sex diff. Male overall RR 3.91 (95%CI 3.12-4.89; p=0.001). Driven by wrestling & football. Football male IPR 2.09 (95%CI 1.09-4.01; p=0.024). Only 2% injuries, male shoulder dislocations/separations IPR 3.23 (95%CI 1.04-10.00; p=0.021).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Kerr 2017 [120]; 16, 57% Moderate	Concussion in Football , basketball, softball/baseball, American Football, Track, wrestling, cheerleading, volleyball	No Demographic info included. Middle school age.	57 game & practice concussions across 76,384 AEs; IR 0.75/1,000 AEs. American Football (2.61/1,000 AEs) & female football (1.30) had highest concussion rate. Sex-comparable sports overall sig. sex diff.; female 0.66 vs male 0.18/1,000 AE's. Findings were retained in sport-specific analyses, but only found sig. in football. Female football IR 1.30 vs. male football 0.15/1,000 AE's. Overall concussion rate higher in games than practices (1.15 vs 0.63/1,000 AEs). This finding was retained per sport, although only girls' basketball reported a sig. finding. In games, female basketball had higher concussion rate than female football (2.61 vs. 2.47/1,000 AE's). Game IRR 1.83 (95%CI 1.06-3.15). Sex-comparable sports; female IRR3.73 (95%CI 1.24, 11.23). Female football IRR 8.46 (95%CI 1.04-68.77). Overall games IRR 1.83 (95%CI 1.06-3.15).
[174]; 20, 71% Moderate	Acute & overuse injuries in Lacrosse	1498; 1098 male, 408 female. Age divisions U9-U15. No specific demographic info included.	Sig. sex diff. for overall IIR; male 12.7/1000AE (95%CI 11.1-14.3) game/practice, female 8.7(95%CI 6.5-10.9) & in game IIR; male 30.2/1000AE (95%CI 25.3-35.1, female 17.1(95%CI 11.1-23.1) & time-loss IIR in game male 4.5/1000AE (95%CI 2.6-6.4, female 3.3(95%CI 0.7-6.0). No sig. sex diff. in practice IIR; male 6.6/1000AE (95%CI 5.2-7.9, female 5.6 (95%CI 3.5-7.7). Overall male RR; 1.5 (95%CI 1.1-1.9), Game male RR 1.8 (95%CI 1.2-2.6). Trunk IPR male IPR 4.2 (95%CI 1.0-16.8). Head female IPR; 2.3 (95%CI 1.2-4.5). Highest proportion of body parts injured; male knee (14.5%), trunk (14.1%)*, ankle (9.5%), female head/neck (20.3%)*, ankle (15.3%, hand/wrist (10.2%). *=sig sex diff. No sig. for diagnoses-contusion & sprain similar in both.
Kolstrup 2016 [130]; 20, 71% Moderate	Acute & overuse injuries in football	45,606 children & adolescents played, (71% boys & 29% girls). 1831 injured players (60% male & 40% female). Mean age injured male was 15.1 (1.8) yrs, mean age injured female 14.7 (1.5) yrs, p<0.001. Players from Europe (89%), North America (4%, Asia (3%, Africa (2%) & South America (2%).	Overall IR 15.3 (14.6;16.0)/1,000 player hrs. Overall sig. sex diff.; female 20.3 (18.9;21.8)/1000 player hrs vs. male 13.1 (12.3;13.9) p<0.001. IR ankle & foot injuries in higher in female 7.02, than male 3.75 p<0.001. IR of knee & lower leg higher in female 4.30 than male 2.30 p<0.001. *diff. augmented in 11–15-year-olds. When comparing genders only in the 11-15-year-olds, all incidences were highest among girls. Conversely, in the group of 16-19-year-olds, all sig. gender diff. reveal a higher incidence in the group of boys. Female RR 1.5 (p<0.001). Female higher proportion of injuries to knee/lower leg-23.8%, (vs. male-19.0%, p<0.01). Male higher proportion of fracture-6.8%, (3.3% female, p<0.001). Male; higher proportion hip & thigh injuries, 14.6% (12.4;16.9, than girls, 7.4% (5.5;9.7, p<0.001). *Youngest females have higher IR than any other group. *IR decreased with age.

Lincon 2011 [176]; 17, 61% Moderate	Concussion in Football, basketball, lacrosse, softball, baseball, American Football, wrestling, field hockey, cheerleading.	No demographic info included. High school sports in the USA	Girls had sig. higher rate (per 1,000 AE's) of concussion in sex comparable sports. Basketball IR-f 0.16, m 0.10; Football IR-f 0.35, m 0.17; Softball/baseball IR f 0.11, m 0.06. Basketball RR 1.7 (95%CI 1.3-2.2), Football RR 2.1 (95%CI 1.6-2.6) & Softball/baseball 1.9 (95%CI 1.2-3.0).
Maciejewski 2016 [205]; 19, 68% Moderate	Acute & overuse injuries in Judo	4 major divisions: 76 junior men (17.67±1.08 yrs), 52 junior women (17.64±1.13 yrs), 52 boys (11.97±2.40 yrs), 12 girls (11.88±2.60 yrs).	No sig. sex diff. in IR. Boys 84.03 (47.20-120.86, girls 37.03 (14.29-88.37). Jnr male 106.56 (73.11-140.00), Jnr female 112. 40 (71.49-153.31). Sig. sex diff. for upper body IR Jnr men 54.64 (30.70-78.59) Jnr female 42.64 (17.44-67.83). Boys OR 3.13 (95%CI 0.62-15.76). Jnr female OR 1.20 (95%CI 0.59-2.43). Upper body injury Jnr male OR 4.17 (95%CI 1.57-11.08).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Marar 2012 [177]; 18, 64% Moderate	Concussion in Baseball/softball, Basketball, Cross-country & track & field, American Football, Gymnastics, Swimming & diving, Wrestling, Hockey, Football, Volleyball, Lacrosse	No demographic info included. High school sports in the USA	Overall injury rate of 2.5 per 10,000 AEs. The injury rate sig. higher in competition (6.4) than practice (1.1). Football had highest concussion rate (6.4, followed by boys' ice hockey (5.4) & boys' lacrosse (4.0). In sex comparable sports, girls had sig. higher concussion rate (1.7) than boys (1.0). In football girls had sig. higher IR (3.4/10,000 AEs) than boys (1.9). Basketball girls had sig. higher rate of concussion (2.1) than boys (1.6). Softball players had sig. higher rate of concussion (1.6) than baseball players (0.5). Overall competition to practice RR 5.7 (95%CI 5.2-6.3). Concussions represented a greater proportion of total injuries among boys' ice hockey (22.2%) than all other sports studied (13.0%) (injury proportion ratio [IPR], 1.7 (95%CI, 1.4-2.1; p<0.01). In sex comparable sports; girls to boys RR 1.7 (95%CI 1.4-2.0). In football girls to boys RR 1.8 (95%CI 1.4-2.3) & concussions represented a greater proportion of total injuries among girls (15.4%) than boys (11.1%); IPR 1.4 (95%CI=0.96-2.0; p=0.08). Basketball girls to boys RR 1.3 (95%CI 1.03-1.8; p<0.03). Softball players to baseball RR 3.2 (95%CI 2.1-5.4; p<0.001). Concussions represented a sig. greater proportion of total injuries among softball players (13.4%) than baseball players (5.4%) (IPR 2.5 95%CI 1.6-3.9; p=<0.001). Majority of concussions resulted from participation in football (47.1%, 912, followed by girls' football (8.2%, 159, boys' wrestling (5.8%, 112, & girls' basketball (5.5%, 107). In more than 40% athletes in sports other than girls' swimming & girls' track, concussion symptoms resolved in 3 days or less. Athletes most commonly returned to play in 1 to 3 wks (55.3%, with 22.8% returning in less than 1 wk & 2.0% returning in less than 1 day. In basketball; boys had quicker symptom resolution times & return-to-play outcomes than girls; however, neither diff. was sig.
Marshall 2020 [209]; 16, 57% Moderate	All injuries in cross country (high school).	N=681 patient cases (415 females, age 15.2±1.2 yrs, 266 males, age 15.4 2±1.3 yrs). The number of exposed clinical practice sites (i.e., high schools with student-athletes participating in cross-country & at risk for injury) was not different between sexes for each academic year (female 19.2±14.9, male	No sex diff. in injury type by anatomical location or diagnosis. There was a trend towards a greater proportion of female injuries being to the ankle & hip & a diagnosis of tendinopathy.

	18.0±14.0; p=.86). 2009-19.	

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Messina 1999 [121]; 17, 61% Moderate	Acute & overuse injuries in Basketball	973 male athletes. 890 female athletes. 75 of the 100 boy's schools completed the study, 80 of the 100 girls' schools completed the study with an additional. The average number of players per team was 13 for the boys & 11 for the girls. The average number of games per season was 31 for the boys' teams & 29 for the girls' teams. The average number of practices per season was 77 for the boys' teams & 67 for the girls' teams.	Overall Male IR 0.56/athlete/season. Female IR 0.49/athlete/season (p<0.0015). Overall Male IR 3.2/1,000 plyr hrs, Female IR 3.6 (not sig.). Practice Male IR 1.8/1,000 plyr hrs, Female IR 2.0 (p<0.09). No sig. diff. for type of injury. Lower extremity IR higher in females (p<0.003, knee IR females 0.1/athlete/year, male 0.06 (p<0.0001). Face IR higher in males (p<0.023). Rate of severe injury; male 0.027/athlete/year, female 0.038 (not sig.). Based on exposure time, risk of knee injury was 2.3 times greater in female athletes than in male athletes (p<0.001).
Mitchell 2015 [179]; 16, 57% Moderate	Knee-Patellofemoral instability injury (PFII) in American Football, Football, Volleyball, Basketball, track & field, Baseball/softball, field hockey, gymnastics, Lacrosse, Ice Hockey, swimming & diving, cross country	No demographic info reported. High school sport	Overall IR 1.95/100,000 AEs. Highest rates; girls' gymnastics (6.19/100,000 AEs, boys' football (4.10). Overall IR; female 1.66, male 2.15. In sex-comparable sports females; 1.47, males 0.88. Football IR female 2.92, male 1.22 (p<0.05). Overall female RR 0.77; (95%CI, 0.62-0.94). In sex comparable sports; female RR 1.67 (95%CI 1.19-2.34). Football female RR 2.40 (95%CI 1.39-4.24). Sex-comparable sports showed similar proportions of PFIs relative to total knee injuries overall. PFIs accounted for a larger proportion of total knee injuries in baseball (12.6%) vs girls' softball (4.8%); IPR, 2.63 (95%CI 1.06-6.55). Contact injuries male IPR 1.53 (95%CI 1.24-1.88). Player-player contact, male IPR 2.45 (95%CI, 1.70-3.55). Non-contact injuries female, IPR 1.56 (95%CI, 1.23-1.98).

Mitchell 2016 [178]; 16, 57% Moderate	Acute knee Injuries in American Football, Football, Volleyball, Basketball, track & field, Baseball/softball, field hockey, gymnastics, Lacrosse, Ice Hockey, swimming & diving, cross country, cheerleading.	No demographic info reported. High school sport	Overall IR 5.1/100,000 AE's. Overall IR; males 5.9, females 4.4 ($p<0.05$). Sex comparable sports overall IR; female 5.5, male 2.5 ($p<0.05$). Overall male, RR 1.4 (95%CI 1.2-1.5). Sex comparable sports overall female RR 2.2 (95%CI 1.8-2.7). Player-player contact male IPR 1.7 (95%CI 1.4-2.1). Non-contact mechanisms female IPR 1.4 (95%CI 1.2-1.6).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Miyake 2016 [199]; 19, 68% Moderate	Acute & overuse injuries in Badminton	133 (94 male, 39 female) badminton players in junior high school (18 male, age 13.4±0.7yrs, height 161.7±7.9cm, weight 49.5±7.6kg; 16 females, 13.8±0.8yrs, 159.4±6.1cm, 49.9±6.8kg), high school; 26 male (age 16.2±0.5yrs, 169.1±5.2cm, 58.0±5.6kg); 16 female (age 16.1±0.7yrs, 162.3±5.7cm, 54.7±5.0kg), or university; 50 male (age 20.0±1.2yrs, 172.5±6.4cm, 65.7±6.6kg); 7 female (age 20.7±1.1yrs, 163.4±3.1cm, 59.6±5.3kg).	Practice IR (/1000 hrs) higher in females than males & increased with age. Sex diff. in practice IR (1000 hour) (Junior male 0.9/1,000 hrs female 1.3/1,000 hrs. High School male 1.5/1,000 hrs female 2.4/1,000 hrs. University male 2.5/1,000 hrs 5.1/1,000 hrs) ($p< 0.001$). No sig. diff. ($p=0.527$) in match or practice in IR by AE's.
Monfort 2015 [180]; 16, 57% Moderate	Acute lower limb Injuries in Basketball & football	No demographic info reported. Data included from High School RIO tool for academic yrs 2005-2006 to 2011-2012. 9 sports, including basketball & football, from 100 schools strategically chosen to provide a nationally	Female IR sig higher for both ball-handling & defending actions for both sports ($p<0.05$). Female knee IR & all-NC IR across sports & activities ($p<0.05$). Female higher ankle IR when ball handling in football ($p<0.001$). Football defending to ball-handling IR between competition & practice; female OR 1.88 (95%CI 1.01-3.48; $p=0.047$). Male No sig. Comp vs. Practice. Non-contact ankle injuries female basketball RR 1.58 (95%CI 1.01-2.50; $p=0.045$).

		representative sample. 6,431,032 football & basketball AEs.	
Muller 2017 [203]; 17, 61% Moderate	Acute & overuse injuries in (elite) Skiing	51 male, age 11.6±1.5yrs, height 153.4±9.0cm, weight 42.3±8.1kg, BMI 17.8±1.9, APHV 13.7±0.5yrs. 31 female, age 11.8±1.3yrs, height 154.9±7.7cm, weight 43.8±7.9kg, BMI 18.1±2.2, APHV 12.0±0.5yrs.	Acute IR 0.63/athlete, acute II 0.86/1,000 hrs (males, 0.9/1,000 hrs of training; females, 0.79/1,000 hrs of training). No sig. sex diff. in traumatic injuries between males & females (p=0.617, or the three groups of maturity (p=0.643; Figure 3, or the four relative age quartiles (p=0.3). Overuse IR rate 0.21 overuse injuries/athlete II 0.28/1,000 hrs of training (males, 0.4/1,000 hrs of training; females, 0.09/1,000 hrs of training). No sig. sex diff. in overuse injuries (p=0.81), the three groups of maturity (p=0.696), or among the four relative age quartiles (p=0.22). 42.7% athletes (35, 14 females, 21 males) had acute injuries. 10.7% athletes had overuse injuries (13, 2 females, 11 males).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Nelson 2007 [181]; 17, 61% Moderate	Acute & overuse ankle Injuries-Boys' American Football, football, basketball, wrestling, & baseball; girls' football, volleyball, basketball, & softball	905 injuries. No demographic info included. High school sport.	Overall, ankle injury rates were higher in competitions than in practices (RR 2.58; 95%CI 2.26, 2.94; P.001). Overall, girls had an ankle injury rate that similar to boys (5.39 vs. 5.15/10 000 A-Es). In sports played by both sexes (football, basketball, & baseball or softball, boys had higher rates of practice-related ankle injuries, but girls had higher rates of competition-related ankle injuries. Proportion of ankle injuries higher female athletes (32.5%) than male athletes (18.4%). Football; injuries to the ankle were most frequent injury in both sexes, but the proportion of ankle injuries to total football injuries higher among female (31.5%) than male (23.5%). Basketball; male basketball had highest ankle IIR of all sports studied (7.74/10,000AE's) (vs. female 6.93, No sig. sex diff., slightly higher proportion in male than female basketball (39.7% vs. 36.5%, more male injuries sustained by forwards (the primary rebounding position) than female (46.2% vs. 26.0%, conversely in females greater proportion of ankle injuries tended to occur to guards (49.2% vs. 41.8%). Overall competition injury RR 2.58 (95%CI 2.26, 2.94; p<0.001). Female ankle IPR 1.77 (95%CI 1.53-2.05; p< 001). Football accounted for 33.6% all ankle injuries (boys 15.7%, girls 17.9%). Football female IPR 1.35 (95%CI 1.00, 1.81; p<05). Basketball playing position 'forwards' male IPR 1.78 (95%CI 1.21-2.62; p<01), basketball 'guards' female IPR 1.18 (95%CI 0.88-1.58; p<0.29). No sig. but in football male injuries from contact with another player, in females contact with equipment/apparatus.
O' Connor 2017 [182]; 16, 57% Moderate	Concussion in 27 American high school sports	Demographic info not included. High school age. 2004 sports related concussions analysed	Overall SRC rate 3.89/10 000 AEs all sports. Overall SRC rate highest American Football (9.21/ 10 000 AEs, followed by boys' lacrosse (6.65/10 000 AEs) & girls' football (6.11/10 000 AEs). During competitions, American Football (19.87/10 000 AEs, boys' lacrosse (17.51/10 000 AEs) & girls' football (17.16/ 10 000 AEs) had highest SRC rates. During practice, American Football (6.78/10 000 AEs), boys' wrestling (4.75/10 000 AEs) & girls' lacrosse (3.44/10 000 AEs) had highest SRC rates. Sex comparable sports overall SRC rate significantly higher in girls than in boys (2.64 vs. 1.69/10,000AEs) & in competition (girls-7.07, boys-4.86) & practices (girls-1.49, boys-0.85). Within specific sex-comparable sports, girls had increased SRC rates cf. with boys for softball (3.57/10,000AEs) or baseball (0.86/10,000AEs, basketball (4.44 vs. 2.52/10,000AEs, football (6.11 vs. 3.98/10,000AEs, & indoor track & field (0.52 vs. 0.14/10,000AEs). Overall SRC rate higher in competition than in practice (RR 3.30 [95%CI 3.02-3.60]). Collective overall SRC rate higher in girls than in boys (RR 1.56 [95%CI 1.34-1.81]). During competition, girls RR1.46 (95%CI 1.20-1.77) times SRC rate of boys, increasing to RR 1.75 (95%CI 1.39-2.20) times SRC rate during practices. Distributions of number of symptoms reported between male & female in sex comparable sports No sig. diff. (p=0.09). Most athletes with SRCs reported symptom resolution < 7 days (40.7%, 814) or 8 to 14 days (21.7%, 234). Among sex-comparable sports, the proportion of SRCs with symptom resolution time within 7 days was sig. higher in boys than in girls (44.4% [142] vs 32.2% [122]; IPR 1.38 (95%CI 1.14-1.67). In contrast, the proportion of SRCs with symptom resolution time greater than 14 days was sig. higher in

			girls than in boys (33.0% [125] vs 24.4% [n78]; IPR 1.35 (95%CI 1.06-1.72). The proportion of SRCs with symptom-resolution time greater than 28 days was also higher in girls than in boys No sig. (14.8% [56], vs 10.3% [33]; IPR 1.43 (95%CI 0.96-2.15). Overall, 211 (10.5%) players RTP time less than 7 days. Among sex-comparable sports, proportion of athletes with SRCs RTP less than 7 days was sig. higher in boys than in girls (14.1% [45] vs 8.4% [32]; IPR 1.67 (95%CI 1.09-2.56). Conversely, 23.0% (461) of all athletes & more girls than boys (28.2% [107] vs 14.7% [47]; IPR 1.92 (95%CI 1.41-2.62) RTP greater than 28 days.
O' Connor 2020 [212]; 16, 57% Moderate	All injuries in junior tennis	Injuries in Irish national junior tennis program (n=82) over two yrs were collected in a prospective cohort study. Age 8-22yrs.	54% players sustained an injury over the study period. No sex diff. in injury rates IRR 1.05 (95%CI 0.72-1.5). Trend towards females having a greater proportion of shoulder injuries (female 23.5%, male 10.9%), males having a greater proportion of knee injuries (male 29.1%, female 7.8%).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Olsen 2006 [136]; 18, 64% Moderate	Acute & overuse injuries in youth Handball	90 teams; 75 female teams (900 players) & 15 male teams (180 players). The players on these teams were all amateurs, depending on ability & ambition, they practiced 1-5 times/wk & played between 20 & 50 matches during season. Injured players mean age 16.4±0.5yrs (range 15-17).	No sig. sex diff. in overall injury rate (female 14.5, male 8.3 injuries per 1,000 player hrs). No further sex analysis. Female RR 1.8 (95%CI 0.9-3.4).
Pierpoint 2016 [198]; 16, 57% Moderate	Acute & overuse injuries in high school Track & Field	1317 female injuries reported. 1168 male injuries reported. No further demographic info reported. High school sport.	Sig. sex diff. in IIR; overall-Female 0.99/1,000 AE's male 0.72 & in practice-female 0.93/1,000 AE's male 0.58. (No sig. in competition IIR). Stress fractures & shin splits accounted for 2.1% & 3.3% boys' injuries, respectively. Stress fractures & shin splits accounted for 4.6% & 4.8% girls' injuries, respectively. A sig. greater proportion of female injuries were chronic/overuse in nature (~40%, male ~30%). Female overall RR 1.37; (95%CI 1.27-1.48). Female practice RR 1.60 (95%CI 1.46-1.76). Female competition RR 0.93 (95%CI 0.80-1.07). Female chronic/overuse IPR 1.32 (95%CI 1.14-1.52).
Pieter 2010 [206]; 17, 61% Moderate	Acute & overuse injuries in Karate	302; 218 male, 84 female. Ages 7-15yrs.	No sig. sex diff. in IIR 1000 AE or 100 ME; male 99.74/1000AE, female 115.11 & male 13.30/100ME, female 15.35. No sig. sex diff. in overall IR male 34.86/100 athletes, female 38.10. Female were not considered to be at a higher risk of injury. No sig. sex diff. on anatomic location or type of injury. Overall female RR 1.093 (95%CI 0.788-1.516, p=0.596).
Powell 2000 [128]; 16, 57% Moderate	Acute & overuse injuries in Baseball/Softball, Basketball & football	8988 reportable injuries: 4559 male (50.7%) & 4429 female (49.3%). High school sport.	Sig. sex diff. in overall injury for Softball 16.7/100p plyrs, Baseball 13.2 (p< 0.001). Baseball players, shoulder/arm category accounted=19.7%; Softball players, the shoulder/arm=16.3% injuries. Baseball injuries evenly divided between practice (49.4%) & games (50.6%, Softball, practices accounted for 55.9% injuries (p<0.001). Player position & injury category similar between Softball/Baseball. No sig. overall in Basketball. Girls' basketball a higher proportion of major injuries than did the boys' teams (p<0.05). Female basketball more surgeries (p<0.02). Female basketball had a higher proportion of game-related injuries (46.8%) than did the boys' teams (42.0%) (p<0.01). Sig. sex diff. in football; general trauma injuries & fractures male (29.9%, female (24.9%) (p<0.01, reinjuries in female (10.4%, male (8.4%) (p<0.04), injuries requiring surgery female (3.09%, male (2%) (p<0.001). Overall Softball IDR 1.27 (95%CI 1.15-1.39). Female overall football IDR 1.14 (p<0.001). Female knee Basketball IDR 1.44. Female knee

			surgery Basketball knee surgery IDR 2.65. Female Basketball ACL surgery IDR 4.15. Female football knee injuries IDR=1.46, Female football knee surgeries IDR 3.66 & female football ACL surgeries IDR 3.41.
Pytiak 2018 [184]; 18, 64% Moderate	Acute & overuse injuries in Baseball/Softball	299 injuries; male=214, female=75. High school sport.	Overall IIR softball 1.22/1000AEs, baseball 1.01/1000AEs ($p<0.05$). Sex diff. in elbow IIR; Baseball 0.92/10,000AE's, Softball 0.43 ($p<0.05$). Greater proportion baseball injuries (9.2%) than softball injuries (3.6%). Elbow injuries were sig. more likely to occur in competition than practice in baseball; this was not shown for softball. Baseball injuries were more likely to require surgery than softball injuries (6.2% vs 0.0%; $p=0.02$). Overall Softball RR 1.21 (95%CI 1.15-1.29). Elbow Baseball RR 2.12 (95%CI 1.64-2.77). Baseball elbow IPR 2.58 (95%CI 1.99-3.33). Baseball competition elbow injury RR 2.24 (95%CI 1.71-2.93). Softball competition elbow injury RR 1.14 (95%CI 0.71-1.81). Baseball ligament sprains; IPR 1.70 (95%CI 1.02-2.84). Softball tendinitis IPR 0.62 (95%CI 0.40-0.96). If all injuries occurring during pitching were removed from both baseball & softball; no sex diff. (non-pitching baseball RR 1.19 [95%CI, 0.88-1.62]).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Rauh 2000 [200]; 17, 61% Moderate	Acute & overuse injuries in cross country	n=3,233. 1,202 females (from 99 teams). 2,031 males (from 100 teams). Age 14-18. From 23 high schools.	Overall IIR 13.1/1,000 athletic exposures (AEs). Sig sex diff. for overall IIR; females 16.7/1,000 AEs, males; 10.9/1,000 AEs ($p < 0.0001$). Female had sig. ($p < 0.0001$) higher IIR for initial injury (10.4/1,000 AEs v 7.6) & subsequent injuries (48.5/1,000 AEs v 35.5), particularly at same body location (44.1/1000AEs v 32.2). New injury highest overall IIR was at the shin (1.9/1,000 AEs) & highest reinjury at the same body location (53.9/1,000 AEs). Female had sig. higher initial IIR than males for shin (2.8/1,000 AEs v 1.4), hip (0.7/1,000 AEs v 0.4), & foot injuries (1.0/1,000 AEs v 0.5), & higher reinjury rates for knee (50.8/1,000 AEs after initial injury v 33.5), calf (75/1,000 AEs v 13.6), & foot (84.1/1,000 AEs v 29.2) injuries. Overall, higher IIR during practices (9.2/1,000 AEs) than meets (7.8/1,000 AEs) IRR 1.2 (95%CI 1.0-1.4). In males, injuries in practice sig. greater than in meets RR; 1.5 (95%CI 1.1-2.0). No sig. diff. between practice & meet in among females. Female had higher rates of injuries than males for meets; IRR 2.1 (95%CI 1.5-3.1) & practices IRR 1.4 (95%CI 1.2-1.6).
Schroeder 2015 [186]; 14, 50% Moderate	Overuse Injury in American Football, Boys wrestling, Boys ice hockey, Girls field hockey, Girl's gymnastics, Cheerleading, Lacrosse, Swimming, Track & Field, Football , Volleyball, Basketball, Baseball/softball	No demographic info reported. High school sport.	Sports with highest % all injuries that were overuse; male & female swimming (55.7 & 44.7%), track & field (28.5 & 36.8%). Sports with lowest % all injuries that were overuse male ice hockey (1.4%), male volleyball (2.4%). Overuse injuries more likely in practice than competition. When all 20 sports were evaluated, IIR higher in females (1.88/10,000AE's) than males (1.26). Sex-comparable sports IIR higher in female (2.08) than male (1.44). Practice IIR higher in female (2.27) than in male (1.34). Across all sports overall injury; female RR 1.50 (95%CI 1.39-1.61). Sex comparable sports overall injury; female RR 1.45 (95%CI 1.32-1.58). Sex comparable sports, female RR 1.69 (95%CI 1.56-1.83). Overall, overuse injuries represented 13.3% all injuries in girls but only 5.5% all injuries in boys.
Stracciolini 2014 [187]; 16, 57% Moderate	Acute & overuse injuries	n=2133. 1151 females, 982 males.	Sig. sex diff.; overall overuse female 62.5% male 41.9%, traumatic female 37.5%, male 58.2%; lower extremity female 58.8% overuse, males 56.2% traumatic; hip/pelvis female 90.9% overuse, males 58.3% traumatic; spine female 93.9% overuse, males 18.8% traumatic. Injury location differed by sex; females 65.8% lower extremity injury, 15.1% upper extremity, 6.7% hip/pelvis & 11.3% spine, male 53.7% lower extremity injury, 29.8% upper extremity, 3.7% hip/pelvis & 8.2% to the spine. Sig. sex diff. for injury diagnosis; fracture male 19.5%, female 8.2%, PFPS male 4%, female 14.3%, OCD male 8.6%, female 4.3%, instability male 4.3%, female 7.3%, SH fracture male 7%, female 4.1%. On average, male & female patients participated in a total of 2.4 activities. A higher proportion of male patients played contact/collision sports (68.9%, female 43.3%; $p<0.001$). Data between sexes not adjusted for sport type; therefore, some patterns observed likely due to demand of sport e.g., female spine overuse in gymnastics.

Stracciolini 2015 [188]; 18, 64% Moderate	Overuse Injury in multiple team & individual sports	1614; mean age=14.2, BMI z score 0.5. 872females; mean age=14.2, BMI z score 0.4. 742 males; mean age=14.2, BMI z score 0.6.	Sig. sex diff. for all injury sites; Head male 3.4%, female 0.7% (p<0.001), Chest male 2.4%, female 0.6% (p<0.002), Upper extremity male 30.5% female 14.2% (p<0.001), Lower extremity male 53.4% female 66.8% (p<0.001), Hip/pelvis-male 3.8% female 7.1% (p<0.004), Spine male 7.7% female 11.3 (p<0.013). 45.9% sex diff. can be accounted for by distribution of background & characteristics (age, BMI, prior injury, type of sport). Team sports increased odds of being seen for overuse (vs traumatic) injury in both males (OR 5.329 [95%CI 3.026-9.383]; p=0.001) & females (OR 1.27 [95%CI 0.824-1.979]; p<0.274). 63% females had overuse injuries vs. 40% males (p<0.001). Analyses accounted for participating in sports that are associated with high overuse injury rate.
Swenson 2009 [193]; 16, 57% Moderate	All recurrent injuries in Baseball/softball, Basketball, American Football, Football, volleyball, Wrestling	High school athletes. No specific demographic data included. RIO - from 2005-2008, 13 755 injuries during 5 627 921 AEs	Overall IR 24.4/10 000AE's. Recurrent injuries accounted for 10.5% all injuries. American Football players had highest rate of recurrent injury (4.36/10 000 AE's). Sig. sex diff. for recurrent injuries in football; female 2.55 male 1.83. cf. with the other 7 sports, Basketball had greater proportion of recurrent injuries: Male (IPR 1.24 [95%CI 1.03-1.50]) & Female (IPR 1.53 [95%CI 1.27-1.86]). In sex comparable sports sig. diff. only in football, female; RR 1.39 (95%CI, 1.07-1.82). In 6/9 sports, recurrent injuries sig. more likely to occur in competition than practice, especially in American Football; RR 4.69 (95%CI 4.02-5.46) & female football; RR 3.89 (95%CI 2.69-5.60).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Swenson 2010 [192]; 20, 71% Moderate	Acute fractures in American Football, volleyball, basketball, football, wrestling, baseball, softball.	No demographic information. RIO from 2005–2009, 18,316 total injuries during 7,740,400 AEs.	Fractures were 4th most common diagnosis after ligament sprains, muscle strains, contusions. The highest rate of fractures was in American Football (4.61/10 000AE's) & the lowest in volleyball (0.52). Basketball boys 1.86/10,000AE's, girls 1.39. Football boys 2.17, girls 1.62. Baseball (15.4%) & softball (13.4%) had highest proportion of injuries that were fractures. Basketball boys RR 1.35 (95%CI 1.06-1.72) football boys RR 1.34 (95%CI 1.05-1.71). Overall competition (vs. practice) RR 3.21 (95%CI 2.93-3.52). This held for every sport except volleyball RR; 0.84 (95%CI, 0.40-1.77). In sex comparable sports boys practice RR (1.39; 95%CI 1.09-1.78); competition RR 1.17 (95%CI 0.97-1.41).
Swenson 2012 [191]; 19, 68% Moderate	Sports related fractures in American Football, volleyball, football, basketball, baseball/softball, wrestling.	No demographic info included. High school sports in the USA. RIO - during 2008-09 to 2010-11, ATs from the 11,625 injuries during 5,640,505 AEs. 2103 Fractures reported. 9% all injuries over the reporting period.	Overall rate of 1.82 fractures/10,000 AEs. Highest IR-football (4.37, male ice hockey (3.08, & male lacrosse (2.59). Overall male had significantly higher IR in competition (5.59 vs. 1.98) & practice (1.98 vs. 0.56). Male sustained 79.1% all fractures. Overall male competition RR 2.82 (95%CI 2.45-3.24) & practice RR 2.43 (95%CI 2.07-2.86). Using linear regression, we found the proportion of all injuries that were fractures was inversely correlated with the athlete's age (p=0.02) but not with Athletes' age-and gender-adjusted BMI. Overall Male IPR 1.57; (95%CI 1.42-1.74), Male lacrosse IPR 2.38 (95%CI 1.47-3.85), & Male football IPR 1.81 (95%CI, 1.42-2.30). No sig. sex diff. for basketball baseball/softball, track & field or volleyball. Recurrent fractures no sig. sex diff. overall or in gender-comparable sports.
Swenson 2013 [190]; 16, 57% Moderate	Acute ankle injury in American Football, volleyball, football, basketball, swimming, baseball/softball, wrestling, field hockey, ice hockey, gymnastics, track & field.	No demographic info included. From RIO - during 2005/06 to 2010/11, 7807 ligament sprains (30.5% all injuries) of which 4108 were ankle sprains.	Overall rate 2005-11 3.13/10 000 AE's (Boys IR 3.14/10 000 AEs & girls 3.11/10 000 AE). Sports with the highest IR; boys' basketball (5.16/10 000 AEs, girls' basketball (5.03) & girls' gymnastics (4.88). The highest competition IR boys' football (15.01/10 000 competition AEs, girls' basketball (9.45, girls' football (9.41, & girls' gymnastics (9.36). Highest practice IR boys' volleyball (3.83/10 000 AEs, girls' gymnastics (3.82, girls' volleyball (3.79, & boys' & girls' basketball (3.79 & 3.13, respectively). Rates were higher for girls than for boys RR 1.25 (95%CI 1.17-1.34) in sex-comparable sports. Rates were higher in competition than practice for boys RR 3.42 (95%CI 3.20-3.66) & girls RR 2.71 (95%CI 2.48-2.95). Girls were sig. more likely to sustain ankle sprains than boys in football (RR 1.46 95%CI 1.29-1.66), softball/baseball (RR 1.65 95%CI 1.29-2.12), track & field (RR 2.18 95%CI 1.44-3.31). Overall, ankle sprain rates were higher in competition than in practice for boys' RR 3.42 (95%CI 3.20-3.66) & girls' RR, 2.71 (95%CI 2.48-2.95) sports.
Swenson 2013 [189]; 16, 57% Moderate	Acute & overuse knee injuries in American Football, football, gymnastics, volleyball, basketball,	No demographic info included. RIO – during the 2005/06–2010/11, 5116 knee injuries	Overall rate of 2.98 knee injuries/10,000 AE. Highest IR in American Football 6.29/10,000 AE's, female football (4.53) & female gymnastics (4.23). Most commonly involved structure was the MCL (36.1%), patella/patellar tendon (29.5%), ACL (25.4%), meniscus (23.0%), LCL (7.9%), & PCL (2.4%). American Football & girls' football had highest rate of ACL injury (1.17/10,000 AEs) female gymnastics (1.14) & female basketball (1.07). American Football only sport with meniscal IR over 1/10,000 AEs & highest MCL

	baseball/softball, lacrosse, swimming & diving, & track & field.	reported during 17,172,376 AEs.	IR (2.42/10,000 AE). Overall competition RR 3.53 (95%CI 3.34-3.73). In sex comparable sports female RR 1.52 (95%CI 1.39-1.65). Female ACL injury RR 2.38 (95%CI 1.91-2.95). Female IPR 1.30 (95%CI 1.11-1.53). Football female RR 1.71 (95%CI 1.50-1.95), basketball female RR 1.89 (95%CI 1.62-2.20, baseball/softball female RR 1.68 (95%CI 1.28-2.21, track & field female RR 1.49 (95%CI 1.08-2.06). Overall ACL female RR 1.01 (95%CI 0.87-1.17). In sex comparable sports ACL female RR 2.38, (95%CI 1.91-2.95). ACL softball female RR 4.99 (95%CI 1.86-13.36), basketball female RR 4.54 (95%CI 2.99-6.89) & football female RR 2.33 (95%CI 1.67-3.26). Not including ACL in this paper analysis or discussion.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Tirabassi 2016 [194]; 16, 57% Moderate	Acute &overuse injuries in wrestling, American Football, football, gymnastics, volleyball, basketball, baseball/softball, ice hockey, lacrosse, swimming & diving, & track & field, cross country.	No demographic info included. RIO - 2005/6-2013/14	Overall MDQ IIR 11.8/100,000AEs. MDQ IIR Competition 28.0/100,000AEs, Practice 6.3/100,000AE's. In sex comparable sports sig sex diff; overall male MDQ 14.4 vs. female 8.0. Highest MDQ IIR; American Football 26.5/100,000 AEs, gymnastics (18.6) wrestling. Other sports with IIR above 10/100,000 AEs; female football (16.5), male lacrosse (15.8), male ice hockey (12.3), & female basketball (11.2). MDQ IIR higher in female basketball 11.2 (male 6.9), cross-country 5.6 (male 2.1), football 16.4 (male 10.5) & track & field 4.8 (male 1.8). Competition (vs. training) RR 4.4 (95%CI, 4.1-4.7). Overall MDQ male RR 1.8 (95%CI, 1.7-1.9). MDQ basketball female RR 1.6 (95%CI 1.3-2.0), female cross-country RR 2.6 (95%CI 1.0-7.5), female football RR 1.6 (95%CI 1.3-1.9), & female track & field RR 2.6 (95%CI 1.7-4.0). In football; proportion of all MDQ injuries that were concussion 12% in female athletes & 6% in male athletes. In male athletes, fracture was the most common diagnosis in 3 sports, sprain/strain most common diagnosis in 6 sports. In female athletes; sprain/strain most common diagnosis in 9 sports.
Yard 2008 [135]; 16, 57% Moderate	Acute & overuse injuries in football.	No demographic info included. RIO - 2005-07 1524 football injuries during 637446 AEs.	Overall study injury rate 2.39/1000 AEs. Higher in competition (4.77) than practice (1.37). No sig. sex diff. in overall IIR (female-2.44 & male-2.34). Female sig higher competition IIR (5.34 vs. 4.26). Male sig. higher practice IIR (1.51 vs. 1.21). During competition, females sustained complete knee ligament sprains requiring surgery at a rate of 26.4/100 000 AEs (male 1.98/100 000 AEs) (RR 13.3 [95%CI, 3.15-56.35]) & higher than female practice (2.34/100 000 AEs). Competition (vs. practice) RR=3.49; (95%CI, 3.15-3.87). Female (vs. male) competition RR=1.25; (95%CI, 1.10-1.42). Male (vs. female) practice RR 1.24 (95%CI 1.06-1.46). Male sustained a larger proportion of contusions than females (IPR 1.60 [95%CI 1.19-2.15]), while females sustained a larger proportion of complete ligament sprains (IPR 2.72 [95%CI 1.33-5.60]). Male hip injuries (vs. female) IPR 3.27 (95%CI 1.70-6.28). Female (vs. male) knee injuries IPR 1.42 (95%CI 1.09-1.84). Female (vs. male) knee ligament sprains IPR 3.41 (95%CI 1.39-8.39). Female (vs. male) competition knee ligament sprain RR 13.3 (95%CI 3.15-56.35). Female competition (vs. female practice) competition knee ligament sprain RR 11.3 (95%CI 4.31-29.58). Overall, females accounted for 51.6% football-related injuries. Most frequent injuries=incomplete ligament sprains (male 24.2% & female 29.2%) & incomplete muscle strains (male 18.8% & female 17.1%). Concussions-similar proportions (male 9.3% & female 12.2%). Overall, males & females sustained ankle (22.0% & 24.7%, respectively, knee (15.4% & 21.8%, respectively, head/face (13.2% & 14.5%, respectively, & thigh/upper leg (14.6% & 11.7%, respectively) injuries most often. Similar rates between sexes of time loss injuries for <1wk, 1-3 wks & >3 wks. Male injuries resulting in >3 wks' time loss; concussions (11.0% all male >3 wks' time loss, incomplete ankle ligament sprains (9.3%), complete knee ligament sprains (8.1%), foot fracture (5.9%), shoulder fracture (5.8%), & arm fracture (5.6%). Female injuries >3 wks' time loss-complete knee ligament sprains (28.4%), ankle fracture (9.0%), incomplete knee ligament sprains (8.3%), concussions

	(7.4%), & incomplete ankle ligament sprains (5.1%). Males & females sustained a similar proportion of concussions (9.3% & 12.2%, respectively) (IPR 1.31 95%CI 0.91-1.88).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
COMBINED AGE GROUPS			
Achenbach 2018 [137]; 17, 61% Moderate	Acute & overuse injuries in handball.	Twenty U17 teams (8 female, 21 male) from 16 different nations. 200; age 16.3±0.8yrs played 7.1±0.6 matches in 3 days; injury data from 200 u-17 players (120 Male, 80 Female) were evaluated. Ten senior teams from 16 different nations. 100; age 26.1±6.2yrs played 11.1±1.6 matches in 5 tournament days; injury data of 100 senior players (40M, 60F) evaluated.	Overall (Senior & U17) 77 (26%) players sustained 87 injuries yielding; overall IIR 286.1 injuries/1000h. U-17 players 205.7/1000h (v senior 395.5/1000h). Male athletes 330.2 injuries/1000h & Female athletes 234.9/1000 hrs (not sig.). Senior players OR 0.29 (95%CI 0.17-0.50; p<0.01). Male athletes OR 0.76 (95%CI 0.45-1.28, n.s). 63.2% resulted from non-contact mechanism, 16.1% from overuse injuries. Thigh was body site most affected by contact injuries (66.7%), & ankle was body site most affected by non-contact injuries (83.3%). Sex analysis not done within age group, injury type or body site. (Overall, 82 injuries (82.8%) required medical attention. Fifteen (17.2%) injuries were time-loss injuries; incidence 49.3/1000 hrs.
Beynnon 2005 [166]; 18, 64% Moderate	Acute ankle injury in football, basketball, lacrosse & field hockey.	663 high school (416 female), 238 college (128 female) athletes. Female athletes mean age 16.5 yrs (range, 13-22 yrs), height 166.1cm (range, 149.9-200.7 cm) & weight 59.0 kg (range, 40.5-85.5 kg). Male athletes mean age 17.2 yrs (range, 13-24 yrs), height 178.6cm (range, 152.4-203.2 cm) & weight 73.6 kg (range, 45.0-121.5 kg).	Overall, 29 female athletes (5.3%) & 14 male athletes (3.9%) sustained injuries. Female IR 0.97/person days (95%CI 0.65-1.39) & male IR 0.68/person days (95%CI 0.37-1.13) No sig. (p=0.20), Female RR 1.51 (95%CI 0.79-2.86). Overall high school IR 0.80 (95%CI 0.53-1.14), college IR 0.99 (95%CI 0.54-1.65), College RR 1.16 (95%CI 0.61-2.21). High school: Female IR 0.90 (95%CI 0.55-1.40), male IR 0.63 (95%CI 0.29-1.19), female RR 1.53 (0.69-3.41). College: female IR 1.15 (95%CI 0.53-2.19), male IR 0.78 (0.25-1.82), female RR 1.50 (0.50-4.48). Sport specific IRs (no sig se diff.); Basketball - female 1.90 (95%CI 0.95-3.40), male 0.42 (95%CI 0.05-1.54), football female 0.73 (95% 0.24-1.70), male 1.15 (95%CI 0.50-2.26), Lacrosse female 0.62 (95%CI 0.23-1.37), male 0.44 (95%CI 0.12-1.13). Overall female RR 1.51 (95%CI 0.79-2.86). Female basketball RR 4.11 (95%CI 0.91-18.60; p=0.046), female football RR 0.69 (95%CI 0.23-2.14; p=0.52) female lacrosse RR 1.40 (95%CI=0.39-4.96; p=0.60). College RR 1.16 (95%CI 0.61-2.21; p=0.64). Sex analysis within high school & college athletes no sig. diff. reported (specific data not included).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Opar 2014 [183]; 16, 57% Moderate	Acute hamstring injury in track & field.	48,473 athletes registered to participate in the Penn Relays Carnival (2002-04 inclusive). Male = 25,232 (junior 912, high school 14,514, college 9113, masters 693), female = 23,241 (junior 912, high school 14,372, college 7915, masters 42). Competition levels; junior high school, age≤13 yrs; high school, age 14–18 yrs; college/elite (including pre-Olympic/professional athletes) age 19–40 yrs; or masters age >40 yrs.	Overall IR, male=3.0/1,000 participants, female IR=1.7. High school IR male=2.9/1,000 participants, female IR=1.7/1,000. Overall male HSI OR 1.79 (95%CI 1.23-2.63; p=0.002). Excluding masters athletes, male HSI OR 1.68 (95%CI 1.14-2.47; p=0.009). No junior high school or masters women reported HSIs during study period. High school female HSI OR 0.55 (95%CI 0.33-0.92; p=0.021). No junior high school athletes were diagnosed with HSI. Masters HSI (v high school) OR 4.26 (95%CI 1.95–9.33; p< 0.001). Masters HSI (v college/elite-level) OR 3.55 95%CI 1.60–7.89; p=0.001). High school HSI (v college/elite-level athletes) OR 0.83 (95%CI 0.57–1.22; p= 0.342).
Opar 2015 [155]; 16, 57% Moderate	Acute & overuse injuries in track & field	48,473 athletes registered to participate in the Penn Relays Carnival (2002-04 inclusive). 25,232 males (junior 912, high school 14,514, college 9113, masters 693), 23,241females (junior 912, high school 14,372, college 7915, masters 42). Competition levels;	Minor orthopaedic injuries; 5.71/1,000 participants, major orthopaedic injuries 0.18/1,000 participants. Minor orthopaedic injuries male OR, 1.36 (95%CI 1.06-1.75; p=0.017). Secondary analysis excluding all masters athletes; overall male OR 1.10 (95%CI, 0.91-1.33; p=0.303), major orthopaedic injuries male OR 0.71 (95%CI 0.16-3.17; p=0.651). Minor orthopaedic injury male OR 1.32 (95%CI 1.02-1.69; p=0.032). College/elite female athletes (ref high school female athletes) OR 0.71 (95%CI, 0.52-0.98; p=0.036). College male athletes (ref college female athletes) OR 1.77 (95%CI 1.13-2.79; p=0.012). College/elite athletes (ref high school) injuries OR 0.81 (95%CI 0.66-0.99; p=0.041). College athletes injuries (ref masters) OR 0.49 (95%CI 0.27-0.88; p=0.001). High school athletes major orthopaedic injury (reference group masters) OR 0.05 (95%CI 0.00-0.56]; p=0.003). High school athletes minor orthopaedic injury OR 0.43 (95%CI 0.22-0.85; p=0.012) (reference group masters).

	junior high school, age≤13 yrs; high school, age 14–18 yrs; college/elite (including pre-Olympic/professional athletes) age 19–40 yrs; or masters age >40 yrs. 436 injuries sustained from competing athletes. (Medical & orthopaedic injuries reported, medical injury data not considered within this review).	

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Roos 2015 [185]; 17, 61% Moderate	Overuse injuries in baseball/softball, basketball, American Football, football, cross country, track & field, swimming & diving, tennis, volleyball.	Collegiate (17-23yrs) & High school (14-16yrs) age athletes. No demographic info. included.	In sex-comparable sports females had higher rates of overuse injury than males. College females highest rate of overuse injury (7.32/10,000 AEs), & HS boys lowest rate of overuse injury (1.42/10,000 AEs). Rate of overuse injury higher in college athletes (5.36/10,000 AEs) than in HS athletes (1.64/10,000 AEs) ($p<0.05$). Among sex-comparable sports, High School athletes female RR 1.55 (95%CI 1.43-1.68). College athletes female RR 1.25 (95%CI 1.16-1.35). College athletes overuse RR 3.28 (95%CI 3.12-3.44) (ref High School). Highest rates of overuse injury (expressed as injuries/10,000 AEs) were female cross country (college, 19.59; HS, 6.73), male cross country (college, 13.67; HS, 4.02), female outdoor track & field (college, 15.76; HS, 3.82), & male outdoor track & field (college, 13.53; HS, 2.13). College football only sport where male overuse injury rate higher than female (5.10 vs 4.19 per 10,000 AEs). Among sex-comparable sports female RR slightly higher in HS athletes (1.55 [95%CI 1.43-1.68]) than college athletes (RR 1.25 [95%CI 1.16-1.35]).
Vaandering 2022 [142]; 17, 61% Moderate	All injuries in volleyball.	2018 Canadian Youth National Volleyball Tournament 114 requiring 114 players in all age categories (U14, U15, U16, U17, & U18) & divisions of play (Division 1-5) were invited to participate (9616 players). Participants n=1,876 players [466 males, 1391 females, mean age 16.2 yrs±1.26)] consented to participate (19.5%).	IIR female 6.78 injuries/1000 AEs (95%CI 5.27-8.72), male 4.30 (95%CI 2.55-7.24), female IRR 1.47 (95%CI 0.80-2.69); point estimate suggests clinical relevance. Highest proportion of injuries in females head (40.96%), ankle (15.66%), knee (14.85%). Highest proportion of injuries in males finger (33.33%), head (16.67%), knee (16.67%). Overall concussion IIR 1.58 concussions/1000 AEs (95%CI 1.06-2.36). Females had a higher proportion of concussion diagnosis, female 27.71%, male 16.67%. Concussion female RR 2.62 (95%CI 0.76-9.04); point estimate suggests clinical relevance.

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; HR: hazard ratio; IRR: incidence rate ratio; RR: relative risk; IQR: interquartile range.

Table 2.7: Participant characteristics and main findings for the Tier-2 athlete studies.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
ADULT			
Dane, 2004 [219]; 11, 39% Limited	Acute & overuse injuries – not reported separately. Football, basketball, volleyball (Team). Running, wrestling (Individual).	329 males (72%); 127 females (28%). 17-28 yrs. Male; 106 football, 58 basketball, 62 volleyball, 45 runners, 58 wrestlers. Female; 55 basketball, 47 volleyball, 25 runners.	No sig. sex diff. in any sport in percentages of injured athletes. Basketball: male IR 84.5% vs female 78.2%. Volleyball: male IR 80.7% vs female 74.5%. Running: male IR 60% vs female 52%. No sex analysis for anatomical location of injury.
Gill, 2021 [232]; 13, 46% Limited	Acute & overuse injuries in Australian Rules football.	1635 patients attending ED study site during 10-month study; (242; 14.8% female). Age 20.2±7.8) yrs (49.1% patients <18 yrs old). Females 20.7±8.1 yrs, males 20.1±7.8 yrs.	68.9% game injuries. Sprain/strain injuries; female 50.4% all injuries, male 38.8% ($p<0.05$). Skin lacerations female 1.7%, male 8.0% ($p<0.05$). Hand or finger injuries; female 34.3%, male 23.4% ($p<0.05$). Cervical spine injuries; female 6.6%, male 2.5% ($p<0.05$). Shoulder injuries; female 5.8%, male 11.5% ($p<0.05$). Thorax/abdomen/pelvic injuries; female 2.1%, male 5.7% ($p<0.05$). Concussion was diagnosed in 14.1% patients, females 15.7%, males 13.8% (NS). Knee injuries; female 9.9%, males 7.0% (NS). Patellofemoral dislocations female 2.9%, male 0.6% ($p<0.05$). ACL injuries, female & male (not sig.).
Hollander, 2018 [213]; 16, 57% Moderate	Acute & overuse injuries – not reported separately. Indoor & outdoor hockey.	232; 68% 157 male, 75 female. 190 played both seasons; some players exclusively played during outdoor (36) or indoor season (6). Mean age ±SD of players was 20.7±4.7 yrs with sig. diff. between male (20.0±4.8 yrs) & female (22.0±4.3 yrs) ($p<0.01$). BMI (female 22.8±2.3, male 23.1±2.8 not sig.).	Overall IIR: male 3.8/1000 hrs (95%CI 3.1-4.5) vs female 3.5/1000 hrs (95%CI 2.9-4.2) not sig. Match IIR: male 9.8 (95%CI 7.9-11.6) vs female 9.6 (95%CI 7.8-11.4) not sig. Practice IIR: male 2.7 (95%CI 2.2-3.2) vs female 2.7 (95%CI 2.2-3.2) not sig. Indoor IIR: male 2.7 (95%CI 2.15-3.2) vs female 5.00 (95%CI 4.1-5.9) & outdoor IIR: male 4.5 (95%CI 3.7-5.4) vs female 2.8 (95%CI 2.3-3.4). More male players injured than female players (40.5% vs 27.0%, $p<0.01$). Data collected over 12 months.
Hollander 2021 [233]; 19, 68% Moderate	Running injuries.	550 injured recreational runners: 49.6% women; 37.0±12.8 yrs; 23.3±3.0 kg/m.	141 knee (25.7%), 119 lower leg (21.6%), 85 foot/toes (15.5%), 71 hip/groin (12.9%) injuries. Strongest associations between risk factors & injury locations for sex (female) with hip/groin & thigh injuries, & midfoot striking with Achilles tendon injuries. Hip female RR 2.22 (95%CI 1.43–3.45). Thigh female RR 2.65 (95%CI 1.45–4.87). Achilles tendon injury male RR 1.92 (95%CI 1.09–3.38). Individuals with midfoot strike pattern RR 2.27 (95%CI 1.17–4.41).

Mintz 2021 [242]; 14, 50% Moderate	All injuries in track & field.	NEISS (ED) database 2004-15. Age >18 yrs. 2004-15. 42,947 ED visits for track & field-related injuries in the US, with an estimated 23,509 (54.7%) male, & 19,438 (45.3%) female. Most ED visits for 18–22-year-olds, male 12,288 (52.3%), female 9,441 (48.6%).	No sig. sex diff. overall or by severity, mechanism of injury or anatomical location of injury. Trend towards higher proportion of male injuries 116requiring hospitalisation (male 2.9%, female 1.7%; p=0.32). Trend towards higher proportion of female injuries to the lower limb (female 70.8%, male 63.7%). Trend towards higher proportion of male injuries to the upper limb (male 15.7%, female 8.7%).
Morrissey 2020 [236]; 15, 54% Moderate	All injuries in adult ice hockey.	NEISS (ED) database 2007-16. Age >18 yrs. 2007-16. 1,653 ED visits for ice hockey injuries in US. Patients represented an estimated 68,786 total ice hockey related injuries, male 62,990 males (92%), 5,796 females (8%). Most commonly injured age group was youngest cohort studied, aged 19–25 yrs old (31,385, 46%).	Female head injury IPR 2.4 (95%CI 1.7–3.3, p < 0.001) (female 32%, male 13.3%). Male facial injury IPR 3.7; 95%CI 2.0–13.3, p < 0.01) (male 19.3%, female 5.2%). Male laceration injuries IPR 3.5 (95%CI 2.3–6.2; p < 0.001) (male 28.1%, female 8.1%). Female concussion IPR 2.3 (95%CI 1.3–3.5; p < 0.001) (female 14.3%, male 6.3%). Primary injury mechanisms for females; falls (29.5%), puck contact (24.3%) & player contact (11.1%), for males; falls (19.3%), player contact (17.3%) & puck contact (14.7%). Although not a top injury mechanism, male stick injuries IPR 3.9 (95%CI 1–169; p=0.07). (n = 4,712, 7.5%) than females (n = 110, 1.9%).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Rugg 2021 [231]; 18, 64% Moderate	Snowboarding injuries.	National registry data – snowboard related emergencies 2005-18.	Male injuries per season declined sig. across seasons (p=0.001), female injuries remained stable, therefore proportionally increased (p=0.035). Contusions, strains or sprains more prevalent in females (45.5%, male 37.4%; p<0.001), males more frequently sustained fractures (26.3%, females 23.5%; p=0.017), wounds (17.1%, females 11.4%; p<0.001) & dislocations (5.2%, females 3.6%; p=0.006). Head injuries most prevalent in both genders (21%), but shoulder & chest injuries more common in males (15.1%, females 9.2%; p<0.001% & 6.8%, females 4.4%; p<0.001) & in females back (13.1%, males 10.2%; p<0.001%) & pelvis (4.2%, males 2.9%; p=0.009) injuries were more prevalent.
Sugimoto 2020 [237]; 19, 68% Moderate	Crossfit related injuries.	Chart review at Sports Medicine clinic 2003-16. N=115. 55 male, 24.4±10.8 yrs, 173.9±10.7 cm, 77.1±17.2 kg, 25.2±4.4 kg/m2. 60 female, 26.0±10.1 yrs, 164.8±7.6 cm, 65.7±14.4 kg, 24.3±5.4 kg/m2.	Injury proportion; upper extremity male 32.7%, female 21.7% (p<0.182), trunk/spine injury male 30.9%, female 20.0% (p<0.178). Lower extremity injury male 34.5% female 58.3% (p<0.011); female OR 2.65 (95%CI 1.25-5.65). Specific body parts/joints; knee male 23.6% female 30.0% (p<0.442), spine male 47.8% female 20.0% (p<0.257) hip/pelvis male 5.5% female 13.3% (p<0.151). Shoulder joint male 23.6% female 10.0% (p<0.049); male OR 2.79 (95%CI 0.98-7.95). When considering age; athletes aged <19 yrs & younger had greater trunk/spine CrossFit injury proportions (<19 yrs 37.5%, over 19 yrs 18.7%; p<0.027), OR 2.61 (95%CI 1.10-6.21). Athletes aged <19 yrs & younger had greater spinal CrossFit injury proportions (<19 yrs 37.5%, over 19 yrs 17.7%; p<0.027), OR 2.86 (95%CI 1.19-6.87). No sex analysis by age group. There were no statistically sig. BMI diff. between any injured & uninjured athletes.
ADOLESCENT			
Chatha 2020 [214]; 12, 43% Limited	Concussion in football.	NEISS database. Concussion injuries occurring during football in paediatric patients (2-18 yrs old) from 2008-16. 3,285 injuries included 45% (1469) occurring in female players 49% (1617) in male players. Average age of the cohort was 13.5 yrs.	Based on national estimates, the football -related concussion rate was 2.51%/year. An average of 202 concussions occurred in male players each year & an average of 184 concussions in female players each year. No sig. sex diff. in overall prevalence or incidence.
Chun 2021 [218]; 18, 64% Moderate	Concussion in high school sports.	92,966 athletes from 63 schools. High school athletes. No further demographic information included. 2011-17.	6.4% all participants sustained a concussion. The pooled concussion rate for boys' sports was higher overall (1.00/1,000 AE, 95%CI 0.97-1.03) than for girls' sports (0.91/1,000 AE 95%CI 0.87-0.95) (p< 0.01). In sex matched sports girls had a

			higher rate of concussion than boys. Judo; boys 1.18 (95%CI 1.02-1.33), girls 1.92 (95%CI 1.68-2.17) (p< 0.01), football; boys 0.69 (95%CI 0.66-0.77), girls 1.10 (95%CI 1.01-1.19) (p< 0.01), basketball; boys 0.57 (95%CI 0.50-0.64), girls 1.09 (95%CI 0.98-1.19) (p< 0.01), & volleyball; boys 0.15 (95%CI 0.11-0.19), girls 0.54 (95%CI 0.47-0.60) (p< 0.01). The diff. in concussion rate between girls & boys participating in wrestling over the 6-year study period was not statistically sig. (P = 0.21).
Delaney 2014 [220]; 19, 68% Moderate	Concussion in American Football, ice hockey & football.	170; Female 47 (ice hockey 19, football 28, male 123 American Football 76, ice hockey 22, football 25). Mean age; American Football 21.1±1.1, ice hockey 21.3±1.0, football 20.5±1.2 yrs).	Sex diff. in injury mechanisms. In hockey; males more blow from an elbow (FE test p=0.02, Conversely, females more contact with the boards (FE test p=0.03). In football, females were more likely to suffer a concussion after contact with the ball (FE test p=0.02). No sig. sex diff. for injury mechanisms among different positions within the same sport. No sig. sex diff. for time lost. Across sport, males lost a median of 11 days (IQR, 7-18, females lost a median of 14 days (IQR, 10-21). In hockey, males lost median 10 days (IQR, 8-18, females lost a median of 14 days (IQR, 10-21). Football, males lost median 11 days (IQR, 7-21, females lost median 14 days (IQR, 10-21).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Deloes 1995 [221]; 15, 54% Moderate	Acute injury in ice hockey, basketball, handball, wrestling, hiking, alpine skiing, athletics, fitness training, judo, football, alpinism, volleyball, apparatus gym.	16,120 injuries; 77% male (12,412). 14-20 yrs.	Sig. sex diff. across all sports; male 4.6/10,000 hrs & female 2.6. In sex comparable sports; male 4.9/10,000 hrs & female 3.2. Standardised for exposure; male 4.3/10,000 hrs & female 4.5. Sig. sex diff. in 5 sports; Basketball-female 4.9/10,000 hrs & male 3.5, Alpine Skiing-female 3.9 & male 3.0, Volleyball-female 3.8 & male 3.0, Apparatus Gymnastics-female 2.9 & male 1.5, Hiking-female 2.5 & male 3.6.
Forward 2014 [222]; 19, 68% Moderate	Acute injuries (assumed due to data from hospital database) in ice hockey.	Overall, 33,233; female=2,637 (7.9%) & male=30,596 (92.1%). Age 13.7±2.1yrs. Adolescent-Female 558 11-12yrs, 916 13-14yrs, 845 15-17yrs, total=2319. Male 6756 11-12yrs, 10,888 13-14yrs, 9435 15-17yrs, total=27,079. 12 females & 53 males left ED without being seen.	No sig. sex diff. for proportion of injured players overall. Top 3 injury types; female-soft tissue, sprain/strain & fracture, male-soft tissue, fracture & sprain/strain. Sig. sex diff. for proportion of soft tissue injuries; female 39.8%, male 32.6%. Sig. sex diff. for proportion of fractures; female 18.2%, male 27.1%. Sig. sex diff. for proportion of upper extremity injuries; female 39.2%, male 45.2%. Sig. sex diff. for proportion of injuries from checking; female 25.7%, male 42.8% & from falling; female 25.3%, male 14.8%. Admission to hospital in 2.6% female players, 3.1% males (p=0.18). Age specific break down of sig. sex diff.: Proportionately more soft tissue injuries in female players 11-12yrs (female 40%, male 34%, 13-14yrs (female 42%, male 32%, 15-17yrs (female 39%, male 31%). Proportionately more sprains/strain in female players 15-17yrs (female 26%, male 17%). Proportionately more fractures for male players 11-12yrs (female 22%, male 27%, 13-14yrs (female 17%, male 33%, 15-17yrs (female 16%, male 24%). No sig. sex diff. for concussion but pattern observed; male players even number across age groups with downward trend in older age. Female increased 11-17 with peak 13-14yrs.
Hoge 2020 [230]; 17, 61% Moderate	Wrestling injuries presenting to ED.	NEISS database 2005-19.	Annually, female patients presenting to US Eds increased sig. (p< 0.001) from 2005 (N = 1,500; 95%CI 923-2,078) to 2019 (N = 3,404; 95%CI 2,296-4,513). 50.1% (CI 44.1-56.2) of females sustaining wrestling-associated injuries were 14-18 yrs. Injury characteristics, including body parts affected & final diagnoses for patients 14-18 yrs of age, are stratified by patient gender. Males sustained greater proportions of head & neck injuries 24.6% (95%CI 23.2–26.0), female 18.5% (95%CI 13.2-23.9) (P = 0.018). Diagnoses: females sustained greater proportions of sprains & strains 48.8% (95%CI 41.2-56.3), males 34.4% (95%CI 31.6-37.1) (p< 0.001), no sex diff. by location of sprain/strain, shoulder most common in both

			female (17.1% [95%CI 12.8-21.5%]) & male (20.3% [95%CI 19.0-21.7]) wrestlers. Males sustained greater proportions of fractures 15.7% (CI 14.-16.7) females 10.6% (95%CI 7.5-13.7) (P = 0.003).
Hosea 2000 [223]; 16, 57% Moderate	Acute ankle Injuries in basketball.	6840 males (504 collegiate, 6336 scholastic players) 4940 females (364 collegiate & 4576 scholastic players).	Overall ankle injury, female RR 1.25. Grade I ankle sprains female RR 1.26 (p=0.0001). Collegiate ankle injuries female RR 1.33 (p=0.059). Scholastic ankle injuries female RR 1.24 (p=0.0006). The relative risk for an ankle injury doubled in both genders from scholastic to collegiate level, 2.19 for females & 2.05 for males. An injury was defined as a traumatic event suffered by the athlete that required evaluation & treatment by the athletic trainer, team physician, or both.
Honrado 2021 [234]; 14, 50% Moderate	Dance related injuries.	NEISS ED database 2014-18. N=4152 patients with a dance-related injury (83% female). 76% age 10-18yrs, 12.8% age 19-30yrs, 7.7% 31-60yrs, 3.3% 61+yrs.	The injuries occurred most commonly at the knee (22.5%; n = 935), ankle (15.7%; n = 650), & foot (10.2%; n = 424) & were diagnosed as sprain/strain (42.6%; n = 1767), fracture (10.3%; n = 428), & contusion (8.1%; n = 336). Diagnosis by body area, most common injuries were ankle sprain/strain (12.7%; n = 527), knee sprain/strain (10.4%; n = 431), & knee dislocation (4.3%; n = 179). Male patients had more fractures (13.6%, n = 94) than female patients (9.7%; n = 33.4), & male patients had fewer sprain/strains (34.9%; n = 242) than their female counterparts (44.1%; n= 1525). Overall IR female 12.4/100,000 people, male 3.0.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Khodaee 2020 [235]; 16, 57% Moderate	Acute lacrosse injuries.	NEISS ED database 1997-2015. Overall, 7,587 lacrosse-related injuries included. Males accounted for 75.5% injuries. The overall average age was 16.0±5.0 (range 5 to 71) yrs (males 16.1±5.2; females 15.7±4.1).	75% injuries were from males. Upper extremity injuries accounted for a larger proportion of injuries males than in females (IPR; 41.6% vs. 26.0%; $p < 0.001$). Lower extremity injuries accounted for a larger proportion of injuries in females than males (IPR; 34.7% vs. 21.9%; $p < 0.001$). Shoulder (including the clavicle) injuries accounted for a larger proportion of injuries in males than females (IPR; 12.3% vs 3.5%; $p < 0.001$). Females sustained larger percentage of knee (IPR; 10.5% vs 6.4%; $p < 0.001$) & ankle injuries (IPR; 17.0% vs 7.1%; $p < 0.001$) cf. to males, respectively. Females sustained a higher proportion of sprains/strains than males (IPR; 36% vs 21.9%; $p < 0.01$), males sustained a higher proportion of fractures (IPR; 21.3% vs. 10.8%, $p < 0.01$). Concussion diagnosis similar proportion between sexes (IPR; 6.1% male & 6.2% female; $p > 0.05$). There were also sex diff. in the rate of trunk injury (male 9.2%, female 7.2%; $p<0.05$), Head & neck injury (male 17.1%, female 19.2%; $p<0.05$) & face (male 10.2%, female 12.9%; $p<0.05$).
Owoeye 2020 [215]; 20, 71% Moderate	Acute & overuse injuries in basketball.	N= 518 players (16±1.4 yrs; 38.6% females), from 63 teams. N=200 females age 16±1.1 yrs (range: 13-18). N=318 males 16±1.5 yrs (range 11-18). Prospective cohort study. Players were observed through one competitive high school or club basketball season.	Overall IR 14.4 (99% CI 12.2-17.0) injuries/1000 h, female 13.8 (99% CI 11.2-16.8), male 14.8 (99.% CI 11.7-18.8); Female IRR 0.93 (95%CI 0.68-1.26). No sig. sex diff. in injury location, diagnoses or type. Trend towards higher incidence overuse injuries in male athletes 7.3 (99%CI 5.3-10.0), female 5.4 (99%CI 4.0-7.4) & higher incidence of acute injuries in female athletes 8.3 (99%CI 6.1-11.4), male 7.5 (99%CI 5.8-9.9). Trend towards higher incidence of acute (male 2.3 [99%CI 1.4-3.5], female 1.8 [99%CI 0.8-4.0]) & overuse (male 5.2 [99%CI 3.6-7.6], female 3.2 [2.1-4.9]) knee injuries in male athletes & a higher incidence of acute (female 4.7 [99%CI 3.3-6.7], male 3.7 [99%CI 2.8-6.7]) & overuse (female 1.5 [99%CI 0.8-2.7], male 1.3 [99%CI 0.8-2.2]) ankle injuries in female athletes.
Leppanen 2017 [224]; 17, 61% Moderate	Overuse injuries in basketball & floorball.	387 (male=211, female 176). 101 female basketball players; mean age 14.5±1.4yrs, height 168.4±6.6, weight 60.9±9.5kg, BMI 21.4±2.9, menarche onset=91. 100 male basketball players; mean age 15.2±1.6yrs, height 179.3±9.5, weight 68.9±13.2kg, BMI 21.3±3.1. 75 female floorball players; mean age 16.1±1.5yrs, height 166.5±5.6, weight 60.3±6.6kg, BMI 21.8±2.1, menarche	Overall study IIR-1.51/1,000 hrs (95%CI 1.35-1.78). Most common knee IIR-0.54 (95%CI 0.42-0.67, lower back IIR-0.32 (95%CI 0.22-0.42). Sig. sex diff. for overall IIR-female 1.94 (95%CI 1.59-2.29, male 1.23 (95%CI 0.96-1.49). Foot IIR female-0.27 (95%CI 0.14-0.41, male 0.10 (95%CI 0.03-0.18). Shin/calf IIR female-0.24 (95%CI 0.12-0.37, male 0.03 (95%CI 0.00-0.07). Muscle/tendon injuries were most common amongst both sexes (56%). Female players sig. higher joint/ligament IIR-female-0.39 (95%CI 0.23-0.54, male 0.09 (95%CI 0.02-0.16). Overall female IRR=1.58 (95%CI 1.20-2.09). Foot injuries female IRR=2.69 (95%CI 1.14-6.94). Shin/calf injuries female IRR=8.30 (95%CI 2.17-53.62). Joint/ligament injuries female IRR=4.43 (95%CI 1.88-11.87). Basketball female IRR=1.61 (95%CI 1.07-

		onset=73. 111 male floorball players; mean age 16.9±1.3yrs, height 178.6±6.5, weight 70.1±8.7kg, BMI 22.0±2.3. 56 players dropped out across the 3 yrs.	2.46). Floorball female IRR=1.55 (95%CI 1.06-2.27). Injury classifications across the study; 44% all injuries were severe. 31% were moderate.
Pasanen 2018 [225]; 18, 64% Moderate	Acute injuries in floorball.	186 players (75 female, 111 male). F age 16.1±1.5) yrs, height 166.5±5.6)cm, weight 59.4(range 45.5-80.0)kg, BMI 21.5(range 17.8-27.2, Menarche, no/yes 2/73. M age 16.9±1.3)yrs height 177.5 (range 164.5-199)cm, weight 68.3 (range 52.4-98.5)kg. BMI 21.7(range 17.2-29.8).	Sig. sex diff. for overall IIR; female 2.40/1,000 hrs male 1.70, Game IIR; female 36.46/1,000 hrs male 19.42 & joint/ligament injury IRR; female 1.39/1,000 hrs, male 0.82. (Most common site of injury; ankle-37%, knee-18% & thigh-14%). Game (cf. to practice) IRR 21.21, (95%CI 15.30-29.82). Female overall IRR; 1.41 (95%CI 1.01-1.98). Female Game IRR; 1.88 (95%CI 1.12-3.19). Female joint/ligament IRR; 1.70 (95%CI 1.07-2.73).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Polites 2014 [226]; 18, 64% Moderate	Acute injuries in ice hockey.	168 injuries; female=26 (15%), male=142. Age <12=30(18%), 13-14=46(27%, 15-18=92(55%).	Sig. sex diff.; extremity injuries-male 50.7%, female 11.5%, TBI-male 16.9%, female 53.9% & spine injuries-male 9.2%, female 26.9%. Male players were most commonly injured by intentional contact (40.9%) & female players by unintentional contact (42.3%). Extremity injury male OR=7.89; (95%CI, 2.27-27.45). TBI female OR=5.46; (95%CI, 2.26-12.21). Female spine OR=3.66; (95%CI, 1.30-10.32).
Quatman 2009 [239]; 16, 57% Moderate	Acute & overuse injuries in weightlifting.	3,713 (3,102 male 611 female). Age 14-30. (High school injuries reported independently)	No sig. sex diff. in fracture injuries from strength training (p=0.37). Accident-related injuries female OR=1.69; (95%CI=1.37 to 2.08). Accidental foot injuries Female OR=2.44; (95%CI=1.75, 3.45). Accidental hand injuries male OR=2.14; (95%CI=1.49, 3.07). Trunk injuries male OR=1.55; (95%CI=1.25 to 1.96). Foot injury female OR=2.63; (95%CI=2.04 to 3.45). Leg injury female OR=1.54; (95%CI=1.05 to 2.22).
Reid 2012 [240]; 17, 61% Moderate	Acute & overuse injuries in track running.	4496; male 2012, female 2486. Age groups; 10-12yrs=770, 13-14yrs=1552, 15-18 yrs=2174	There was no sig. increase in the annual rate of track-related injuries over the study period (p=0.717). Elementary school age-Male laceration OR, 2.29 (95%CI, 1.49-3.52, male fractures/dislocations OR, 1.31 (95%CI, 1.07-1.60). Female sprain/strain OR, 1.32 (95%CI, 1.11-1.57). Elementary school age-Male trunk injury OR, 2.04; 95%CI, 1.65-2.53, male upper extremity OR, 1.30 (95%CI, 1.07-1.58). Female lower extremity OR, 1.63 (95%CI, 1.41-1.90). High school age-Male pelvic injury OR, 2.43; (95%CI, 1.92-3.07). Female ankle injury; OR, 1.80 (95%CI, 1.48-2.18).
Robinson 2014 [227]; 16, 57% Moderate	Acute & overuse shoulder injuries in American Football, football, basketball, wrestling, & baseball; volleyball, & softball.	2798 injuries. High school athletes. No demographic info included.	Shoulder injuries=8.4% all injuries over study period. Overall, Shoulder IIR across all sports 2.15/10,000AE's. No sig. sex diff. in the number of injuries requiring surgery; over 90% total sample did NOT require surgery. Over 40% male & 45% female athletes returned to sport within 1 wk. Boys basketball (10.6%), boys American Football (10.5%) & Girl's football (25.1%) resulted in >3wks medical disqualification. Across all sports, boys had double risk of injury during competition vs practice as cf. with girls (RR: 3.75 [95%CI: 3.46-4.05] vs 1.78 [95%CI:1.41-2.24]). In sex comparable sports competition as cf. with practice was equal (male RR: 2.68 [95%CI: 2.16-3.32] vs female RR 2.86 [95%CI: 2.17-3.78]). When comparing all 9 sampled sports; male fracture IPR=2.20 [95%CI: 1.05-4.59]. No sig. sex diff. in sex comparable sports only. Injury mechanism in all 9 sports; male contact with another person IPR=3.07 [95%CI: 2.35-4.01], female contact

			with the playing apparatus IPR=4.84 [95%CI: 2.77-8.48], female noncontact IPR=4.14 [95%CI: 3.32-5.16]. No sig. sex diff. in sex comparable sports.
Schallmo 2017 [228]; 19, 68% Moderate	Concussion in American Football, football , volleyball, basketball, wrestling, baseball, softball.	Injured student-athletes had a mean age (and standard deviation) of 15.9±1.2 yrs.	In sex-matched sports, girls IR sig. ($p<0.05$) higher than boys. During the 2005-2006 academic year, boys' American Football players (rate ratio, 3.26 [95%CI, 2.67 to 3.99]; $p<0.0001$) & girls' football players (rate ratio, 1.75 [95%CI, 1.30 to 2.34]; $p=0.0002$) demonstrated the highest concussion rates cf. with all other sports.
Sokka 2020 [216]; 19, 68% Moderate	Acute injuries in youth (9- to 14-year-old) football (football) players.	Ten football clubs. 730 players. 567 males 12.3±1.1 yrs, 151.7±10.0 cm, 41.2±8.7 kg, age at starting football 5.4±1.3 yrs; 163 females 12.4±1.1 yrs, 151.6±9.2 cm, 41.6±7.7 kg, age at starting football 6.7±1.5 yrs participated in 20-wks follow-up study (January–June 2015).	278 players sustained a total of 410 injuries: 22 % (n = 89) females, 78 % (n = 321) males. Overall IIR 6.47(95 % CI 5.84–7.01)/1000h football. 25.91(95%CI 22.41–29.40)/1000h football in games & 3.60 (95 %CI 3.10–4.10) in practices. Female overall IIR 7.20 (95 %CI 5.71–8.70), male 6.29 (95 %CI 5.60–6.98). Male IRR 0.86 (95%CI 0.64–1.17). Sex diff. in injury location: Ankle injuries, male 1.68, female 2.83, male IRR 0.58 (95%CI 0.35–0.96; $p=0.033$). Female ankle IRR 1.85 (95 %CI 1.18–2.91; $p=0.007$), female non-contact ankle injury rate IRR 2.78 (95%CI 1.91–4.02; $p < 0.001$). Shoulder injuries male 0.04, female 0.16, male IRR 0.24 (95%CI 0.09–0.65; $p=.005$).

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Tsushima 2019 [229]; 20, 71% Moderate	Concussion in American Football, football, volleyball, basketball, wrestling/martial arts, baseball, softball, cheerleading, water polo, tennis, track & field & cross country.	5,938 males & 4,396 females, grades 8-12. 15.5 ±1.3 yrs. Participants 250 male & 262 female 13-year-olds, 1,295 male & 1,057 female 14-year-olds, 1,414 male and 1,063 female 15-year-olds, 1,456 male & 1,014 female 16-year-olds, 1,148 male & 796 female 17-year-olds, & 375 male & 204 female 18-year-olds.	In sports played by both sexes, girls had sig. higher concussion risks than boys in three comparable sports: basketball (14.8% vs. 9.1%, football (13.3% vs. 7.7%) & softball (12.8% vs. 6.5%). ARR for girls in all sports, including comparable sports, was 1.5 times greater than boys (ARR=1.50, p<0.0001). Female football RR=1.72 (CI=1.27, 2.32, p=0.0003. Female basketball RR 1.62 (CI=1.20, 2.19, p=0.0018. Softball RR 1.97 (CI=1.31, 2.97, p=0.0004.
Ursej 2019 [241]; 17, 61% Moderate	Acute & overuse injuries in hip hop dance.	Hip hop dancers from Slovenia (129, 114 females, 17.95±4.15 yrs).	Each dancer suffered 0.78 injuries (95%CI: 0.61-0.97) over 3 months. No sig sex diff. (F-0.76 [95%CI: 0.60-0.95] vs. M-0.93 [95%CI: 0.75-1.13]) (Mann-Whitney Z-value: 0.68, p=0.52). 3.12 injuries per dancer per year. 8.7 injuries/1000 hrs (95%CI: 6.9-1.1); M-10.33 (95%CI: 5.1-14.2, F-8.44 (95%CI: 3.5-15.1). Separating MSK-injuries (OSTRC scores >40) from MSK-problems (OSTRC: 1-40, 17% dancers suffered an MSK-injury, while an additional 33% reported MSK-problems, with no sig. sex diff. between (Chi square: 0.62, p=0.73). Injury that occurred during period of 6-months before study baseline was a strong predictor of MSK-injury & MSK-problem during study. Previous injury increased risk for occurrence of MSK-problem over the course of study by more than 2.5 times (OR: 2.62, 95%CI: 1.61-4.58, & risk for occurrence of MSK-injury by more than 3.5 times (OR: 3.76, 95%CI: 1.87-4.59).
Willick 2021 [238]; 16, 57% Moderate	MTB injuries.	Injury surveillance data from National Interscholastic Cycling Association (NICA) 2018 & 2019 seasons. Injuries tracked in 41,327 student-athlete-yrs, identifying 1,750 unique injuries during 1,155 injury events.	1,155 injury events in 41,327 student-athlete-yrs (79.7% male, 20.3% female), resulting in injury event proportion 2.8%. 1.52 unique injuries/injury event (1,750 unique injuries in 1,155 injury events). Injury event proportion higher in female riders than male riders (female 3.21%, male 2.69%; p=0.009). Sex diff. lower limb injury; 37.8% injuries female riders, 28.3% male riders (p=0.003).
Zynda 2022 [217]; 15, 54% Moderate	Basketball injuries.	NEISS database. 2012-18 patients aged 7-11 yrs (childhood) & 12-17 yrs (adolescence).	Females higher finger IIR (child female 13.8 [95%CI 10.5-17.1]/100,000 athlete days, male 8.0 [95%CI 6.4-9.6]; p=0.002) (adolescent female 17.4 [95%CI 14.3-20.5], male 11.5 [95%CI 9.1-13.9]; p=0.0032). Female higher rate of ankle strain/sprain IIR in child basketball players; 6.1 (95%CI 4.9-7.3), male 3.4 (95%CI 2.6-4.2), no sex-diff. in adolescents. No sex-based diff. in concussion or knee strain/sprain among childhood group; however, prevalence of each increased

		from childhood to adolescence, & the rate of increase for head injuries & knee injuries was sig. higher in female cf. with male athletes (p<0.0001 for both).

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; HR: hazard ratio; IRR: incidence rate ratio; RR: relative risk; IQR: interquartile range.

Table 2.8: Participant characteristics and main findings for the Tier-1 athlete studies.

Reference & Quality score	Focus	Participants characteristics, age (mean ±SD)	Findings/Comment
Meixner 2020 [244]; 15, 54% Moderate	Fractures & dislocations in sports & recreation.	NEISS-AIP data for 2005 to 2013. 18 sports & recreational activities in United States; American Football, baseball, basketball, softball, football, volleyball, ice hockey, ice skating, snow skiing, toboggan/sledding, inline skating, skateboarding, gymnastics, racquet sports, swimming, waterski/surfing, track & field, & combative sports.	Est total ED visit incidence 7.35/1000 people, 20.6% fractures, 3.6% dislocations. Annual ED visit incidence 1.51 fractures & 0.27 dislocations/1000 people. Sex diff. in fracture incidence overall, male OR 1.21 (95%CI 1.14-1.27), with bimodal peaks at 10 to 14 & >84 yrs. Highest incidence of fractures American Football (22.5%). Majority of fractures occurred in arm/hand (55.9%; 2,326,129). Sex diff. in dislocation incidence overall, male OR 1.50 (95%CI 1.38-1.62). Highest incidence of dislocations in basketball.
Stogner 2020 [245]; 17, 61% Moderate	Hand injuries in 42 different sports.	364 injuries - retrospective analysis. No other demographic info included. Sports categorised ball sports with a bat, ball sports without a bat, gymnastics, martial arts, climbing, outdoor sports, precision sports, cycling, equestrian sports, skating, water sports, winter sports & miscellaneous.	364 hand injuries, caused by 42 different types of sport. Highest prevalence in cycling (101, 28%), American Football (66, 18%) & equestrian sports (46, 13%). Highest prevalence category "ball sports without a bat" (114, 31%), cycling (101, 28%) & equestrian sports (46, 13%). Overall, males affected more often than females (118 females, 32%; 246 males, 68%). Sex diff. in equestrian sports, 84% (n=39) of equestrian-related hand injuries affected female study population (p< 0.001) & 33% equestrian sports accounted for most of female hand injuries. Sex diff. in ball sports without a bat (101 males, 89%; p< 0.001)*. *Due to American Football-associated injuries. No sex diff. for LOS or the need for operation respectively (p=0.306; p=0.703).
COMBINED ADOLESCENT & ADULT DATA			
Xiao 2022 [247]; 14, 50% Moderate	Nasal bone fractures in sports & recreation.	158,979 nasal fractures (25% female) over 10 yrs (2009-2018). Age 20.4 (SD12.3). Age groups; 0-12 (19.3%), 13-18 (36.2%), 18-65 (43%), >65 (1.5).	Ball sports account for 64% nasal bone fractures. Bicycles account for 5% the overall cohort. The most common causes of fracture in adults' basketball (n = 18 653, 26%) & in children baseball (n = 22 129, 25%). In males basketball accounted for highest proportion (25%) of nasal bone fractures, in females softball accounted for highest proportion (25%) of injuries. No further sex analysis.
Aman 2016 [243]; 19, 68% Moderate	Acute injury in motorcycle, handball, squash, floorball, hockey, golf.	Insurance company data. Mean number of licensed athletes was 1,162658 per year, 27% licensed athletes females. Mean±SD age for injured athletes in all sports during 4 yrs was 21.7±8.6 yrs. Mean age injured	Mean number of injuries 11,868 per year, 47,470 in total over 4 yrs. 10.2 injuries/1000athlete yrs. females 8.4, males 10.9. Female had sig. higher incidence of injury in; motorcycle (females 109.1, males 65), handball (females 71.1, males 57.2), Rugby (females 48, males 28.4), floorball (females 11.6, males 8.3), squash (females 28.6, males 3.4), golf (females 0.2, males 0.1). Male had sig. higher incidence of injury in ice hockey (females 29.4, males 48.7), basketball (females 5.0, males 6.1). Overall male RR 1.3. Among

		males 22.2±8.6 yrs & injured females 20.3±8.6 yrs.	popular sports with high numbers of licensed athletes & with more than 200 injuries per year; automobile sports female RR 2.1, motorcycle female RR 1.7, floorball female RR 1.4, handball female RR 1.2). Ice hockey male RR 1.7 & basketball male RR 1.2.
Aman 2019 [246]; 15, 54% Moderate	Acute injury in ice hockey, floorball, handball, football.	Insurance company data. 92,162 registered injuries in total for all four sports (70,538 male injuries & 21,624 female injuries) during study 2006-2015, & in ice hockey (2006-2012). Males 21±7 yrs, females 19±6 yrs.	Highest IR (/1000 player yrs); Floorball female knee injury 4.8; Football female knee injury 7.8; Handball male hand/finger injury 9.4, male knee injury 10.5, female knee injury 20.7; Ice hockey male dental injury 9.3. Highest IR of concussion in Handball (male 1.3, female 2.0) & ice hockey (male 2.3, female 3.1). Females higher injury RR overall in floorball (1.4) & handball (1.1). Females higher RR for; knee injury in floorball (2.2), football (1.5) & handball (2.0), Cspine injury in floorball (1.9), football (1.9, handball (1.3) & ice hockey (1.3), SRC in floorball (2.1), football (1.2), handball (1.5) & ice hockey (1.3).

OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval; HR: hazard ratio; IRR: incidence rate ratio; RR: relative risk; IQR: interquartile range.

2.4.3 Injury data collection, definitions and rates

Injury data collection methods included injury report forms [71-76, 78, 79, 85-87, 91, 92, 97-107, 111-113, 117, 128, 136, 142, 155, 169, 183, 196, 205, 206, 211-213, 215, 216, 223], clinical records [77, 81, 108, 110, 114, 120, 130, 137, 164, 166, 188, 210, 222, 225, 226, 229, 232, 233, 237, 240], insurance scheme data [221, 243, 246] and national online injury surveillance systems [70, 76, 83, 89, 90, 94, 95, 101, 105, 109, 115, 116, 118, 119, 122-126, 131-135, 138-141, 143-151, 153, 154, 156-163, 165, 168, 171, 176-179, 186, 189-193, 197, 198, 201-203, 207, 209, 214, 217, 218, 228, 230, 231, 234-236, 238, 239, 242, 244, 245, 247, 250]. Data collection periods varied from a single tournament to 20 years.

2.4.4 Athlete classification

Of 180 studies assessed, eight studies included tier-5 athletes [71-78] (see Table 2.4), 40 studies included tier-4 athletes [70, 76, 79-98, 100-117 70, 83, 90, 115, 116, 122-125, 131, 138, 143-151, 153, 154, 156-160, 207] (see Table 2.55), 98 studies included tier-3 athletes [70, 83, 90, 119-121, 123-128, 130-136, 138-165, 169-194, 198-203, 205-212] (see Table 2.6), 30 studies included tier-2 athletes [213-242] (see Table 2.7), and five studies included tier-1 athletes [243-247] (see Table 2.8).

2.5.5 Study participant characteristics and sports

Tier-5 studies (see Table 2.4) provided data for rowing, martial arts, aquatic sports, gymnastics, winter sports and team-based ball sports. All studies presented season data and investigated senior athletes. Sample sizes ranged from 24 to 2,567 athletes and age ranged from 19-24 yrs. Within these studies, females represented 40-50% of participants and 43% of the total 3,243 athletes across all studies.

Tier-4 studies (see Table 2.5) provided data for tennis, martial arts, athletics, ballet, cricket, triathlon, aquatic sports, gymnastics, winter sports and team-based ball sports. Data were collected through the season and during tournaments. Sample size for tier-4 studies ranged from 42 to 17,101 athletes with age ranging from 11-35 yrs. Within these studies, females represented 35-62% of participants and 46% of the total 45,472 athletes across all studies.

Tier-3 studies (see Table 2.6) provided data for rowing, martial arts, aquatic sports, equestrian, military recruits, mountain biking, tennis, athletics, badminton, and team-based ball sports. Ninety-four studies presented through season data and four studies presented tournament data. Forty-three studies investigated senior athletes, forty-nine studies adolescent athletes and six studies include data combined from adolescent and senior athletes. Thirty-four studies in tier-3 athletes reported participant characteristics (sample size ranged from 49 to 358,319 athletes with age ranging from 11-40 yrs). Within these studies, females represented 18-91% of participants and 22% of the total 985,186 athletes across all studies. Studies that did not report sample size did report injury data consistent with methods of this review.

Tier-2 studies (see Table 2.7) reported injury data from running, martial arts aquatic sports [229], gymnastics [221], winter sports, CrossFit, wrestling, dance, cycling and mountain biking, and team-based ball sports. Tier-2 included data from senior athletes and adolescent athletes. Data were collected through season and

during tournaments. Data from over 45,281 female athletes and 96,027 male athletes (32% female) were included.

Tier-1 data (Table 2.8) included multiple different recreational sports, and all data were from injuries occurring throughout the year. Studies included senior athletes and adolescent athletes. Tier-1 studies did not report sample sizes but reported injury data consistent with methods of this review. Across the tier-1 studies data for a total of 641,582 injuries were reported with 27% from females.

2.4.6 Studies with sex specific injury location diagnoses

Analyses in cohorts from 'sex comparable' sports (males and females playing by the same rules) revealed sex-specific injury profiles. There was moderate evidence that female athletes were at increased risk of knee (RR 2.7 (95% CI: 1.4 to 5.5)), foot/ankle (RR 1.25 (95% CI: 1.2 to 1.3) to RR 8.0 (95% CI: 1.0 to 63.0)), bone stress injury (RR 3.4 (95% CI: 2.1 to 5.4)) and sports related concussion (RR 1.3 (95% CI: 1.0 to 1.8) to RR 8.5 (95%CI 1.0 to 69.0)). Conversely, male athletes had 1.4 (95% CI: 1.3 to 1.6) to 2.3 (95% CI: 1.3 to 3.9) times increased risk of hip/groin injuries and 2.4 (95% CI: 1.8 to 3.2) times greater risk of hamstring injury than female athletes, particularly in sports that are explosive in nature. Male athletes were also 1.8 (95% CI: 1.4 to 2.7) to 2.8 (95% CI: 2.5 to 3.2) times more likely to sustain acute fractures than female athletes, particularly in competition. Table 2.3 outlines studies by injury location and tier.

2.4.6.1 Lower extremity

Three moderate quality studies [82, 105, 210] showed a significantly higher rate and incidence of lower limb injury in male athletes in athletics (male 22 ±3.4 v female 16.2 ±3.2 injuries/1,000 registered athletes) [105] (tier-4), football (soccer) (male 7.3 v female 5.8/1,000 player hours) [82] (tier-4) and mixed martial arts (male 8/100 v female 2/100 AE) [210] (tier-3). Ten moderate quality studies [75, 97, 107, 156, 158, 188, 198, 235, 237, 238] across all tiers of the participation pyramid showed that a higher proportion of female injuries are to the lower limb and one study across multiple sports in adolescents [170] showed that lower limb injuries were 1.5 times (RR 1.5 (95% CI: 1.12 to 2.15)) more likely to be season ending in female athletes. This was driven by knee ligament injuries resulting in surgery, particularly in football (knee surgery Injury Proportion Ratio [IPR] 3.57 [95% CI: 1.20 to 10.6]).

2.4.6.1.1 Hip/groin

Nine studies [71, 79, 80, 82, 84, 105, 139, 143, 151] of moderate quality showed a significantly higher rate and incidence of hip/groin injury in male athletes in athletics [105] multiple team based sports [71, 79, 80, 82, 84, 139, 143, 151], gymnastics [143, 151] and tennis [143, 151]. There was moderate quality evidence [79, 135, 143] that in male athletes, a greater proportion of overall injuries were to the hip/groin region; IPR 1.07 (95% CI: 0.73 to 1.56) [143], 3.27 (95% CI: 1.70 to 6.28) [135] and the risk of hip/groin injury in male athletes was 1.41 (95% CI: 1.28 to 1.55) [151] to RR 3.01 (95% CI: 1.54 to 5.87) [251] greater than female athletes in similar sports. Five studies [187, 188] reported higher rates of hip/groin injuries in female athletes ([female 6.7% vs male 3.7%], [female 7.1% vs

male 3.8%] and female 50% vs male 6.7%]); these data were specifically related to overuse/gradual onset injuries in adolescent (tier-3) athletes [187, 188] and recreational runners [233] and acute injuries in basketball (tier-3) [139] and Crossfit (tier-2) [237]. The meta-analysis of 11 studies for hip/groin showed an overall significant RR of 1.30 [95% CI:1.21 to 1.40] for males, however showed considerable heterogeneity (I^2=89%) across studies (Appendix V, Supplementary Figure S1).

2.4.6.1.2 Hamstring

Male athletes were up to 2.42 times [124] more likely to sustain hamstring injury than female athletes. Moderate quality evidence across eight studies for team [82, 105, 123, 124, 146, 183] and individual sports [99, 123, 208] consistently showed male athletes to have a higher rate and incidence of hamstring injuries. The highest incidence and rate of hamstring injury was reported in (tier-4) male football players (male 1.52 vs female 0.79/1,000 player hours) [82] (tier-4) track and field athletes (male 22.4 ±3.4 vs female 11.5 ±2.6 injuries/1000 registered athletes respectively [99]. No studies reported a higher rate or incidence of hamstring injury in female athletes. The meta-analysis of five studies for hamstring injuries showed an overall significant RR of 1.67 [1.48-1.88] for males (see Figure 2.2).

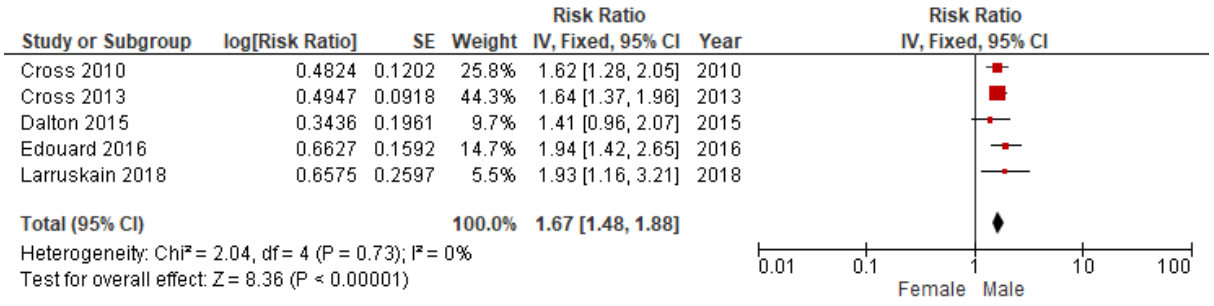


Figure 2.2: Meta-analysis for hamstring injuries in males.

2.4.6.1.3 Knee injury

Dependent on sport, female athletes had up to 2.72 times (RR 2.72 (95% CI: 1.35 to 5.51)) [109] increased risk of knee injury. Moderate quality evidence across nine studies [82, 109, 158, 178, 179, 187, 189, 232, 246] showed a meaningfully higher rate of knee injury in female athletes, with the highest rates reported in elite skiers (female 10.1 v male 3.7 injuries/100 athletes/season) [109]. Three studies [158, 179, 187] reported a higher incidence of patellofemoral pain with an incidence rate of up to 19.35/100,000 AEs (vs 14.15 in males) and the highest prevalence in female outdoor track, field hockey, lacrosse and softball [158]. Knee injuries accounted for a greater proportion of overall injury burden in (tier-3) adolescent female athletes (IPR knee 1.3 (95%CI: 1.11 to 1.53) [189] (female 16.7% v male 5.9%) [201] and in higher performance female athletes (knee ligament injury; male 3%, female 9%; p<0.05) [82]. Further, one study [217] (tier-2) in children and adolescents highlighted that the relative increase in prevalence of knee injuries from childhood to adolescence was greater in females than it was in males. The meta-analysis of 14 studies for knee injuries showed an overall significant RR of 1.61 [95% CI: 1.39 to 1.86] for females (see Figure 2.3).

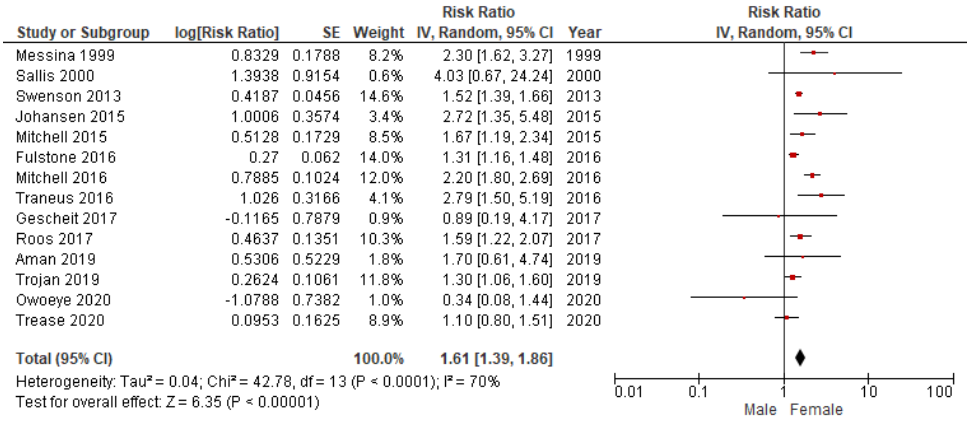


Figure 2.3: Meta-analysis for knee injuries in females.

2.4.6.1.4 Foot/ankle injury

The risk of foot/ankle injury was 1.25 (RR 1.25 (95% CI: 1.17 to 1.34)) [190] to 7 times (RR 7.95 (95% CI: 1.00 to 63.1) [76] greater in female athletes compared to male athletes in the same sports. This pattern was consistent in 12 moderate quality studies across the participation pyramid [76, 89, 115, 117, 161, 166, 180, 190, 215, 216, 223, 224]. Incidence rates were highest in basketball competition (9.45/10,000 AE's) and gymnastics competition (9.36/10,000 AE's) [190] and football (2.83/1,000 hrs) [216]. One study specifically highlighted female non-contact ankle IRR 2.78 (95% CI: 1.91 to 4.02) [216]. Three moderate quality studies [70, 153, 159] reported notably higher rates of ankle injury in male athletes specifically in basketball [153, 159], ice hockey [153] and running [70, 233], in addition, one study [161] across multiple sports reported that foot injuries in male athletes were more likely to result in surgery (RR 2.43 (95% CI: 1.71 to 3.44)) or to be season ending (RR 1.89 (95% CI: 1.48 to 2.41)) than was observed in female athletes. One study in taekwondo [78] reported no meaningful sex difference in foot and ankle injuries but there was a trend towards IIR being higher in male athletes. The meta-analysis of 17 studies for ankle injuries showed an overall significant RR of 1.20 (95% CI: 1.09 to 1.33); p<0.001) for females (see Figure 2.4).

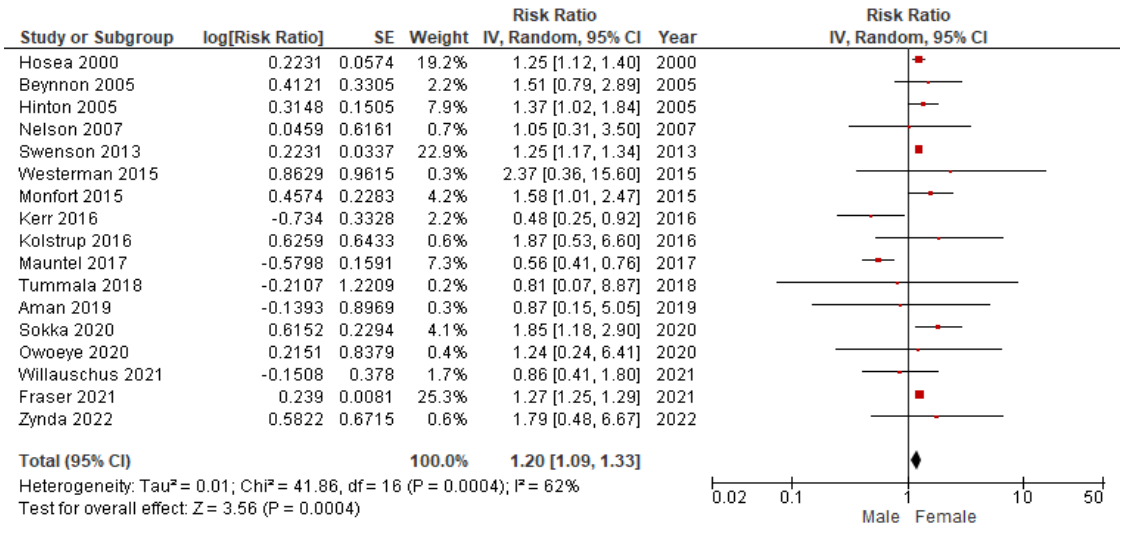


Figure 2.4: Meta-analysis for foot/ankle injuries in females.

2.4.6.2 Upper extremity

There was moderate quality evidence in 20 studies [83, 97, 119, 149, 154, 162, 175, 184, 187, 188, 201, 205, 207, 222, 231, 232, 235, 237, 245, 250] of a notably higher incidence of upper limb injuries in male athletes in different sports and across all tiers of the participation pyramid. The highest IIR in specific sports were 54.64/1,000 AEs (versus female 42.64) in adolescent judo [205] and 6.40/10,000 AE's (versus female 2.12) in NCAA ice hockey [149]. One study in adolescent athletes [119] across multiple sports reported male athletes to be at 2.38 (95% CI: 1.32 to 4.55) times increased risk of upper limb injuries compared to female athletes. More specifically, two studies reported that male athletes were at increased risk of shoulder injuries in sex comparable sports (RR 4.67 (95% CI: 3.56 to 6.14)) [149] and in water polo (RR 3.0 (95% CI: 1.3 to 7.0)) [140]. Four moderate quality studies [106, 109, 139, 216] reported that female athletes had a higher rate of upper limb injury. These studies included softball, canoe-kayak, rowing, sailing, swimming, tennis, triathlon, fencing, biathlon, judo, snowboarding [106], football (shoulder injuries male 0.04/1000h football vs female 0.16/1000h; male IRR 0.24 (95% CI: 0.09 to 0.65); p=0.005) [216], skiing [106, 109] and wrestling (shoulder injury female 62.5% vs male wrestlers 0.0%) [139]. Only one study [230] in tier-2 wrestling reported no difference in upper limb injuries between males and females, specifically at the shoulder. Data specifically for shoulder injuries from 11 studies were suitable for the meta-analysis but showed considerable heterogeneity (I^2=80%) across studies (Appendix V, Supplementary Figure S2).

2.4.6.3 Trunk

Six moderate quality studies reported a higher incidence of trunk injuries in females. There were three studies in senior athletes including; rib stress injuries in Olympic level tier-5 rowers [72] (females 11.94% vs male 6.98%; p<0.05), all trunk injuries in tier-4 tennis (most common injury in female players 41%) [102], and back (female 13.1%, male 10.2%; p<0.001) and pelvis (female 4.2%, male 2.9%; p=0.009) injuries in tier-2 snowboarding [231]. There were three studies in adolescent athletes including; multiple different tier-3 sports (spine female 11.3%, male 8.2%) spine female 11.3% male 7.7%; p=0.013) [188] and specifically in tier-2 ice hockey (spine injuries male 9.2%, female 26.9%; female spine OR 3.66 (95% CI: 1.30 to 10.32)) [226]. Eight moderate quality studies reported a higher incidence of trunk injuries in male athletes in Australian Rules football [232], lacrosse [119, 125], weightlifting [239], water polo [140, 186], swimming [144], tennis [97] and track running [240]. Male athletes were between 1.73 (95% CI: 1.17 to 2.54; basketball) [125] and 4.31 (95% CI: 2.07 to 8.96; lacrosse) [125] increased risk of trunk related injuries. Four moderate quality studies reported no significant sex difference in the rate or proportion of trunk injuries in Crossfit [237], ice hockey [90], swimming and diving [73] and skiing [98]. No meta-analysis was conducted due to the lack of sufficient data reporting similar types of trunk injuries.

2.5.6.4 Concussion

Female athletes were reported to be at 1.3 (95% CI: 1.03 to 1.8) (basketball) [177] to 8.46 (95% CI: 1.04 to 68.77) (football) [120] times greater risk of concussion than male athletes in the same sports. Moderate quality evidence across twenty studies [87, 120, 138, 142, 145, 148, 150, 157, 160, 167, 169, 176, 177, 182, 218, 226, 229, 232, 243] showed a meaningful higher incidence of sports related concussion in female athletes in sex comparable team sports. Four studies [145, 148, 160, 182] more specifically showed that the highest IIR were consistently seen in competition, with the highest rates reported in football (female 19.38 vs male 9.69/10,000 AE's) [160] (female 1.10 vs male 0.69/1000 AEs) [218] and judo (female 1.92 vs 1.18/1000 AE) [218]. Six studies [120, 129, 142, 172, 177, 236] reported injury proportion ratios, in female athletes sports related concussion accounted for a greater proportion of total injuries than in male athletes. IPR ranged from 1.4 [177] to 2.5 [172, 177, 236]. One study [119] in lacrosse reported a higher incidence of concussion in male athletes (0.29/1,000 AE's vs 0.10 in females; RR 2.99 (95% CI: 1.65 to 5.79). One study [230] in wrestling reported a higher incidence of head/neck injuries in male athletes and one study [90] in ice hockey reported a higher incidence of head/neck injuries in female athletes but neither study specifically reported on concussion. Two studies [214, 217] reported no sex difference in the rate of concussion in football [214] (tier-2) and basketball [217] (tier-2 adolescent). The highest volume of studies for sports related concussion were from tier-3 athletes. The meta-analysis of 14 studies for sport related concussion showed an overall significant RR of 1.84 (1.76 to 1.92) for females (see Figure 2.5).

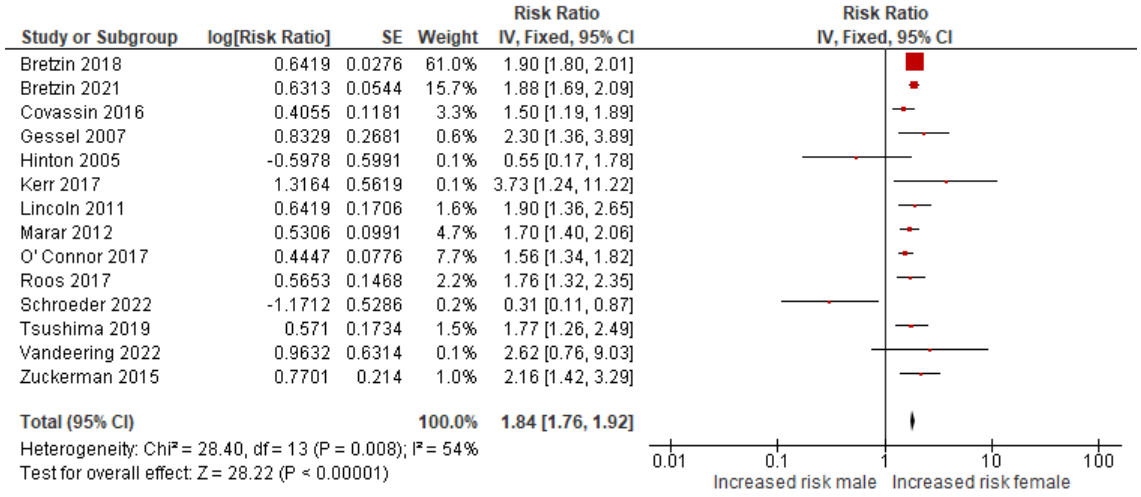


Figure 2.5: Meta-analysis for sport related concussion in females.

2.4.6.5 Fractures

2.4.6.5.1 Stress fractures

There was moderate quality evidence that female athletes have 1.75 [168] to 3.37 [88] times greater risk of bony stress injury than male athletes. Five studies [88, 105, 156, 168, 198] including team and individual sports from adolescent and senior athletes (tier-3) consistently reported a higher rate and incidence of bony stress

injury in female athletes. Overall, incidence ranged from 2.2/100,000 AE [168] to 9.13/100,000 AE [156]. In specific sports the highest reported IIR was in cross-country running (female IIR 28.59/100,000 AEs vs male IIR 16.14/ 100,000 AEs) [156]. The meta-analysis of four studies for stress fractures injuries showed an overall significant RR of 2.03 (95% CI: 1.77 to 2.34) for females (see Figure 2.5).

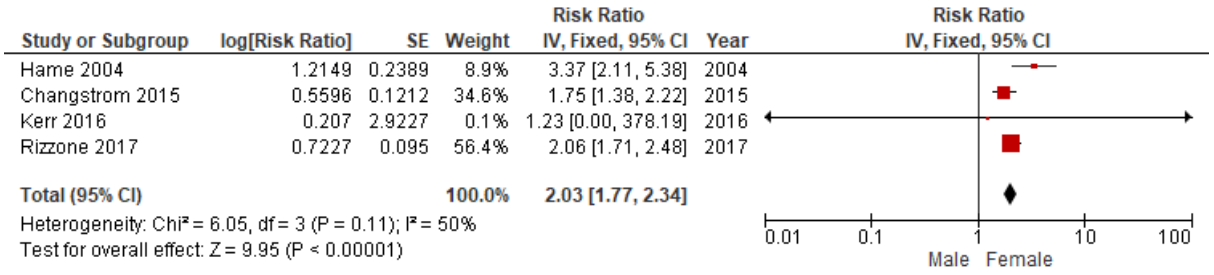


Figure 2.5: Meta-analysis for stress fractures in females.

2.4.6.4.2 Acute fracture

Conversely to stress fractures, moderate quality evidence in 15 studies [71, 88, 127, 129, 130, 187, 192, 222, 230, 231, 234, 235, 240, 244, 250] highlighted a significantly higher rate of acute fracture in male athletes across multiple different sports. Three studies [127, 129, 230] reported that acute fracture accounted for a significantly greater proportion of total injuries in male athletes in football (IPR 1.9 (95% CI: 1.11 to 3.26) [127], basketball (IPR 2.49 (95% CI: 1.59 to 3.91) [127, 129], lacrosse (IPR 21.3% vs. 10.8%; $p < 0.01$) [235] and wrestling (male 15.7% (95% CI: 14.0 to 16.7), female 10.6% (95% CI: 7.5 to 13.7)) [230]. Male athletes were 1.8 (RR 1.8 (95% CI: 1.37 to 2.7)) [88] to 2.8 (RR 2.8 (95% CI: 2.45 to 3.24) [192] times more likely to sustain acute fracture than female athletes with the highest risk in competition. Male athletes had an OR of 1.21 (95% CI: 1.14 to 1.27) [244] for acute fracture across all recreational sports. Acute fracture data were from studies in all tiers of the participant classification framework. The meta-analysis of five studies for acute fractures injuries showed an overall significant RR (1.43 (95% CI: 1.23 to 1.67); $p<0.001$ for males (see Figure 2.6).

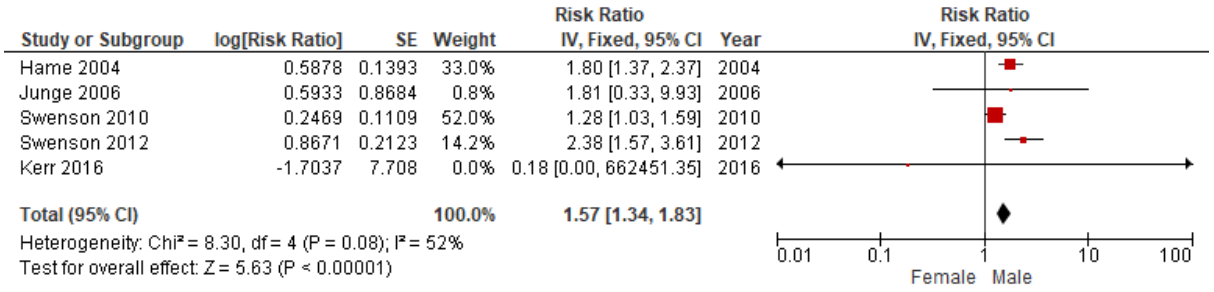


Figure 2.6: Meta-analysis for acute fractures in males.

2.5.6.6 Overuse injuries

Sixteen moderate quality studies [79, 96-98, 106, 133, 146, 158, 164, 168, 185-188, 198, 215] reflected a greater burden of overuse injury in female in athletes. Nine of these studies were in adolescent athletes [164, 168, 186-188, 198, 201, 203, 209]. Reported injury rates were IR 24.0/100 athletes (95% CI: 20.7 to 27.3) (males IR 10.5 (95% CI: 7.4 to

13.7)) in skiers [98] and 16.8/10,000 AE (males 14.9; p<0.0001) across multiple sex comparable sports [96]. The proportion of overuse injuries in females compared to males varied by study; female overuse IPR 1.32 (95% CI: 1.14-1.52) [198], female 18% vs male 4% [97], female 53.1% vs male 47.5% [106] female 18% vs male 11% (p<0.006) [79], female 13.3% vs 5.5% male [186], female 62.5% male 41.9% [187, 188], lower leg – male 24.9%, female 31.7%, medial tibial stress syndrome – male 10.1% vs female 18.0% [133]. One study in tier-3 adolescent athletes that had specialised in a single sport reported that females were 1.50 (95% CI: 1.14 to 1.99); *p*<0.004) times more likely than males to sustain an overuse injury [164]. These studies included tennis [97], skiing [98], sex comparable senior sports [96, 106], water polo [186] adolescent athletes that have specialised in a single sport [164] and high school sports [133]. One study highlighted that the ratio of acute to overuse injury was higher among male athletes (RR 3.67 (95% CI: 3.02-4.46)) than female athletes (RR 1.53 (95% CI: 1.28 to 1.83)) [96]. One tier-4 study [82] in football reported a higher rate of overuse injury in male players (male 4.19/1000 player hours, female 2.84; *p*<0.01; RR 1.48 (95% CI: 1.12 to 1.95)). Three moderate quality studies in adolescent athletes in swimming [201], cross country [209] and skiing [203] and one study in senior athletes across multiple sports [139] reported no significant sex difference for overuse injuries. These studies were all in tier-3 athletes. No meta-analysis was conducted due to the lack of sufficient data by body location for overuse injuries.

2.5 Discussion

The focus of this review was to identify where differences in injury are most apparent between the sexes in sport. There was evidence to support sex specific injury profiles. Injury is rarely the result of one single factor, rather the interaction of multiple factors. Recent studies [28, 50, 252, 253] have highlighted that biological sex interacts with gender, social and environmental factors, all of which play a role in how athletes are injured and must be considered in the overall web of determinants.

2.5.1 Lower extremity injuries

On balance there was higher volume of moderate quality evidence that female athletes were more susceptible to lower limb injuries overall than there was for male athletes, which was consistent with recent reviews [254-256] that highlighted the lower limb injury burden in female athletes. More specifically in this review, the higher rate of knee and foot/ankle injuries was consistent with other studies [257]. Changes that occur at the onset of puberty may influence this injury profile in female athletes. Males experience an increase in strength, neuromuscular performance, and power within one year of peak velocity of growth in height and weight not typically seen in females [258]. These differences in natural development may leave girls with decreased lower limb control and could be addressed through strength training and skill redevelopment as girls come through puberty and adolescence.

Male athletes appear to have a significantly greater propensity for hamstring injury than female athletes by as much as 62% to 64% [79, 123, 124], with high rates of reoccurrence, particularly in sports and playing positions with explosive physical demands. This was consistent with Zech et al. [259] who reported a higher incidence of thigh injuries in male athletes. An increasing incidence of hamstring injuries has also been observed in elite male football players [260], and there is evidence [261] that male athletes are generally more susceptible to muscular injury than female athletes. There is a growing body of research investigating mechanisms of [262] and risk factors for hamstring injury [263, 264] and although male sex is not cited as a risk factor the data predominantly comes from male cohorts. The injury mechanism typically associated with hamstring injury involves eccentric overloading and/or overstretching with a combination of hip flexion and knee extension [265]. Consistent with other studies [259, 266, 267], data in this review showed that male athletes were more susceptible to hip/groin injuries. There is evidence of sex differences in groin anatomy and pelvic and hip joint morphology that may contribute to the higher incidence of hip/groin injuries in male athletes [268]. Subtle differences in attachment sites of adductor and abdominal musculature alter the line of muscle action in certain positions and possibly making the tendons more vulnerable to higher tensile forces in male athletes [269]. When playing sports in positions that require a combination of kicking and a rapid change of direction, the male adductor complex may be put into an 'at injury risk' position.

Neuromuscular training programmes with the inclusion of specific components targeting adductor [270] and hamstring [271, 272] muscles have been effective in protecting against all lower limb injuries [273, 274], particularly to the knee in high demand pivoting sports that require rapid change of direction. In female athletes these programmes are more effective for preventing ACL and other knee injuries when implemented before or during adolescence [273-276]. Adolescence could therefore be a window of injury prevention opportunity in female athletes. Given the volume of evidence supporting targeted neuromuscular training programmes in reducing the risk of injuries that affect both male and female athletes, these programmes should be an integral part of all coach and player education and training behaviour. Organisations that influence the provision of sport from financial support to technical development should prioritise neuromuscular training programmes.

2.5.2 Upper extremity injuries

There was a higher volume of moderate quality evidence reporting that male athletes across all levels of the participation pyramid had a greater risk of upper limb injuries than female athletes in both team and individual sports, particularly shoulder injuries. The greatest risk was seen in judo, ice hockey and water polo, all considered contact collision sports [277] with some demand on the upper limb and shoulder. Those studies that did report female athletes with a higher incidence of upper limb injury were lower contact/individual sports that may be categorised as 'reverse chain' or 'above shoulder height with or without throwing' in terms of playing demands on the shoulder [277]. These sports typically include repetitive

upper limb movement patterns and may therefore be more likely to result in overuse injuries, which would be consistent with the findings is this review relating specifically to a higher rate of overuse type injuries in female athletes. The Bern consensus statement [277] for shoulder injury prevention in sport provides best practice guidance on risk factors and considerations for primary and secondary prevention of shoulder injuries in all sport.

2.5.3 Trunk injuries

On balance, the incidence of injuries to the trunk in male and female athletes could be considered equivocal from the data in this review. Studies that showed a higher incidence in male athletes were from sports that primarily load the lower limb. Studies that showed a higher incidence in female athletes were more balanced across sports that primarily load the upper limb or the lower limb. Previous research has shown a bi-directional relationship between lower limb [278, 279] and upper limb [280] characteristics/injury and trunk related pain, injury, mobility or deficit. Dynamic activities that occur during sport and involve any acceleration/deceleration movement of the limbs require a stable base in the lumbo-pelvic region to absorb and transfer load [281]. It is widely accepted that lumbo-pelvic stability is created by appropriate dynamic neuromuscular control of the trunk musculature. Studies have demonstrated an association between deficiencies in trunk muscles and incidence of lower limb injuries [282-284]. Studies investigating sex differences specifically in the fatiguability of anterior and lateral trunk muscles have reported inconsistent results [284-286]. Regardless, the findings in this current review further highlighted the importance of neuromuscular training programmes which include core strength and balance exercises for both male and female athletes.

2.5.4 Sports related concussion

The higher incidence of sports related concussion in females [259, 287, 288] is not well understood. A number of contributing factors have been cited but not thoroughly investigated, such as more honest reporting in females [259, 289-291], inadequate neck strength [288, 292], structural differences in the brain [293] and hormonal profile [287, 294-297]. The limited volume of research investigating concussive injury mechanisms in female sports has contributed to a lack of evidence informed preventative strategies and physical preparation protocols. Studies identified using our search terms consistently reported that female athletes have greater symptom severity and take longer to recover from sport related concussion [135, 148, 167, 169, 177, 298]. This highlighted a potential need for specific guidelines for rehabilitation and return-to-play for female athletes to optimise recovery from concussion. Further, a recent review [13] showed that there is a considerable under-representation of female participants (19.9%) in the studies that inform the recommendations of the most influential concussion consensus and position statements and that 40.4% of the studies include no female participants at all. There is likely a complex interaction between intrinsic biological sex with extrinsic, sociocultural or gendered behavioural differences. This is currently a poorly understood area, and research is needed to advance knowledge and inform prevention and rehabilitation protocols.

2.5.5 Fractures

Female athletes were at increased risk of bone stress injuries across multiple sports, a finding consistent with a review examining sex differences in running related injuries [299]. This sex difference may be underpinned by the interaction between energy availability, the menstrual cycle and bone health; the female athlete triad [300], as described within the relative energy deficiency in sport (RED-S) model [301]. Adolescence is widely accepted as a critical period for achieving peak bone mass [302] and an increasing body of evidence [301, 303, 304] supports the critical role of nutrition and energy availability in developing and maintaining bone health. Education to reinforce the importance of, and strategies for, achieving optimal bone strength during adolescent years is a key preventative strategy. Strength training has been shown to promote the development of bone strength [302, 305] in adolescent female athletes and in older cohorts [306-308].

2.5.6 Overuse injuries

There was moderate quality evidence in this review of a higher incidence of overuse injuries in female athletes, particularly in adolescent athletes. One study [164] reported that after controlling for all variables, athletes categorized as highly specialized in one sport had 1.46 (95% CI: 1.04 to 2.04) times greater odds of an overuse injury than low specialized athletes (p=0.03).

Overuse injuries result from acute micro trauma and repetitive stress and tend to occur when inadequate time is provided for the body to recover from and adapt to the stress. Previously identified factors associated with overuse injury include under recovery, excessive loading and under preparedness (or underloading of the musculoskeletal system) [309]. These factors can compromise an athlete's ability to tolerate and adapt to the training load. Athletes who are exposed to sports with repetitive movement patterns either due to the nature of the sport or the high training volume in a single sport may be at increased risk of overuse injury due to repeated loading of the same structures. Although risk factors associated with overuse injury could be present in both male and female athletes, the previously highlighted sexually divergent changes that occur during adolescence could make females more susceptible to overuse injury if not considered in the overall balance of sports participation e.g., strength development to tolerate training loads.

Due to the influence of circulating estrogen on joint laxity and neuromuscular control [310], the menstrual cycle has been studied as a risk factor for ACL injuries and a limited number of studies have investigated the overall risk of injury across the menstrual cycle [311, 312] with inconclusive results. However, loss of the menstrual cycle for >3 months (amenorrhea) has consistently been shown to put female athletes at increased risk of injury, particularly bone stress injury [301].

2.5.7 Limitations and future research

Although quality assessment was undertaken using the Downs and Black checklist, a detailed domain based RoB assessment was not carried out on the studies included in this review. Non-randomised study designs

are typically at increased susceptibility to bias and preliminary analysis using the ROBINS-E tool confirmed that studies in this review were likely at “high risk of bias”. This is an important consideration when interpreting the findings of the review.

In assigning data to the different tiers across the participant classification framework, where studies did not explicitly state the level of participation, tier classification was assumed based on the data presented. There was substantial heterogeneity across the studies included in some of the meta-analyses, somewhat limiting the generalisability of the results. The findings of this review should therefore be considered carefully.

Studies in this review did not consistently include training histories and protocols so it was unclear whether these injury patterns exist in the presence or absence of injury prevention strategies. Future sports injury epidemiological research should include sex analysis and include more factors outlined in the IOC consensus statement [313] to improve consistency of injury data collection. Given the lack of clarity on the impact of female physiology on injury, reproductive status and menstrual cycle phase should also be included as standard in injury and illness data collection as outlined in the ‘Female Athlete Health Domains’ supplement to the IOC consensus statement [314]. It should also be noted that there was inconsistent use of 'sex' (biology) and 'gender' (social construct) within the literature, studies within this review did not report how participant sex and/or gender information were obtained, and it was assumed that athletes included in the studies of this review were unlikely to have under-gone sex verification. Therefore, the results may represent a combination of differences due to biological sex and gender.

Areas for future research include surveillance and analysis of injury mechanisms across female sport and investigations into the underlying mechanisms in female athletes’ susceptibility to sports related concussion and prolonged recovery. Increased inclusion of female research participants and female-only studies with appropriate methodological design will enable sports training and injury prevention recommendations and strategies to be based on relevant research.

2.6 Conclusions

There were clear sex-specific body location injury patterns in sports across the participation pyramid; the meta-analyses showed that female athletes had a higher risk of knee and foot/ankle injuries, sports related concussion and bone stress injuries. Male athletes were at higher risk for hamstring, injuries and acute fractures than female athletes. Data showed that overall injury rates were generally higher in male athletes across the participation pyramid, or that there was no significant sex difference. However, it is important to be aware of sex specific injury patterns and tailor approaches accordingly. Sex is only one factor that will determine injury risk, therefore must be considered in combination with other individual risk factors and

with the demands of each sport. Further research is needed to develop prevention protocols for sports related concussion.

Chapter 2 and 3 linking section

The systematic review and meta-analysis in chapter two provides evidence of where the differences between the sexes are most apparent in sport related injury profiles. Male athletes are at increased risk of hamstring injury, acute fracture and hip/groin injuries. Whereas female athletes are at increased risk of bone stress injury (BSI), foot/ankle and knee injuries and sports related concussion (SRC). Females also appear to report greater symptom severity and take longer to recover following SRC [315-317].

Five themes have emerged in the literature as possible underlying reasons for these differences:

1. Females are more honest at reporting the injury and the symptoms than males [259].
2. Female sex hormones may affect outcomes following concussion [294, 295].
3. Weaker neck muscles in females may result in decreased absorption capability [318].
4. Differences in Cerebral Blood Flow (CBF) pre- and post-injury [319].
5. Females have smaller neuroanatomy more susceptible to damage and dysfunction [293].

Female athletes have been understudied in concussion research [320] it therefore remains unclear whether the observed difference between males and females represents a gendered reporting bias or a true sex difference. It likely includes a complex interaction between cellular, hormonal, anatomical, morphological, social and environmental factors.

Concussion has received increased profile through social and traditional media which has emphasised the risks of the injury in male collision sports (e.g., Rugby, American Football, Ice Hockey). Chapter two highlights that that concussion frequently occurs in other sports, particularly football (soccer) and this finding is consistent with other studies specifically investigating concussion risk across sport [321]. Given that female participation in team sports is growing, and sport is becoming a viable career option for females, it is reasonable to anticipate that the overall burden of sport related concussion in females will also increase. It is therefore important that the reasons underlying the apparent increased incidence and poorer outcomes in females are better understood so that education, prevention and management strategies support both male and female athletes and are based on relevant data.

Further research is needed to understand how much concussion knowledge and attitudes ~~(i.e., reporting behaviour)~~ influence the gender difference observed in the epidemiological concussion data.

Chapter 3. Differences between the sexes in concussion knowledge and attitudes in community football (soccer) players in New Zealand.

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3.1 Overview

Objective: To evaluate self-reported concussion knowledge and attitudes in community football players in New Zealand, comparisons were made between the sexes and those with and without history of concussion. **Design:** Cross-sectional cohort study. **Methods:** The Rosenbaum Concussion Knowledge and Attitudes Survey (RoCKAS) was used for data collection and analysis. Players were recruited through the New Zealand Football registered player database. **Results:** Seventy-four players had data eligible for inclusion (55% female). Thirty-four players (45%) had previously diagnosed concussion (56% female). Mean concussion knowledge index (CKI) scores were significantly (p=0.002) higher for male (20.8 ±1.4) than female (19 ±3.7) players. There were no significant sex differences in mean concussion attitude index (CAI) scores (female 63.7 ±11.4, male 65.6 ±6.0; p=0.427). Surprisingly, 32% female and 39% male players indicated they would play on with symptoms of concussion. Comparison within female players showed a significantly higher CKI (20.3 ±1.7 versus 18.8 ±2.6; p=0.025) and higher CAI (66.5 ±4.9 versus 64.1 ±5.9; p=0.151) in players with a history of concussion. There were no within male player trends for CKI or CAI. **Conclusions:** Male players had a notably higher concussion knowledge (CKI) than female players. Females with a previously diagnosed concussion also had a notably higher CKI than female players with no concussion history. Therefore, healthcare professionals could play a key education role when managing players with concussion. Given over a third of players indicated they would play on with symptoms of concussion, education should focus on short- and long-term impacts of concussion and potential consequences of concussion.

3.2 Introduction

In New Zealand there are an estimated 35,000 traumatic brain injuries (TBI) every year with ~95% classified as mild TBI (also known as concussion) [322]. Yet the national injury insurance scheme only receives ~22,000

concussion claims per year, suggesting that ~30% concussions may go unrecognised and unreported. Approximately 30-40% those concussion claims are from playing sport [323].

Rugby (union) has been the focus of sports concussion research in New Zealand [324]. Although the risk is lower, other team-based sports, including football (soccer), also carry a risk of concussion [321] which has been less well recognised in New Zealand.

Football is the worlds' most popular sport with over 270 million people involved worldwide [325]. While there are mental and physical health benefits of playing team sport [2] there is a risk of football-related injury, including concussion [326]. Concussion carries risk of long-term consequences including increased risk for cognitive decline and neurodegeneration in later life [6]. Early recognition and management of concussion substantially improves outcomes and recovery time [327, 328]. Awareness of concussion in sport has increased due to social and traditional media raising the profile of the injury. This is particularly true for collision sports in which routine, purposeful body-to-body collisions are a legal and an expected part of the game (e.g., Rugby codes, American Football, and Ice Hockey) where risk of concussion is accepted to be high.

There are sex differences in sports related concussion in team sports (e.g., football, hockey, basketball). Females typically have a greater incidence of concussion [321], report a higher symptom burden and often take longer to recover and return to play [329]. These differences are particularly evident in football [329, 330]. Sex differences may be due to gendered reporting bias where female players are more likely to report injury and ongoing symptoms [259, 331]. Little is known about levels of knowledge and attitudes towards concussion in football in New Zealand and there is limited research investigating potential reporting bias or sex differences among tier two and three [63] (community) football players.

Sustaining a single concussion that is recognised, well managed and given time to fully resolve is unlikely to lead to long-term detrimental consequences. Concussion knowledge and attitudes play a role in prevention, recognition, and management of this injury. In community sport where there are lower levels of medical support, there is greater reliance on players, coaches and caregivers to recognise and report a suspected concussion [330, 332]. Although concussion knowledge does not always translate to desired behaviour [333-335], understanding current levels of concussion knowledge and attitudes in football is an important start point. Identifying knowledge gaps may contribute to developing more effective injury prevention and concussion education strategies tailored to different genders.

This purpose of this cross-sectional study was to evaluate via online survey the self-reported concussion knowledge and attitudes of community football players in New Zealand.

3.3 Methods

Ethical approval for the study that complies with The Declaration of Helsinki was granted on 23 August 2022 by Auckland University of Technology Ethics Committee (AUTEC 22/192).

All 104,000 players registered with New Zealand Football (NZF) in the 2023 season were invited to take part in the study. Participants were recruited via email and social media. NZF provided information about the research to the six regional federations to distribute to affiliated clubs. Potential participants were directed to an online version of the Rosenbaum Concussion Knowledge and Attitudes Survey (RoCKAS) survey via a link shared through player databases and through federation and club social media channels. Responses were monitored and federations sent reminders to their football communities twice throughout the season (17 weeks) to encourage participation. At season end, a third and final reminder was sent to players during the off-season to further increase participation. The survey, completed between 01 February 2023 and 19 February 2024, was anonymous.

Inclusion criteria for participants were; (i) Aged 16 yrs. or older, (ii) Be an amateur (non-professional) player – tier two or three on the participant classification framework [63], and (iii) Have been involved in football within the previous 12 months. Exclusion criteria were; (i) Professional player, (ii) Failing the internal validity index consisting of three true/false items in section one of the RoCKAS with a score of <2 [336].

Data were collected through an online survey (supplementary material) administered on the Alchemer platform (https://app.alchemer.com). Player demographics collected included age group, gender (terminology used was 'female' 'male' 'non-binary' 'Prefer not to say'), level of participation of football, years of playing experience, residing region, and history of previously diagnosed concussion.

The RoCKAS [336] survey was utilised for data collection and analysis. RoCKAS was developed to examine concussion knowledge and attitudes in high school students and has been used in adult populations [337-340]. RoCKAS has been psychometrically tested and has good validity and reliability to measure concussion knowledge and attitudes (knowledge items, r = 0.67; attitude items, r = 0.79) and appears to be less susceptible to social desirability in its measures of attitude than other similar tools [336]. RoCKAS has an Intraclass Correlation Coefficient (ICC) of 0.79 ($p<0.001$) and an internal consistency with a Cronbach's alpha score of $\alpha=0.76$ [341].

RoCKAS consisted of 55 items across five sections. Question format varied; sections one and two were TRUE/FALSE items, and section five was a checklist. A correct answer in these sections scored one point. Collectively these sections included 25 items and were used to determine the concussion knowledge index (CKI) with a total possible score of 25 points. Sections three and four were used to determine the concussion attitudes index (CAI) and included 15 items utilising a five-point Likert scale. Participants scored five points for the safest answer and one point for the least safe answer for a total score range of 15-75. RoCKAS included three items that tested validity of participant responses (items four, ten and 15 in section one). If less than two validity items were answered correctly, the survey was considered invalid and therefore excluded from analysis (see Supplementary Table I). RoCKAS was modified to make terminology in some items reflect the New Zealand context (Supplementary Table II). The primary outcome measures were CKI and CAI.

CKI and CAI were calculated using the RoCKAS scoring key (Supplementary Table I) and tabulated with the other participant items in Excel (Microsoft, USA) for data handling before import into GraphPad Prism v9.31 (GraphPad, USA).

Descriptive statistics were generated to provide general information about the population demographics and to summarise CKI and CAI scores. Data are reported as mean ±standard deviation (SD) for CKI and CAI scores to characterise the sample. Frequency and percentage of correct or safe/desirable answers were calculated. Graphs were plotted in GraphPad Prism v9.31.

Due to sample size, normal distribution could not be confidently assumed. Normality and lognormality were tested using Anderson-Darling, D'Agostino & Pearson's, Shapiro-Wilk and Kolmogorov Smirnov tests, and did not indicate Gaussian distribution. Box plots were presented given non-normally distributed data. The Mann Whitney U test was utilised for pair wise comparisons of total CKI and CAI scores between males and females. Mann Whitney U tests were also performed within males and females to evaluate whether CKI and CAI differed between those who did or did not report a history of medically diagnosed concussion. Alpha level was set at $p<0.05$ for all statistical tests.

3.4 Results

Ninety-seven football players participated in the study (54% female). Twenty-three responses were excluded due to incomplete survey answers, resulting in 74 responses eligible for analysis (55% female) (see Table 3.1). Thirty-four players (45%) reported a previously diagnosed concussion.

3.4.1 Concussion knowledge index scores

The total mean concussion knowledge index (CKI) score was 20.0 ±2.0 (see Table 3.2). Male players had a significantly higher mean CKI than females (20.8 ±1.4 vs. 19.0 ±2.2; p=0.002) (see Figure 3.1). Nearly all players (98% females, 100% males) knew that a person did not have to be knocked out to sustain a concussion (evidenced by section 1, question 5, 'In order to be diagnosed with a concussion, you have to be knocked out'). All (100%) players knew that 'Concussions can sometimes lead to emotional disruptions.' (Section 1 question 16). The question most frequently answered incorrectly was section 1 question 11, 'After a concussion occurs, brain imaging (e.g., CAT Scan, MRI, X-Ray, etc.) typically shows visible physical damage (e.g., bruise, blood clot) to the brain.' Only 24% of female, and 48% of male players, answered this question correctly. All responses for CKI sections 1 and 2 are included in supplementary table 3.3 (see Appendix VI).

Table 3.1: Participant characteristics by age group, participation level, years of playing and previous concussions for 74 amateur football players in New Zealand.

	Female n (%)[a]	Male n (%)[b]
Total	41 (55%)	33 (45%)
Age Group (yrs.)		
15-19	5 (12.2%)	1 (3.0%)
20-24	8 (19.5%)	3 (9.1%)
25-29	9 (22.0%)	3 (9.1%)
30-34	7 (17.1%)	6 (18.2%)
35-39	3 (7.3%)	1 (3.0%)
40-44	6 (14.6%)	4 (12.1%)
45-49	2 (4.9%)	6 (18.2%)
50-54	1 (2.4%)	4 (12.1%)
55-59	0 -	5 (15.2%)
Participation level		
National League	2 (4.9%)	nil
Premier*	13 (31.7%)	11 (33.3%)
U18	1 (2.4%)	nil
Local League	25 (61.0%)	12 (36.4%)
Masters	0 -	10 (30.3%)
Years Playing		
0-1	1 (2.4%)	nil
1-2	1 (2.4%)	nil
2-5	8 (19.5%)	nil
5-10	10 (24.4%)	4 (12.1%)
10+	21 (51.2%)	29 (87.9%)
Previous concussion		
Yes	19 (46.3%)	15 (45.5%)
No	22 (53.6%)	18 (54.5%)

* = Premier League, Championship / Federation League; a = percentage (%) of count for all female participants; b = percentage (%) of count for all male participants

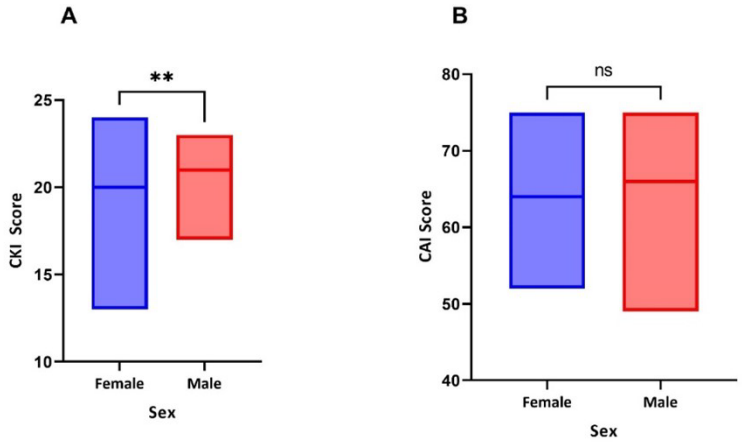

Figure 3.1: Differences in mean scores between males and females for (A) Concussion Knowledge Index (p=0.002) and (B) Concussion Attitude Index (p=0.538).

The question answered most differently between females and males was section 2 question 2, 'It is likely that Player X's concussion will affect his long-term health and well-being.' Only 30% of male players knew

that this could have a long-term impact versus 95% of female players. Only 32% of male players knew that playing with concussion would impact performance. Whereas 95% of female players recognised that this would be detrimental (section 2 question 3, *'Even though Player F is still experiencing the effects of the concussion, her performance will be the same as it would be had she not suffered a concussion'*).

Table 3.2: Mean CKI and CAI scores of all respondents, female and male respondents for each section and total assessment by mean ±standard deviation (SD), statistical significance between male and female respondents.

Section	Total Mean ±SD	Female Mean ±SD	Male Mean ±SD	*p value
1 (max score = 14)	9.76±1.50	9.29 ±1.50	10.3 ±1.17	
2 (max score = 3)	2.53 ±0.53	2.46 ±0.55	2.60 ±0.50	
5 (max score = 8)	7.76 ±0.64	7.7 ±0.67	7.8 ±0.57	
Total CKI (max = 25)	**20.0 ±20**	**19.46 ±2.20**	**20.76 ±1.40**	**p=0.002**
3 (score range 5-25)	21.95 ±2.26	22.15 ±2.15	21.72 ±2.44	
4 score range 10-50)	43.65 ±4.07	43.46 ±3.77	43.88 ±4.39	
Total CAI (possible 15-75)	**65.61 ±5.61**	**65.61 ±5.34**	**65.57 ±5.57**	**p=0.427**

SD = Standard Deviation; *p = difference between male and female players; CKI = Concussion Knowledge Index; CAI = Concussion Attitude Index

3.4.2 Concussion attitudes index scores

The total mean concussion attitudes index (CAI) score was 65.6 ±5.6 (see Table 3.2). Male players did not have a significantly higher mean CAI than females (65.61 vs. 65.57; p=0.427) (see Figure 3.1). Of 18 questions that comprised the CAI score, no question was answered 100% safely by either male or female respondents. The highest percentage of correct responses was for Section 4, Question 1 *'I felt the Coach A made the right decision to keep Player R out of the game'*, 84.8% of male and 80.5% of female players selected the safest responses. Both male and female respondents' lowest rate of safe/desirable responses was for Section 3, Question 1 *'I would continue playing a sport while also having a headache that resulted from a minor concussion'*, with 68% of female and 61% of male players gave a safe response. All responses for CAI Sections 3 and 4 are included in supplementary table 3.4 (Appendix VI). Over 90% male and female respondents correctly identified symptoms of a concussion (see Table 3.3). Difficulty speaking, panic attacks and reduced breathing rate were symptoms most frequently incorrectly identified as concussion symptoms by both male and female players.

Table 3.3: Percentage and number of participants responses to symptoms they identified as concussion symptoms.

Symptom	Female % (n=)	Male % (n=)	Overall % (n=)
Hives	29 (12)	45 (15)	36 (27)
Headache	***100 (41)***	***97 (32)***	***99 (73)***
Difficulty Speaking	95 (39)	100 (33)	97 (72)
Arthritis	24 (10)	30 (10)	27 (20)
Sensitivity to Light	***93 (38)***	***94 (31)***	***93 (69)***
Difficulty Remembering	***100 (41)***	***100 (33)***	***100 (74)***
Panic Attacks	88 (36)	97 (32)	92 (68)
Drowsiness	***98 (40)***	***100 (33)***	***99 (73)***
Feeling in a "Fog"	***95 (39)***	***97 (32)***	***96 (71)***
Weight Gain	48 (20)	48 (16)	49 (36)
Feeling Slowed Down	***88 (36)***	***94 (31)***	***91(67)***
Reduced Breathing Rate	92 (38)	91 (30)	92 (68)
Excessive Studying	20 (8)	18 (6)	19 (14)
Difficulty Concentrating	***98 (40)***	***100 (33)***	***99 (73)***
Dizziness	***100 (41)***	***100 (33)***	***100 (74)***
Hair Loss	24 (10)	24 (8)	24 (18)

True concussion symptoms indicated in ***bold***

3.4.3 Impact of previous concussion

Of 34 players that reported a previously medically diagnosed concussion, 19 (55%) were female and 15 (45%) were male. Female players with a history of concussion had a significantly higher CKI when compared with females with no previous concussion (20.3 ±1.7 vs. 18.8 ±2.6; *p*=0.034). There were no statistically significant differences for any other comparisons (see Table 3.4 and Figure 3.2).

Table 3.4 Differences between males and females Concussion Knowledge Index and Concussion Attitude Index scores by mean ±standard deviation (SD), statistical significance and effect size with and without prior concussion.

	Prior Concussion Mean ±SD	No Prior Concussion Mean ±SD	*p-value
CKI			
Male	20.80 ±1.26	20.71 ±1.65	0.837
Female	20.26 ±1.66	18.75 ±2.61	0.034
CAI			
Male	66.67 ±6.21	64.24 ±5.74	0.263
Female	66.53 ±4.86	64.05 ±5.93	0.151

SD = Standard Deviation; CKI = Concussion Knowledge Index; CAI = Concussion Attitude Index; *p difference within sex by concussion history

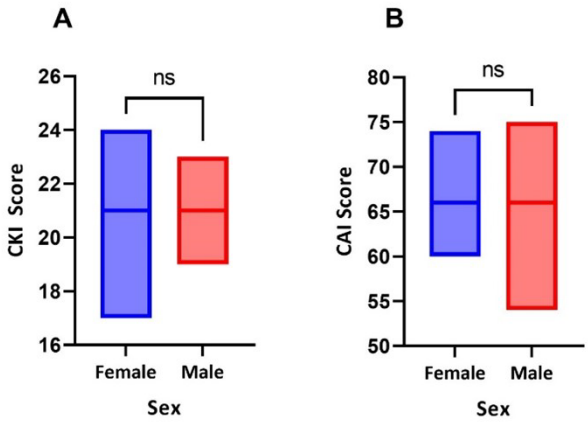

Figure 3.2: Mean scores for males and females with a history of concussion showed no differences by sex for (A) Concussion Knowledge Index (p=0.403) and (B) Concussion Attitude Index (p=0.843).

3.5 Discussion

Female vs male responses (including CKI and CAI scores)

This was the first study to evaluate concussion knowledge and attitudes in tier two and three [63] (community) football players in New Zealand. Male players had a significantly (p=0.002) higher CKI than female players and female players with a history of concussion had a significantly (p=0.034) higher CKI than female players not reporting a history of concussion. There were no other significant findings. Although the RoCKAS is the most utilised tool for evaluating concussion knowledge and attitudes and provides a useful objective measure, what remains unclear is whether there is a minimum threshold index score for knowledge and attitudes that should be achieved or that relates to a specific outcome. Therefore, it is difficult to infer whether the current CKI and CAI scores should be considered 'high' or otherwise. However, the CKI and CAI scores for male and female players were comparable to those reported in similar studies in football [338-340, 342] and other team sports [337, 343, 344] (Appendix VI, Supplementary Table 3.5).

Of local relevance, the study of 533 community Rugby players in New Zealand [343] recorded lower CKI (overall 18.6 ±2.4, women 18.9 ±2.1, men 18.4 ±2.6) and CAI (overall 59.3 ±6.8, women 60.2 ±6.5, men 58.8 ±7.0) scores than football players in the current study. Score differences could be due to the rugby study having a larger sample size from the in-person recruitment strategy which may mean results were more representative of the NZ rugby playing population. This cohort was inclusive of older players whereas the rugby cohort focused on high school players which could also have influenced the higher scores recorded in the football players. It is also plausible that higher CKI and CAI scores in the current study were a result of an increased level of activity around concussion more generally in response to concerns over long-term impacts of concussion and media influence associated with two major sporting events (FIFA Women's World Cup 2023 and the Women's Rugby World Cup 2023) hosted in New Zealand since the previous studies were conducted. In contrast, a relatively recent study [338] in football players in Nigeria reported much lower CKI

(14.0 ±3.0) and CAI (54.5 ±9.4) scores, which may be a reflection of levels of resource available to provide education and medical support in the two countries.

The lower CKI of female players in this study compared to male players could be due to over 50% of female players in this study being under the age of 30 with a range of playing experience from one to ten years, whereas male players were older (55% were over 40 years old) and had a minimum of five years playing experience which may lead to more exposure to concussive injury and concussion education. These factors may have influenced concussion knowledge. CKI scores have previously been positively associated with the number of times concussion education has been received in college age student athletes [345]. These variables were not included in current analyses due to the small sample size and risk of type one error. Interestingly, females make up 22% of the playing population in New Zealand and represented 54% of respondents in this study. Although this introduces an element of bias in the findings, it could also be considered a standalone finding. It has previously been observed that females are more likely to engage in and complete surveys than males and that males and females differ in the types of health conditions that interest them and impact their lives [346]. This over-representation of females could indicate that female players are more interested in or concerned about concussion. This may be somewhat reflected in the lower CKI recorded for female respondents.

Identification of symptoms

Knowing concussion symptoms is critical to recognising the injury. Over 90% of players in this study correctly identified concussion symptoms (see Table 3.4). Symptom knowledge could however be improved further as there were some listed symptoms that were incorrectly identified as concussion symptoms, including: difficulty speaking, panic attacks and reduced breathing rate. This is important to clarify as other medical concerns may present with these symptoms.

The knowledge statement, *'After a concussion occurs, brain imaging (e.g., CAT Scan, MRI, X-Ray, etc.) typically shows visible physical damage (e.g., bruise, blood clot) to the brain'* (Section 1 question 11) was the least correctly answered statement in the current study with only 24% of females and 48% of males answering this correctly. Previous studies in elite female football reported that 53% answered this statement correctly [339], in cycling, 44.9% answered this correctly [347] and in community Rugby in New Zealand where 26% players answered this statement correctly [343]. This result outlined a general lack of understanding of what happens to the brain during concussion and highlights an education opportunity to help players recognise the importance of reporting a suspected concussion. These results also outlined that knowledge around brain imaging was particularly low in the two New Zealand cohorts. There is potential for sports codes to work together on development of consistent concussion education content.

Attitudes towards concussion

The attitude statement *'I would continue playing a sport while also having a headache that resulted from a minor concussion'* (section 3, question 1; Appendix VI, Supplementary table 3.4) has been shown to be a reasonable proxy to indicate concussion reporting intention (CRI) in male ice hockey players [333]. This metric has been used in other research [343]. Although CRI was not the focus of the current study it was interesting to note that, 68% of female players and 61% of male players gave a safe/desirable response to this statement. This was encouraging but also concerning given that over 30% players indicated an intention to play on while experiencing the symptoms of a concussion. This is worthy of further investigation particularly given that it could have been expected that a higher percentage of female players would have given a safer/more desirable response to this statement given the majority (95%) of female players in the current study knew that a history of multiple concussions could have a long-term impact on health and wellbeing (Section 2 Question 2; supplementary table III) and that concussion would be detrimental to performance (section 2, question 3; supplementary table III). This was notably higher than ~30% of male players that reported knowing these points and inferred that male players may lack an understanding of implications of an unreported concussion in the short (immediate performance) and long (health and wellbeing) term. The finding ~~also~~ supported other studies [339, 343, 344] demonstrating that concussion knowledge does not necessarily translate to the safest/most desirable behaviours and that players alone cannot be relied upon to self-report injury. Reporting of concussion is influenced by multiple factors including ability level, gender, not wanting to let teammates down [348], fear of being removed from competition [334], self-denial in the 'heat of competition' and underlying motivations to be involved in sport [349]. In community sport, teams often do not have medical support, and it is likely that many concussions go unrecognised and unreported. Multiple people can play a role in recognising a suspected concussion, including coaches, family (whanau), teammates, sports medicine support staff (if present), officials and team managers. It is therefore important that future research is conducted to determine the level of concussion knowledge of these key people and whether it could be improved to support increased recognition of the injury.

Respondents to concussion surveys are likely motivated to engage in the survey due to their previous injury experience. Forty six percent of all players in the current study reported a history of medically diagnosed concussion (46% all female players and 45% all male players). This proportion was consistent with previous studies in professional football where 43% female [339] and 50% male [340] players reported concussion history. Conversely, community rugby players reported a lower rate (33%) of previously diagnosed concussion [343]. Given higher incidence of concussion in rugby than in football [321], it would be reasonable to expect that the number of previously diagnosed concussions in a cohort of rugby players would be higher than that seen in football players. Concussion education and management strategies have been implemented in community Rugby in New Zealand [350, 351], however, there still seems to be under-reporting of concussion, which is a well-documented challenge [335].

The higher CKI in females with a reported history of concussion when compared with those with no reported concussion history may be due to injury serving as education through experience. Prior concussion has been shown to improve CKI and CAI via players learning more through experience than through education alone [345]. Due to the younger age and lower number of years playing football for female players in this study it is plausible that the experience of having a concussion went someway to filling the education/knowledge gap. This highlighted a valuable secondary prevention education opportunity for healthcare professionals when engaging with community players who sustain concussion.

3.5.1 Limitations

The small sample size overall and in each age group mean the study findings may not be representative of the football community and limited the number of variables that could be investigated and the possibility to draw conclusions for specific populations. It is also noteworthy that this study had a small sample and a low response rate (0.07%) in comparison to other similar studies. This could be attributed to several factors. Firstly, 2023 was the first uninterrupted season since COVID-19, which may have affected participants' motivation to engage in research activities. Studies have shown that post-pandemic periods can lead to lower participation rates due to altered priorities and fatigue from previous disruptions [352]. Secondly, the online recruitment strategy employed for this survey may have been sub-optimal. Online surveys often face challenges such as lower engagement and higher dropout rates compared to face-to-face methods [353]. Additionally, previous research indicates that novel survey topics can initially attract fewer respondents until awareness and interest are built over time [354].

The low response rate (0.75%) also introduces the potential for substantial non-response bias and a bias towards those with an interest in, or some experience with, concussion. However, this was the first study to evaluate concussion knowledge and attitudes of community football players in New Zealand, where much of the previous sports concussion research has focused on rugby.

The proportion of respondents with a history of medically diagnosed concussion highlighted a possible bias in this sample; players engaging with the survey likely had an interest in concussion due to prior experience. Future studies need to adopt a wider recruitment approach and encourage responses from players of all age groups and playing experience.

3.6 Conclusions

Male players had significantly higher concussion knowledge (CKI) than female players. Females with a previously diagnosed concussion had significantly higher CKI than female players with no concussion history. Therefore, healthcare professionals could play a key education role when managing players with concussion. Given over a third of players indicated they would play on with symptoms of concussion, education should focus on short- and long-term impacts of concussion and potential consequences of concussion if not

medically managed. The wider football community should be supported to promote an environment where concussion recognition and reporting is expected and encouraged. Given the response to the statement related to concussion reporting intention, further research is needed to understand motivations underlying the intention to report a concussion or not. It would be valuable for larger studies to further investigate concussion knowledge and attitudes of players and of other members of the football community in New Zealand including coaches, team managers and those involved in side-line care. Finally, given the widespread utilisation of the RoCKAS it would be valuable to compare all studies and evaluate how concussion knowledge and attitudes compare across sports, countries and over time.

3.7 Practical implications

- Concussion education strategies could focus on ensuring that players understand the short- and long-term impact of a concussion and the potential consequences of the injury if not reported and managed.
- Sports medicine and other healthcare professionals engaging with community players have a key education role in further increasing concussion knowledge and promoting a safe attitude towards the injury.
- Concussion knowledge in players may not always results in safe/desirable attitudes towards the injury. This should be considered within education strategies and further investigation is needed to elucidate reasons or motivations underlying attitudes towards concussion.

Chapters 3 and 4 linking section

The observed sex difference in sports related concussion has been attributed to more honest reporting by female athletes [259]. When similar research studies are considered collectively [290, 355-357] the data are equivocal and don't fully support this assertion. Although the study in chapter 3 did not investigate reporting behaviours, it did analyse CKI and CAI by sex in a small sample of community football players in New Zealand. The data reflect that male players had a higher CKI and that CAI was similar between the sexes. It remained unclear if or how CKI and CAI related to reporting behaviours. It is important to explore other factors contributing to the differences in concussion incidence and recovery trajectory observed between male and female athletes.

As previously highlighted, other hypotheses emerging from the literature are:

1) Weaker neck muscles in females may result in decreased absorption capability [358]. This is a modifiable factor that could be developed into prevention strategies, there is an increasing amount of research activity in this area. It is however noteworthy that, there is currently no consensus or conclusive evidence of a relationship between relative neck strength and concussion in male or female athletes;
2) Differences in cerebral blood flow (CBF) pre- and post- injury [319];
3) Smaller neuroanatomy in females that is more susceptible to damage and dysfunction [293]. This is important to understand but not modifiable; and
4) Ovarian hormones, estrogen and progesterone, may affect how females experience concussion [294, 295]. Data in this area are limited, further research could contribute to informing education, prevention and rehabilitation strategies tailored specifically to females.

Sex hormones interaction with the brain

The blood brain barrier (BBB) is a natural semi-permeable vascular membrane that separates the central nervous system (CNS) from the peripheral tissues and functions to protect the CNS from blood borne toxins and pathogens. Limited substances can cross the BBB and enter the CNS [359, 360]. Estrogen and progesterone secreted in the ovaries also act on other systems of the body including the CNS [361, 362]. These hormones are transported to the brain via blood vessels and easily pass through the BBB to exert modulating and regulatory effects on neurotransmitter pathways, neurotrophin activity, the bioenergetics of the brain [363] and ultimately brain function [364, 365]. Further, it is evident that even in the absence of injury the fluctuating nature and changing ratio of these hormones interacting with the brain can cause a variable cluster of cognitive and emotional symptoms in females [37, 366]. When the hormonal balance between estrogen and progesterone is disrupted, females are at greater risk of experiencing neurocognitive dysfunctions [363].

Limited evidence suggests that menstrual cycle phase at point of injury and outcomes in concussion may be closely and bi-directionally related [294-296, 367].

An understanding of hormone profiles, symptomology and the magnitude of change in symptoms across the menstrual cycle in the absence of concussion is needed. An in-field research protocol that includes a suite of data collection tools and does not put unnecessary burden on participants also needs to be tested for use in future studies.

Chapter 4. Magnitude of change in baseline symptom profiles across the menstrual cycle in physically active females – considerations for concussion?

This chapter has been submitted and is under review as:
Hardaker, N.J., Hume, PA., Sims, ST., King, DK., Stewart, T. Magnitude of change in baseline symptom profiles across the menstrual cycle in physically active females – considerations for concussion? Submitted to *Research Quarterly for Exercise and Sport*.

4.1 Overview

Background: Cyclical rise and fall of estrogen and progesterone across menstrual cycles can cause a variable cluster of physical, emotional and cognitive symptoms. Symptoms are non-specific to the menstrual cycle and are congruent with some clinical concerns including concussion. Females report greater symptom severity and longer recovery times with concussion. Fluctuating hormones could mediate the way that females experience concussion, due to congruency of concussion and hormone-related symptoms.
Objective: The aim of this study was to understand magnitude changes in daily menstrual cycle symptoms in eight healthy physically active females with a regular menstrual cycle. Relevance to concussion is discussed. **Method:** This observational cohort study monitored daily symptoms using a menstrual cycle tracking app (WILD AI) for a minimum of three full menstrual cycles. Daily sleep characteristics were monitored as a potential modifier of symptoms. A mixed model analysis was used to investigate changes in symptom scores across the menstrual cycle. Sleep characteristics were included as covariates.
Results: There was a significant association (R^2=0.363, p<0.001) between menstrual cycle day and symptom score; increased symptoms were not limited to the week before menstruation. Higher sleep quality was significantly associated with a lower symptom score (R^2=0.513, p<0.001). Overall symptomology was associated with day of menstrual cycle and was inversely associated with sleep quality.

4.2 Introduction

The menstrual cycle typically lasts 21 to 35 days and results in a cyclical rise and fall of estrogen and progesterone that can be considered in distinct phases. These are: (1) Menses (begins with the first day of bleeding; low levels of estrogen and progesterone); (2) Mid-follicular phase (consistently low levels of progesterone and gradually rising levels of estrogen); (3) Ovulation (1 day, preceded by an estrogen surge); (4) Luteal phase (levels of progesterone rising to a peak and a second smaller peak in estrogen); and (5) Late Luteal phase (a rapid perimenstrual withdrawal of estrogen and progesterone triggering menstruation). These fluctuations in estrogen and progesterone cause biochemical changes that effect

cognitive, emotional and sensory function [368] and result in a variable cluster of symptoms including, fatigue, irritability, headache, emotional volatility, bloating, uterine cramps [37]. Most females have some level of discomfort and experience at least one or more physical, cognitive or emotional symptoms in the week before menstruation [30, 36-38, 41]. Menstrual cycle related symptoms are congruent with symptoms associated with some clinical concerns including concussion. Studies have shown that there are sex and gender differences in both sports [259, 288] and non-sports related concussion [294]; females report greater symptom severity and take longer to recover compared with males [259, 316, 369]. There is currently no objective test for concussion; injury is diagnosed through multi-domain assessment of physical signs, balance, cognition and self-report symptoms [370]. Symptoms remain the primary predictor and measure of recovery [371] and there are several different tools available for reporting and evaluating symptoms [372]. In a sporting context baseline testing is frequently used whereby a concussion assessment inclusive of symptom evaluation is administered to athletes in absence of injury at the start of a sports season [370]. Similar to post-injury reports, females tend to endorse a higher number and greater severity of symptoms at baseline when compared with males [350, 373-375]. This may be due to similarity of symptoms experienced after a concussion and those associated with the menstrual cycle.

Hormone related symptoms experienced during the menstrual cycle in absence of clinical concerns vary within and between females [37, 38]. It is not clear if timing and magnitude of change in these symptoms is enough to impact symptom evaluation for clinical concerns like concussion, this is important to consider and understand so that clinically related symptom assessments can be interpreted more accurately and with context of individual baseline symptom patterns.

This observational study aimed to prospectively investigate daily changes in self-reported hormone related symptoms across the menstrual cycle in un-injured healthy physically active (tier 1-2) [63] females with a regular menstrual cycle. Data presented are part of a broader feasibility study [376].

4.3 Methods

A prospective exploratory observational cohort study was undertaken. Participants were recruited through the university postgraduate research school, workplaces, gyms, sports clubs, and social media. Menstrual cycle data were collected through observational calendar tracking. Basal body temperature (BBT), urinary testing of luteinizing hormone (LH) surge or serum measures of progesterone were not used for confirmation of ovulation or phase verification. Results are based on specific days of a cycle, not a phase of the cycle and are reported to reflected this.

Research was conducted in accordance with the Declaration of Helsinki, and ethics approval was obtained from the Auckland University of Technology ethics committee (AUTEC #21/167).

4.3.1 Participants

Thirty healthy females with a self-reported regular natural menstrual cycle (21 to 35 days) between 25 and 47 years of age were recruited to take part in this study. As the study examined associations across the menstrual cycle, several inclusion/exclusion criteria were utilised to ensure other exogenous and endogenous factors did not influence the endocrine system during the 12 weeks prior to study participation. Inclusion criteria were: (1) Female aged 16 yrs. or older (and had a menstrual cycle for a minimum of 2 years); and (2) Self-reported a natural regular menstrual cycle (21–35 days long). Exclusion criteria were: (1) Commenced any medication in the last 3 months that could alter reproductive hormone concentrations (glucocorticoids – e.g., prednisone; Antidepressant; or Antipsychotic medications); and (2) Current or any previous history of brain injury.

Participants provided written consent and attended a 15-minute introduction session with the lead researcher (NH), where they received detailed instructions on data collection protocols and had opportunity to ask questions.

4.3.2 Measures

Daily menstrual cycle data were gathered using a mobile menstrual cycle tracking application (app) for three to five complete cycles and the Low Energy Availability in Females Questionnaire (LEAF_Q) [47] was administered weekly. All data were collected by participants at home.

4.3.2.1 Low Energy Availability in Females Questionnaire

Low energy availability (LEA) is the state that occurs when there is insufficient energy available to sustain physiological function after subtracting energetic cost of exercise/ training [46], LEA is prevalent in 45% of active females [53]. LEA is known to disrupt hormones, and these effects can occur in as little as four days [46]. Given the study length, the Low Energy Availability in Females Questionnaire (LEAF-Q) was administered weekly to monitor LEA status as a variable that could impact on the menstrual cycle.

LEAF-Q was designed and validated to evaluate physiological symptoms (e.g., fatigue, irritability) of insufficient energy intake [47]. LEAF-Q consists of 25 items rated on a Likert scale across three categories; (1) Sports injuries; (2) Gastrointestinal problems; and (3) Menstrual function. There are three questions relating to menstrual function including recent menstrual history (see Supplementary material I). LEAF-Q was administered weekly via the online data management platform REDCap (Research Electronic Data Capture; https://www.project-redcap.org) [377]. A unique link to LEAF-Q was emailed to participants every seven days. Participants with total LEAF-Q score ≥8 in combination with an injury score ≥2 and/or menstrual dysfunction score ≥4 were considered to have markers of Low Energy Availability (LEA) [378]. LEAF-Q score was collected weekly to give a more accurate indication of LEA status of participants, i.e., presence or absence of LEA over time rather than a single point in time. Information from LEAF-Q was also used to calculate Body Mass Index (BMI kg/m2).

4.2.2.2 Menstrual Cycle Tracking And Symptom Report

The mobile phone application (app) 'WILD AI' (WILD.AI technologies limited; https://www.wild.ai) is a free to download menstrual cycle tracking app with artificial intelligence (AI). This app was used to capture symptoms and sleep characteristics daily for study duration. Participants downloaded the 'WILD AI' app, created a profile and then digitally consented to link their profile to the WILD AI research platform using a unique study code. Training and nutrition advice/notifications available in the app were turned 'off' and the daily symptom report was standardised from an evidence informed pre-determined list of cognitive, emotional and physical symptoms associated with fluctuating hormones across the menstrual cycle (full list outlined in Supplementary material II). Symptoms were rated on a four-point scale as "None (0), Low/mild (1); moderate/medium (3); or high/severe (5)" and a daily symptom score was calculated by summing ratings for each endorsed symptom. Given that sleep can also impact experience of daily symptoms [379-381], self-reported sleep data (quality and duration) were also collected in the app. Sleep quality was rated on a three-point scale as "Poor (1); Ok (3); and Great (5)" and the score was calculated by multiplying the number of hours sleep by the quality rating. Sleep was considered a covariate in data analysis that may influence symptomology.

Adherence to the menstrual tracking protocol was calculated by dividing number of completion days by total number of monitoring days [40]. Engagement with the app was determined by cycle length difference (CLD) between consecutive cycles within each female. If the difference between median and maximum CLD was greater than 10 this was considered low engagement [41] and may impact on result accuracy.

4.3.3 Statistical analysis

Data analyses were performed in R (version 4.3.0) to examine how sleep hours, sleep quality, and symptom severity changed across the menstrual cycle, using a series of linear mixed-effect models. Due to the non-linear relationship of symptoms across the menstrual cycle, the day of the cycle was added to the model as a fixed effect and specified as a 3rd degree polynomial term. Firstly, sleep hours, sleep quality, and symptom severity were each modelled separately as the dependant variable. How interaction of sleep quality and day of the cycle were related to symptom severity was also explored, by including both terms (and their interaction) as fixed effects. In all models, participant was specified as a random intercept, to account for repeated measurements within participants. To aid interpretation of all models, estimated marginal means were produced for every 5-day interval across the cycle. Model fit was reported as marginal R^2, which described the proportion of variance explained by the fixed effects alone, and conditional R^2, which described the proportion of variance explained by both the fixed and the random effects. The model ICC was reported to describe the proportion of variance that was accounted for by the participant (clustering). All models were fit using the *lme4* package, estimated means were produced using the *emmeans* package, and model

diagnostics were visually checked using the *performance* package. Mean (±SD) are reported for descriptive data.

4.4 Results

4.4.1 Participants

Of the 30 participants recruited, four were excluded due to having a prior head injury, two exited the study after contracting long COVID, two exited the study due to the time commitment, one exited the study due to falling pregnant, five were excluded due to being on contraceptives and eight attended the introduction session but did not collect any data. As a result, only eight eligible females completed the study. Participants were 39.4 ±5.8 years old, 170 ±3.7 cm tall, 64.75 ±6.6 kg and had BMI 22.4 ±5.8. Seven participants (87.5%) were over age of 15 at menarche. No participants were smokers or had a history of smoking. Seven participants were tier-one athletes and one participant was a tier-two athlete on the participant classification framework [63]. Mean adherence and engagement were 80% and 1.7 respectively. Based on the LEAF_Q scores, no study participants met the criteria for being 'at risk' of LEA, therefore the LEAF_Q data were not included in any further analysis (Table 4.1). Thirty-one menstrual cycles were included in analyses from April 2022 to March 2023 from eight participants. In addition, the symptom data provided by one of the participants was incomplete and only covered the days of menses. These data were included in the study analyses because the participant did provide sleep data across the menstrual cycle and the symptom reporting was consistent (i.e., only reported on the days of bleeding).

Table 4.1: Individual results of menstrual cycle characteristics, LEAF-Q scores, self-reported sleep characteristics, symptom score and top-rated symptoms by mean (±SD).

Participant	1	2	3	4	5	6	7	8
Menstrual cycle characteristics								
Mean cycle length in days (±SD)	23.8(2.8)	27.0(1.7)	36.0(5.2)	27.0(0.0)	29.5(1.7)	17.5(6.9)	26.8(1.1)	25.75(1.0)
No. of cycles	5	3	3	3	4	4	5	4
Adherence (%)	100	100	92	40	98	94	39	75
Engagement*	1.5	1.5	0	0	2	5	2.5	1
LEAF_Q scores								
Injury	2	3	0-3	0	0	0	5	3-5
Menstrual function	1	0-1	1-2	0	0-1	2-3	0	1-3
Total	4	4-7	2-6	0	0-1	2-5	5-6	4-9[1]
Self-reported sleep characteristics								
Sleep hours Mean (±SD)	7.5(1.0)	7.5(1.4)	7.7(0.9)	8.1(1.0)	6.8(0.9)	8.0(0.8)	5.3(1.7)	7.6(1.1)
Min	4.5	5.5	3.5	5.4	4.0	5.5	0.0	5.0
Max	10.4	10	9.9	10.1	9	11.1	8	10.6
Sleep quality Mean (±SD)	3.1(1.3)	4.2(1.0)	3.0(1.4)	3.6(0.7)	3.3 (1.4)	3.1(1.0)	2.7(0.8)	2.8(0.9)
Min	1	1	1	1	1	1	1	1
Max (1 lowest, 5 highest)	5	5	5	5	5	5	3	5
No. of nights	119	81	108	81	118	70	134	103
Symptom score								
Symptom score Mean (±SD)	2.9(2.8)	1.1(1.4)	7.4(4.7)	2.4(2.7)	1.8(2.7)	3.5(2.4)	3.0(3.0)	8.3(7.0)
min	0	0	0	1	0	0	1	0
max	13	8	19	14	22	14	15	35
Number of symptoms endorsed Mean (±SD)	0.9(0.9)	0.9(0.9)	6.1(3.4)	1.9(1.7)	1.2 (1.5)	1.9(1.5)	1.9(2.3)	4.3(2.7)
min	0	0	0	1	0	0	0	0
max	4	3	14	8	7	6	11	11
No. of days	119	81	108	81	118	70	134	103
Top rated symptoms for each participant								
1	Fatigue	Gas	Stress	Stress	Night sweats	Stress	Spotting	Stress
2	Insomnia	Fatigue	Fatigue	Fatigue	Bloating	Anxiety	Fatigue	Fatigue
3	Stress	Low back pain	Headache	Low back pain	Gas	Mood swings	Insomnia	Injury
4	Bloating	Cravings	Cramp	Headache	Stress	Cramps	Low back pain	Soreness
5	Mood Swings	Soreness	Soreness	Breast tenderness	Insomnia	Mood swings	Acne	Headache

SD = Standard deviation; 1 = LEAF-Q score ≥ 8 but did not have a combination with an injury score ≥ 2 and a menstrual cycle score ≥ 4 therefore not considered to be at risk for LEA; Symptom score - Max number of symptoms that could be endorsed = 34; Max symptom score possible = 170; Adherence – calculated as number of days completing the symptom report divided by number of days in the study [40]; *Engagement calculations - Max Cycle Length Difference (CLD) - median CLD (if greater than 10 = poor engagement) [41].

4.4.2 Symptomology

There were significant associations between symptom score and sleep quality ($p < 0.001$; $R^2 = 0.513$) and between sleep quality and menstrual cycle day ($p = 0.003$; $R^2 = 0.671$, see Table 4.2). Higher sleep quality was associated with a lower symptom score (see Figure 1). There was no significant interaction between sleep quality and day of the cycle on symptom score ($p = 0.279$; $R^2 = 0.531$, see Table 4.2).

Table 4.2. Linear mixed model results presented as a Type III ANOVA table for sleep hours, sleep quality, and symptom severity by day.

Outcome	Parameter	Sum of Squares	Mean Square	df	F	p=	R^2_{con}	R^2_{marg}	ICC
Sleep hours	Day[A]	3.5	1.2	3, 749	0.9	0.433	0.607	0.001	0.606
Sleep quality	Day[A]	17.6	5.9	3, 740	4.7	0.003	0.671	0.006	0.669
Symptom severity	Day[A]	709	237	3, 682	16.9	< 0.001	0.363	0.051	0.328
Symptom severity	Day[A]	366	366	1, 624	32.1	< 0.001	0.513	0.12	0.447
	Sleep quality	276	92	3, 361	8.4	< 0.001			
	Day[A] *Sleep quality	43.9	14.6	3, 630	1.28	0.279			

Note: Degrees of freedom estimated using Satterthwaite's approximation; A = Day entered into model as a 3rd degree polynomial term; R^2_{con} = Conditional $R^{2;}$ R^2_{marg} = Marginal R^2; ICC = Model intraclass correlation coefficient.

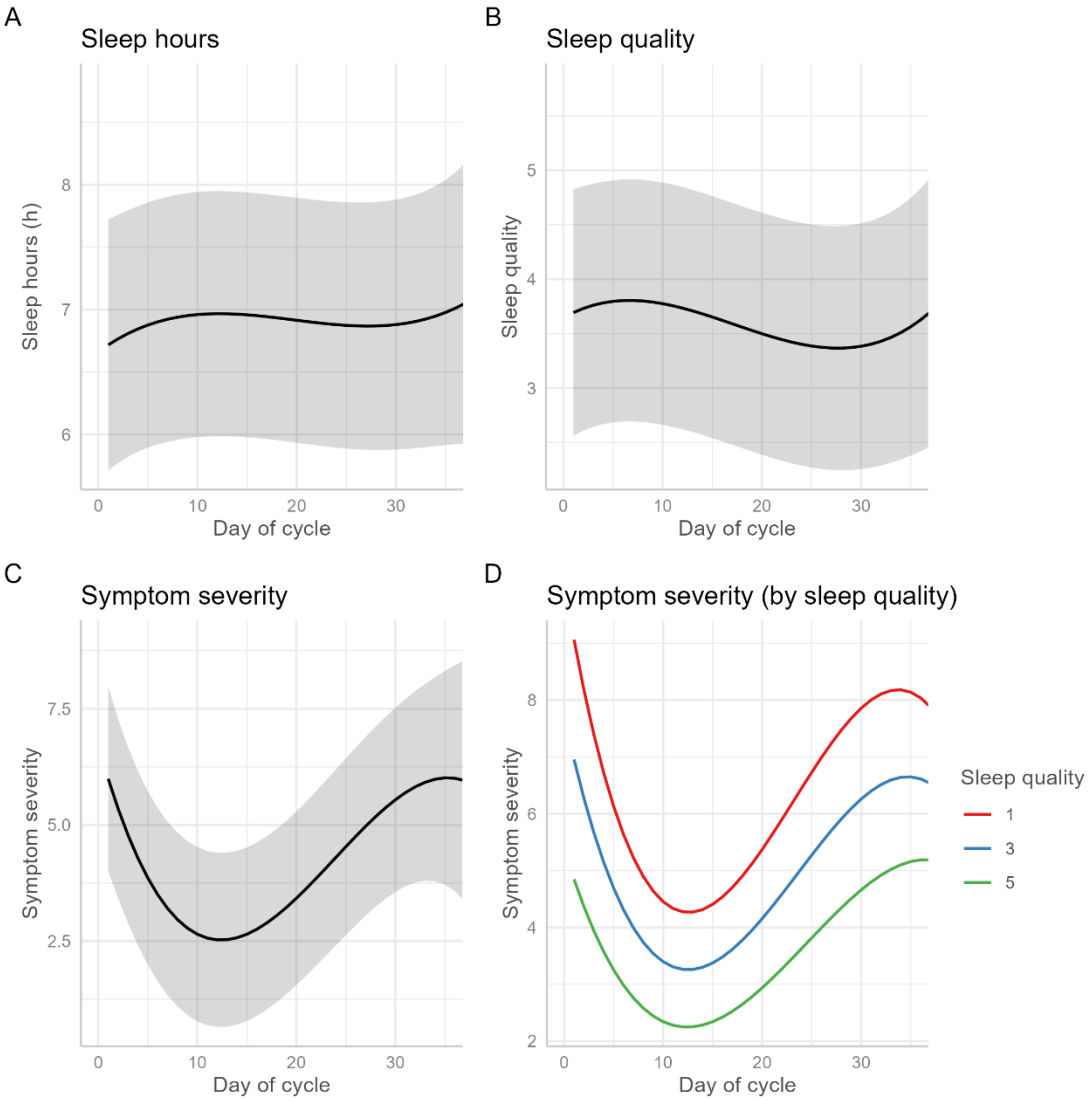

Figure 4.1: **Group level: (A) Sleep hours across days of menstrual cycle; (B) Sleep quality across days of the menstrual cycle; (C) Symptom score across days of the menstrual cycle; and (D) Symptom score by sleep quality across days of the menstrual cycle (1= poor, 3 = ok, 5 = great).**

There was a significant association between symptom score and menstrual cycle day ($F_{(3,682)} = 16.9$; $p < 0.001$; $R^2_{con} = 363$; see Table 4.2 and Figure 4.1C). Wide confidence intervals highlight variability between participants. Figure 4.2 presents symptom score across each individual menstrual cycle for each participant.

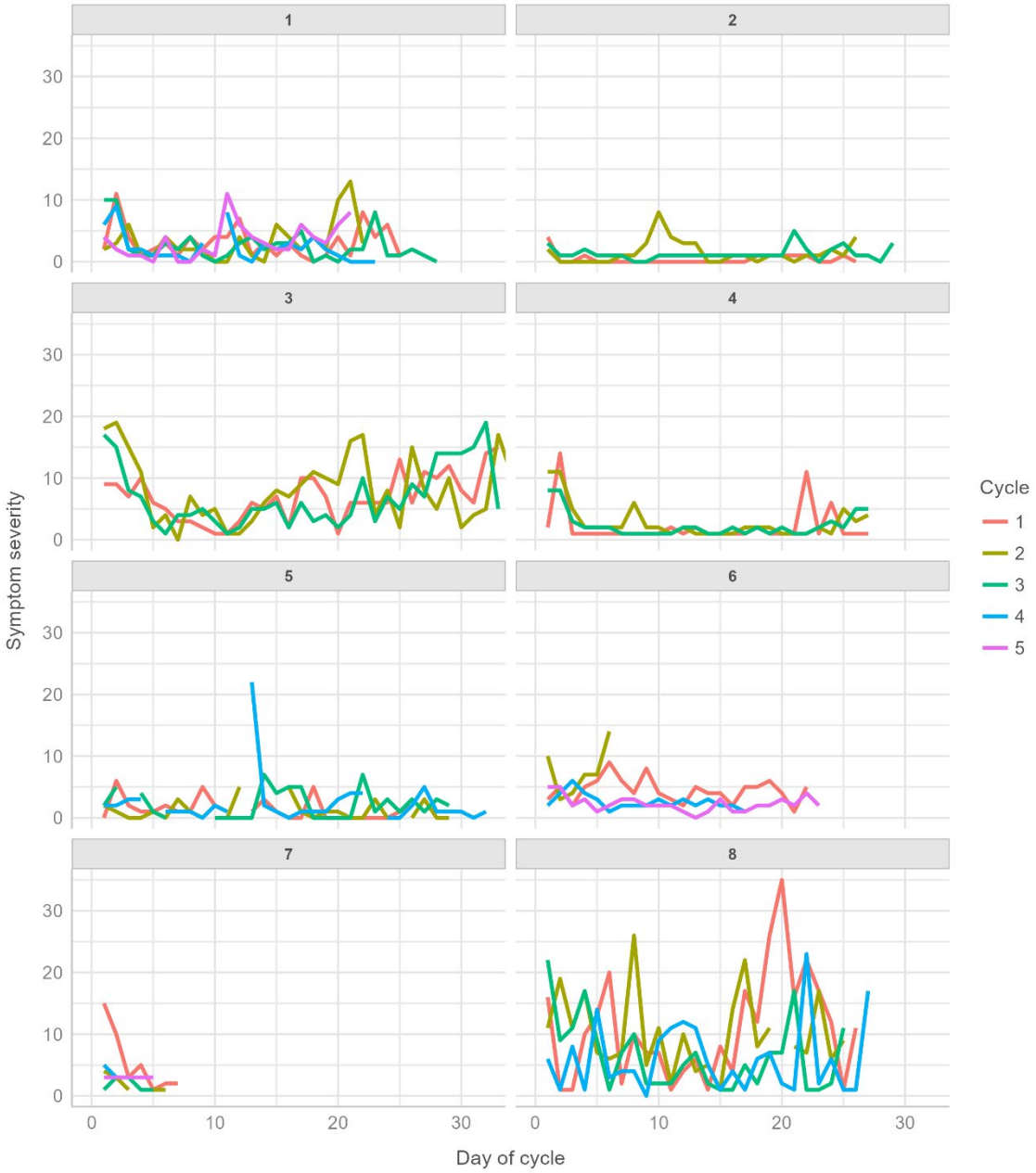

Figure 4.2: **Symptom score across days of the menstrual cycle for each individual menstrual cycle by each participant. Participant number 7 only reported symptoms in the app in the first 7 days of each cycle.**

4.5 Discussion

This observational study examined the daily changes in self-reported hormone related symptoms across the menstrual cycle. Data showed a meaningful association between menstrual cycle day and symptom score with the greatest magnitude of change in day-to-day symptom score observed over the duration of this study being 22 points. The magnitude of change in symptom score across the whole menstrual cycle ranged from eight to 35 points. Data highlighted that symptom score fluctuated across the menstrual cycle and the individual results underscored multiple peaks and troughs in the number and intensity of

symptoms experienced between and within females. Symptoms associated with the menstrual cycle are non-specific and crossover with some clinical concerns including concussion (see Table 4.3).

Table 4.3: Congruent symptoms as reported in literature for concussion [370], menstrual cycle [37].

Symptom	Sports Related Concussion	Menstrual cycle (as captured in WILD AI)
Physical symptom cluster		
Headaches	x	x
Fatigue or low energy	x	x
Nausea or vomiting	x	x
Pressure in head	x	x
Neck pain	x	x
Sensitivity to light	x	x
"Don't feel right"	x	x
Dizziness	x	x
Feeling slowed down	x	
Sensitivity to noise	x	
Balance problems	x	
Blurred vision	x	
Cognitive symptom cluster		
Feeling like "in a fog"	x	x
Confusion	x	
Difficulty concentrating	x	
Difficulty remembering	x	
Emotional symptom cluster		
Irritability	x	x
Depressive mood	x	x
More emotional	x	x
Nervous/Anxious	x	x
Sadness	x	x
Sleep related symptom cluster		
Insomnia	x	x
Trouble falling asleep	x	x
Drowsiness	x	

The symptom crossover is of relevance when considering the observed gender difference in concussion symptomology [382]). It is evident during pre-season baseline testing [373, 375, 383-385] and post-injury evaluation, that females endorse a higher number and greater severity of symptoms than males [316, 386]. This has been attributed to the hormonal fluctuations occurring throughout the menstrual cycle mediating female athletes' experience and reporting of symptoms [386]. Data in the current study support this hypothesis. The individual results showed a degree of consistency within females, and the individual peaks and troughs followed a similar pattern across each individual's menstrual cycle. Fatigue, headache, mood swings and insomnia featured in the five most reported symptoms in this study cohort in the absence of injury. Other physical symptoms that are not associated with concussion including bloating, gas, cramping and breast tenderness also featured in commonly reported symptoms. Table 3 highlights that, 'feeling slowed down',

'sensitivity to noise', 'balance problems', 'blurred vision' and 'drowsiness' are the symptoms that are more specific to concussion and may be key symptoms to monitor in females following concussion.

The American Congress of Rehabilitation Medicine (ACRM) consensus outlines six diagnostic criteria for mild traumatic brain injury [387]. For a diagnosis of concussion, a plausible mechanism of injury combined with one of the two following criteria must be met; one or more clinical signs, or at least two acute symptoms and one clinical finding and *confounding factors do not fully account for the clinical signs, acute symptoms, and clinical examination findings.* Given the day-to-day change in hormone related symptoms shown in the current study, this could be a confounding factor in the clinical evaluation of concussion. It is important to consider this further as symptoms remain the most consistent predictor of prolonged concussion recovery, are an integral part of monitoring progress overtime [371] and are used to inform return-to-activity decisions [370].

A recent evidence review [13] of three international concussion consensus and position statements concluded that female athletes were notably under-represented in studies and data informing clinical guidance for concussion. Interaction of cyclical hormone related symptoms and severity and duration of concussion symptoms remains unclear. Information about menstrual cycle characteristics and related symptoms could be an important constituent in the overall clinical picture when managing females with concussion and should be considered in future research and in clinical practice. These baseline fluctuations may be relevant to interpretation of clinical symptom assessments.

Self-reported sleep characteristics were considered as covariates in the current study. Total sleep hours did not change meaningfully across the cycle, but higher self-reported sleep quality did show a statistically significant association with lower symptom score. The direction of this relationship could not be determined from this analysis (i.e. does poor sleep quality increase symptoms or do symptoms decrease sleep quality) however it was consistent with previous sleep research that highlighted sleep quality variability by menstrual cycle phase [381], and that sleep quality impacted on symptom experience in other clinical concerns [379]. Sleep disturbance and sleep related symptoms are also common following concussion and can impact on recovery outcomes [388]. Post-concussion sleep symptoms also differ by gender; women experience more sleep related symptoms [389] and greater disturbance to sleep architecture [390].

Information about typical menstrual cycle and sleep characteristics and variability could be helpful to clinicians when managing symptom based clinical concerns including concussion. Collectively, the current study and previous research [40, 41] highlight the potential value of mobile phone applications as a simple and accessible way for females to become familiar with individual menstrual cycle and sleep characteristics and patterns. This may be particularly useful for those athletes that participate in sports that are considered 'high risk' for concussion, to aid in clinical interpretation and monitoring of symptoms at baseline and during recovery.

4.5.1 Limitations

The small sample size limits generalisability of these findings. In addition, the symptom data provided by one of the participants that only covered the days of menses could bias their mean data towards the menses phase which may have altered the group level peak in the later phases of the menstrual cycle. However, it may also be plausible that this participant only experienced symptoms during menses, therefore this was a fair reflection of her symptom experience. This study considered data by menstrual cycle day rather than phase and did not include confirmation of ovulation; phase determination using urinary ovulation kits and/or basal body temperature (BBT) measurement which would have allowed detection of anovulatory cycles and a more accurate categorisation of data into follicular, ovulatory and luteal phases for further analysis. Collecting symptom data via the app left symptoms open to user interpretation; on an individual level this is reasonable and will reflect individual changes but at group level there may be inter-individual differences in interpretation of symptoms that limit results. Further, symptoms captured in the app are mostly negative; it would be valuable to capture positive symptoms (e.g. feeling more energized, happier mood) to provide a more complete understanding of baseline 'state'.

Although not necessarily a limitation, it is noteworthy that the mean age of females included in this study was 39 and although cycle length fell within the normal range there were within-female variations. Some females may have been experiencing more hormone fluctuations due to early perimenopause.

4.6 Conclusions

Baseline tracking of the menstrual cycle and related symptoms is useful for female athletes to identify individual patterns. Sleep quality is a noteworthy factor in self-reported symptom score. Improving sleep quality may be a key strategy for symptom management in females. Further research investigating interaction of menstrual cycle characteristics and concussion in female athletes pre, and post injury is warranted. App-based monitoring is an accessible option for clinicians and females to monitor symptom changes over time pre/post or in response to injury.

Chapters 4 and 5 linking section

The findings in chapter 4 demonstrated a substantial change in symptoms and sleep characteristics across the menstrual cycle in the absence of injury and indicated good compliance and adherence to daily app-based reporting. This was useful for practical implementation with female athletes but also demonstrated the feasibility of using app-based reporting for research studies, particularly those studies that require high frequency data collection and over an extended period.

Used in isolation, app-based or other self-report methods of data collection are, at best, a physiological proxy and need to be supported by objective measures. As previously discussed, (Chapter 1 – Female research methodology), phase verification and the timing of testing throughout the cycle are critical in menstrual cycle-based research questions, which includes a serum progesterone measure (>16pg/ml). This can be challenging for participants if multiple measurements are required as this comes with additional time and logistical cost and, for some participants the discomfort associated with the venepuncture procedure presents a barrier. Additionally, this is only a single point in time across the menstrual cycle. In some research questions monitoring of sex hormone concentrations at multiple points would be beneficial to identify patterns, ratios and the rate of daily change across the entire menstrual cycle. It would therefore be valuable to have a reliable non-invasive measure of hormones. The second part of this study compared salivary hormone concentrations against blood-based measures to test the potential for salivary measures to be used as a reliable part of the suite of data collection tools that support monitoring of hormone levels over multiple timepoints. The participants in the chapter 4 and 5 study collected saliva samples daily. However, only two of those samples from different time points in the menstrual cycle from each of eight participants were paired with a blood sample for analysis in chapter 5. The remaining samples were processed and are visually presented in the supplementary material for chapter 5.

Chapter 5. Validity study: Association between salivary and blood hormone concentrations using an automated ECLIA technique – challenges and pitfalls.

This chapter has been published as: Hardaker, N.J., Hume, P.A., Sims, S.T., Stewart, T., King, DA. Association between salivary and blood hormone concentrations using an automated ECLIA technique – challenges and pitfalls. Short communication. Experimental Physiology http://doi.org/10.1113/EP092542

5.1 Overview

Background: Blood-based measures are considered gold standard for evaluating steroid hormone concentrations, particularly for confirmation of hormone profiles in female-specific research. However, saliva sampling offers several advantages: it can be collected independently by participants, is less invasive, and may be a more time and cost-effective solution. **Aim:** This preliminary study explored associations between salivary and blood plasma hormone (estrogen, progesterone and cortisol) concentrations using an automated Electrochemoluminesence Immunoassay (ECLIA) technique. **Method:** Eight healthy, physically active females with a regular natural menstrual cycle provided: a) daily app-based menstrual tracking data; b) daily saliva samples and; c) two blood samples, each at different time points in the third to fifth menstrual cycle estimated using the backward calculation method. Associations between saliva and blood hormone concentrations were analysed using repeated measures correlations (rmcorr). **Results:** Progesterone and estrogen showed positive associations between blood plasma and salivary measures; (rm=0.996, p<0.0001 and rm=0.705, p=0.0507 respectively). Weak non-significant associations for cortisol (rm=0.245, p=0.526) were found. **Conclusion:** With further work to validate the assay and develop salivary reference ranges, it may be possible to use the ECLIA technique for the quantitative determination of progesterone and estrogen in saliva and have application in research for within participant monitoring of changes overtime.

ABBREVIATIONS:

pmol/L=picomols per litre

pg/mL=picograms per litre

nmol/L=nanomols per litre

ng/mL=nanograms per litre

µg/dL=micrograms per decilitre

kg/m2=kilograms per metre squared

5.2 Introduction

There is a data gap in sports science and biomedical research; females have either been excluded from studies or have been included without accounting for sex in the research design or data analysis [12]. Data pertaining to the influence of hormone profiles on female athletic performance, injury risk, and health outcomes are limited, often inconclusive and conflicting across different study designs [391]. Protocols to enhance the quality of female-specific research design, particularly related to the menstrual cycle, have been outlined [54]. The menstrual cycle can vary within and between females [35], making it crucial to confirm menstrual cycle phase on the day of testing with physiological markers. For accuracy, phase verification includes a combination of the calendar-based counting method with urinary testing for the Luteinising Hormone (LH) surge, and blood-based progesterone measurement to confirm ovulation measured as >16 Nmol/L [54].

Given the pulsatile regulation and daily fluctuations of female sex hormones, it may be necessary to measure estrogen (estradiol) and progesterone levels over multiple time points to monitor hormonal patterns. Daily blood sampling, or data collection, over multiple menstrual cycles have time and logistical cost for participants and financial cost for researchers. An optimal protocol should yield good participant engagement and compliance with sample collection and provide ac-curate data relating to the female's hormone profile. Saliva sampling could offer a non-invasive means of hormone quantification that can be collected independently by the participant outside of the laboratory setting. The samples re-quire minimal processing prior to laboratory analysis. The limiting factor with saliva is that hormone concentration is much lower (only 1% to 5%) than in serum making it difficult to detect [392]. Liquid chromatography-tandem mass spectrometry (LC–MS/MS) is considered the "gold standard" for steroid hormone determination in blood-serum when compared with immuno-based clinical methods, which are often utilised due to lower costs [393].

Enzyme-linked immuno-sorbent assays (ELISA) are commonly used to measure sex hormones in blood and saliva for research and diagnostic purposes [392]. ECLIA has demonstrated similar or greater specificity, sensitivity and reliability when compared to other assays in the detection of steroid hormones in serum [394, 395] and urine [395]. Cortisol has been validated with reference ranges of 166 to 507 nmol/L for serological assays and 2 to 22 nmol/L for salivary assays [396]. ECLIAs for measuring estradiol and progesterone have not yet been proven in saliva.

The study aimed to determine the association between saliva and blood plasma measures of estradiol, progesterone, and cortisol in healthy, physically active females using an automated ECLIA technique. The findings from this exploratory study were intended to inform whether this protocol may be used in future female-specific research that requires measurement of hormones over multiple timepoints.

5.3 Methods

A prospective observational cohort study was undertaken. The data presented in this short communication are part of a broader exploratory study of 538 saliva samples across 31 menstrual cycles from April 2022 to March 2023, designed to test and refine the data collection protocol for future research. The small sample size of eight participants was chosen to provide insights and help ensure the robustness and effectiveness of the methodology for larger-scale studies.

5.3.1 Ethics approval

Written informed consent was obtained from all participants. The research was conducted in accordance with the Declaration of Helsinki except for registration in a database, and ethical approval was obtained from the Auckland University of Technology Ethics Committee (AUTEC #21/167).

5.3.2 Participants

Participants were recruited through the university postgraduate research school, workplaces, gyms, sports clubs, and WILD AI (Wild Technologies AI Limited, London UK) social media platforms/promotion. Eight healthy, physically active females with a regular, natural menstrual cycle (21–35 days) between 16 and 50 years of age consented to take part in this study. As the study examined hormones across the menstrual cycle, several inclusion/exclusion criteria were utilised to ensure other exogenous and endogenous factors did not influence the endocrine system during the 12 weeks prior to study participation. Inclusion criteria were: (1) Female aged 16 years or older (and had a menstrual cycle for a minimum of two years); and (2) Had a natural regular menstrual cycle (self-reported as 21–35 days). Exclusion criteria included: (1) Unable to attend the laboratory to provide saliva and blood samples; (2) Commenced any medication in the last 12 weeks that could alter reproductive hormone concentrations (glucocorticoids, e.g., prednisone; antidepressant; or antipsychotic medications); (3) Thyroid disorder and (3) Current or previous brain injury.

Participants attended a 15-minute introduction session with the lead researcher (NH), where they received detailed instructions on data collection protocols and had the opportunity to ask questions.

5.3.3 Measures

Daily menstrual cycle data were gathered for five complete cycles and the Low Energy in Females Questionnaire (LEAF_Q) was administered weekly. Blood and saliva samples were collected simultaneously at two different time points during the third to fifth menstrual cycles to assess the method's sensitivity in detecting differing hormone concentrations. Study participation concluded upon collection of the second set of samples. The two blood and saliva samples were obtained in the lab, while all other data were collected by the participant at home.

5.3.3.1 Low Energy Availability in Females Questionnaire

The LEAF-Q was designed and validated to evaluate the physiological symptoms (e.g., fatigue, irritability) of insufficient energy intake [47]. The LEAF-Q consists of 25 items rated on a Likert scale across three categories; (1) Sports injuries; (2) Gastrointestinal problems; and (3) Menstrual function (supplementary material). The LEAF-Q was administered via the online data management platform REDCap (Research Electronic Data Capture; https://www.project-redcap.org) (Harris, et al. 2009). A unique link to the LEAF_Q was emailed to participants every seven days. A total LEAF-Q score ≥8 in combination with an injury score ≥2 and/or menstrual dysfunction score ≥4 was considered as having symptoms of Low Energy Availability (LEA) (Karlsson 2023) (scoring key provided in supplementary material). LEA is prevalent in 45% of active females [53] and is known to disrupt hormones, and these effects can occur in as little as four days [46]. Given the length of the study, the LEAF_Q was administered weekly to monitor LEA as a variable that could impact on hormone profiles. Information from the LEAF_Q was also used to calculate Body Mass Index (BMI kg/m2).

5.3.3.2 WILD AI - Menstrual cycle tracking

'WILD AI' (WILD.AI technologies limited) is a free to download menstrual cycle tracking app with artificial intelligence (AI). Participants downloaded 'WILD AI', created a profile, and then digitally consented to link their profile using a unique study code to the WILD AI research platform accessible by the lead researcher (NH). The data collected in WILD AI were used to estimate the timing of the expected hormone fluctuations using the backward calculation phase projection method [55], this determined the timing of blood and saliva sample collection. Adherence to the menstrual tracking protocol was calculated by dividing the number of completion days by the total number of monitoring days [40]. Engagement with the app was determined by cycle length difference (CLD) between consecutive cycles within each female. If the difference between the median and maximum CLD was greater than 10 this was considered low engagement [41] and may impact on result accuracy.

Cortisol follows a diurnal rhythm, in concordance with peak morning secretion, [396, 397], on the specified days, participants attended the clinical research laboratory between 6 and 9 am in a fasted state to provide the blood and saliva samples.

5.3.3.3 Blood and Saliva sample collection

Participants provided a 2 ml saliva sample via passive drool (supplementary material) in a sterile cryotube (Thermo Scientific, Nunc, Biobanking and Cell Culture Cryogenic Tubes). Venous blood (~5 ml) was collected into a BD Vacutainer Tube SST II Advance (gold) via standard venipuncture procedures. Blood samples were centrifuged (1,300 x g) at room temperature (~21oC) for 10 minutes. Plasma (~1.5 ml) was aliquoted into two Eppendorf tubes. All samples were clearly labelled with (1) the unique ID number, (2) the date and (3) the exact time of the sample (e.g., F001_070223_06:25) and stored at -80oC within 30 minutes of collection for subsequent analysis.

5.3.4 Transfer of samples

There were two data collection sites (Auckland and Wellington, New Zealand). Samples collected and stored in Wellington were transported to the AUT Roche laboratory by air freight on dry ice one day prior to analysis. All blood and saliva analyses were conducted in the AUT Roche laboratory in Auckland, New Zealand.

5.3.5 Blood and saliva sample analysis

Estradiol and progesterone were prioritised for evaluation and analysed simultaneously from the same sub-sample. Cortisol was measured in a second sub-sample that resulted in cortisol undergoing two freeze-thaw cycles. All samples were brought to room temperature overnight the day prior to analysis. Processing of saliva samples involved 500 µL of each sample being aliquoted into an Eppendorf tube and centrifuged (1,500 x *g*) for five minutes until the supernatant was clear. The plasma and saliva samples were then transferred to Assay Cups (Hitachi, Japan) prelabelled with a unique barcode assigned to each participant. Levels of estradiol, progesterone and cortisol were measured in the plasma and saliva samples using Electrochemiluminescent immunoassay (ECLIA) in the COBAS e801 analyser (Roche Diagnostics) with Estradiol III, Progesterone III (third-generation, monoclonal antibody) kits and Cortisol II (second-generation, monoclonal antibody) kit, respectively. All kits were sourced from Roche Diagnostics (NZ) and standardised before use. An external quality control was run with every batch. The duration of the automated assay was 30 min.

The quantification limits as per the manufacturer were: estradiol (18.4 to 11010 pmol/L), progesterone (0.159 to 191 nmol/L), and cortisol (0.054 to 63.4 µg/dL) [398]. These values were derived from plasma for estradiol and progesterone, and from both plasma and saliva for cortisol. All values were reported in nanomoles per litre (nmol/L), where one nmol equals 1000 pmol.

5.3.6 Data analysis

Data analyses were performed in R (version 4.3.0). To examine the association between blood plasma and saliva measures of estradiol, progesterone and cortisol (separately), a repeated measures correlation was utilised to account for repeated measurements per participant (i.e., two blood tests each with a corresponding saliva sample). The repeated measures correlation is a statistical technique for determining the common within-individual association for paired measures assessed on two or more occasions for multiple individuals and does not violate the assumption of independence [399]. The *rmcorr* R package was used to perform this analysis. Data were reported as correlation coefficients (r_m), and a p-value of <0.05 was considered statistically significant.

5.4. Results

5.4.1 Participants

Of the eight participants who completed the study (Table 1) seven (87.5%) were over the age of 15 at menarche. Seven participants were classified as tier-one athletes, and one was classified as tier-two [63]. Based upon the LEAF_Q scores, no study participants met the criteria for being 'at risk' of LEA, and this did not change over the study. There was good average adherence and engagement from all participants who completed the study. Sixteen saliva samples and 16 plasma-matched samples were analysed (i.e., two blood and two saliva samples per participant).

Table 5.1: Participants age, height, weight, calculated body mass index, reported exercise, menstrual cycle length and LEAF_Q score by mean with standard deviation and range of reported characteristics.

Characteristic for 8 participants	Mean ±SD	Range
Age (years)	39.4 ±5.8	32 to 47
Height (cm)	170.0 ±3.7	165 to 176
Weight (kg)	64.8 ±6.6	58 to 79
BMI (kg/m^2)	22.4 ±2.5	20 to 27
Exercise (hours/week)	6.6 ±3.4	2.5 to 11
Menstrual cycle length (days)	26.9 ±4.4	17 to 42
Adherence	79.8 ±26.1	39 to 100
Engagement	1.6 ±1.7	0 to 5
LEAF_Q score	3.4 ±2.4	0 to 9

SD=Standard Deviation; yrs=years; cm=centimetres; Adherence – calculated as number of days completing the symptom report divided by number of days in the study [40]; *Engagement calculations - Max Cycle Length Difference (CLD) - median CLD (if greater than 10=poor engagement) [41].

5.4.2 Correlation of blood plasma and salivary measures

The plasma hormone concentration for the eight participants were within normal ranges. In comparing the 16 saliva samples with the 16 matched blood plasma samples, there was a non-significant weak correlation for cortisol (rm=0.245, p=0.526, see Figure 1A). There were strong positive correlations between blood plasma and salivary measures of progesterone (rm=0.966, p=<0.0001, Figure 1C) and estradiol (rm=0.705, p=0.0507, Figure 1B). The rmcorr coefficient (rm) represents the strength of the shared intra-individual association between blood plasma and salivary measures. The parallel lines represent the line of best fit (or not) between each participants individual data points with the common regression slope. The degree to which each participant's data was reflected by the common slope of the best-fit parallel lines was appropriately represented in the rmcorr effect size. When the relationship between variables varies widely across subjects, the rmcorr effect size will be near zero with confidence intervals also around zero. When

there is no strong heterogeneity across subjects and parallel lines provide a good fit, the rmcorr effect size will be large, with tight confidence intervals.

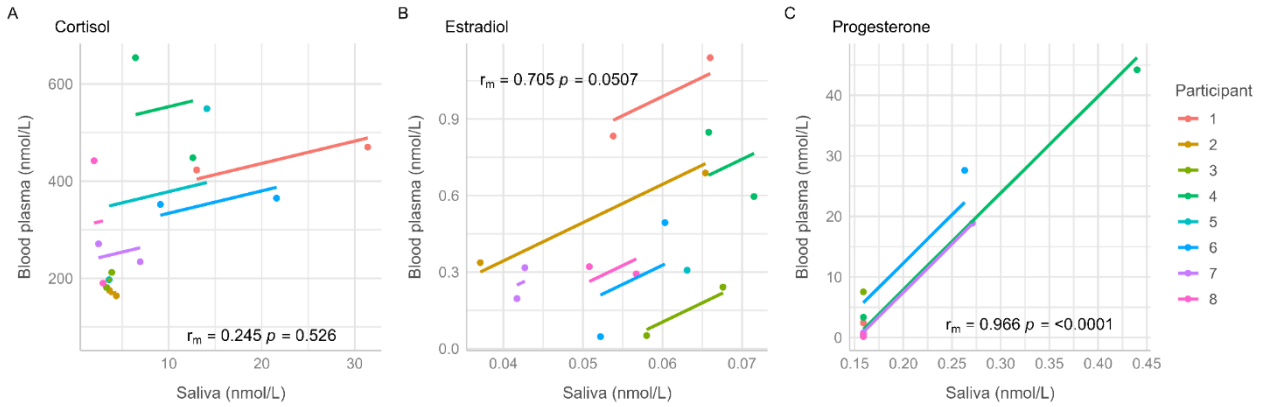

Figure 5.1: Repeated measures correlation plots showing the association between saliva and blood measures of A) cortisol, B) estradiol and C) progesterone, in n=8 female athletes.

5.5 Discussion

The findings of this study were encouraging. Salivary progesterone showed a strong positive correlation with blood plasma measures. This was consistent with other work [392] highlighting that, although salivary immunoassays for evaluation of progesterone need further validation, there is value in further refining these methods. The weaker correlation observed between measures of serum and salivary oestradiol in this study was consistent with a previous study [392]. The concentration of oestradiol in saliva is only approximately 2–5% of that measured in serum and the lowest levels of oestradiol concentration are lower than the lowest levels of progesterone across the menstrual cycle [35], therefore the lower analyte concentrations are harder to detect and require a higher specificity of the assay to minimize interference from other substances in the sample. There are well documented challenges with using immunoassays to measure hormone concentrations even in serum. This is particularly evident in low oestradiol states (e.g., follicular phase, puberty, in males) [400]. Studies investigating the predictive value of salivary or serum oestradiol have highlighted a low correlation and a likely upward bias of oestradiol concentration in the follicular (low oestrogen) phase of the menstrual cycle [401].

It was anticipated that there would be a stronger correlation between plasma and salivary cortisol measures given that the assay has been validated for use in both fluids, and given salivary cortisol is widely utilised for research and diagnostic purposes. In a meta-analysis of previous studies in adults employing immunoassays, higher correlations between serum and saliva have been reported (r=0.43 to r=0.82) when evaluated using Pearson's correlation or Spearman's rho [402]. The lower correlation value (r_m=0.245, p = 0.526) in the current study is likely due to limited power rather than a flaw in the repeated measures statistical approach, which is an important component of menstrual cycle related research [403]. Exploratory

Pearson correlations on averaged trials (r = 0.61, p = 0.1055) and first trials (r = 0.387, p = 0.343) of data in this study suggest a moderate relationship, with non-significance, further supporting this point. Previous studies employed different techniques including radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) [402] which may contribute to the different correlation values recorded. There is also limited research in females [404] suggesting that there are inter-individual differences and there may be high and low 'secretors' of salivary progesterone and cortisol. The concentration of these hormones in saliva differs after correcting for blood hormone values.

5.5.1 Benefits of the ECLIA compared with other assay techniques

Although other methods of evaluating hormone levels exist, many of them are serum based [392, 393]. There are practical advantages of Electro-chemiluminescence immunoassays (ECLIA). These include the wider linear range which allows for single-dilution measurements of concentration, a smaller sample volume requirement for analysis, and the automated multiplex testing platforms that support higher throughput which is more efficient when processing large numbers of samples [394]. ECLIA has also shown higher sensitivity and specificity than ELISA, making it potentially more suitable for detecting low concentrations of analyte [394, 395]. All these features are particularly beneficial for 'in-field' research methodologies; the ability to collect multiple samples over time allows for real-time monitoring of hormone fluctuations and participants can self-collect samples without the need to visit a laboratory.

5.5.2 Challenges and Pitfalls

Menstrual cycle phase was estimated using the backward calculation phase projection method [55]. The use of this method limits the accuracy of phase determination and does not allow confirmation of ovulation. Although participants in this study were provided with careful instructions around sample collection and storage, there was no direct oversight of this from the research team. Poor sample collection can impact on the quality of the sample. A further challenge to sample quality is salivary flow rate (calculated by dividing volume of sample by time taken to obtain in min; mL/min) which has been inversely associated with total protein concentration [405]. Both of these factors can affect the measurement of some target analyte concentrations [406, 407]. Due to the self-collect protocol employed in this study, and the fact that previous work has indicated that flow rate and total protein concentration does not affect steroid hormone measurement in saliva, flow rate was not measured. Cortisol was analysed from a sub-sample that had undergone a second freeze-thaw cycle, as a result, the quality of both samples may have been compromised or, given that salivary cortisol has shown to be stable for up to four freeze-thaw cycles [408] and that plasma may be more vulnerable to freeze-thaw cycles [409], it could be that saliva and serum responded differently to the second freeze-thaw cycle. To minimise the risk of this occurring, it is recommended that, where possible, at the point of collection the samples are aliquoted prior to freezing. Although this was intended as an exploratory study, the small sample size and low number of paired samples bought a degree of uncertainty, and the findings should be interpreted cautiously.

5.6 Conclusions

Analysis of saliva samples using an automated ECLIA technique detected changes in progesterone concentration across the menstrual cycle and showed strong association with serum measures. In a research context, salivary progesterone has the potential to support monitoring of changes and fluctuations in sex hormones. This could improve applied and in-field methodologies when blood sampling is not feasible, or high frequency sampling is required and increases participant burden. There is value in further work with larger studies of female participants to refine and validate this technique for female specific research so that reliable, relevant measures are accessible to participants and can easily be adhered to.

5.6.1 New Findings

- What is the central question of this study?
  Do salivary steroid hormone measures correlate with blood-plasma measures using an automated ECLIA technique?
- What is the main finding and its importance?
  In this exploratory analysis salivary progesterone showed a strong positive association with blood plasma measures and has potential to support monitoring of fluctuations in this hormone.

Chapters 5 and 6 linking section

The findings from chapters 4 and 5 were used to inform the design of the studies reported in chapters 6-8.

Chapter 5 confirmed the acceptability of daily self-collection and storage of saliva samples. However, chapter 5 has shown that further work needs to be done before the automated ECLIA technique can be reliably used to measure salivary hormone concentrations and should not be used for phase verification or confirmation of ovulation. As such, measures of salivary hormones were not used any further in this thesis. In addition, since the data in chapter 5 were collected another study [410] comparing blood and salivary measures of progesterone using an ELISA has been published and concluded that salivary measurements of progesterone are well correlated with capillary blood when taken during eumenorrheic menstrual cycles, however in females with menstrual irregularities including oligomenorrhea anovulation and amenorrhea there was either a weak correlation or no correlation between blood and salivary measures.

Chapter 4 also showed that that baseline symptomology in naturally cycling females in the absence of injury changes at a magnitude that could make symptom monitoring ambiguous in females with concussion, yet health practitioners rely on subjective symptom reports combined with balance measures and neurocognitive testing to diagnose and manage concussion and inform return to play decisions [370, 411]. These assessments can be influenced by the athletes to hide the injury and avoid being removed from play or to get clearance for return to play before they are fully recovered. The possible consequences of this non-disclosure include prolonged symptoms, delayed recovery or a second more severe injury before the brain has had time to fully recover. Conversely, if symptoms are mis-attributed to concussion this could be detrimental to the person's recovery and could mean that do not receive the treatment they need. Currently there is no widely accepted objective test to diagnose and monitor concussion, such a test could address the previously outlined challenges and reduce the associated risks. There are several candidate biomarkers that show potential.

Biomarkers are biological molecules (molecular marker or signature molecule) found in blood, other body fluids, or tissues that are a sign of a normal or abnormal process, or of a condition or disease. Biomarkers are by definition, objective, quantifiable characteristics of biological processes [412]. A concussion specific biomarker would have research and clinical utility. There has been increased interest and application of salivary biomarkers as reliable diagnostic marker of concussion [413-416]. Research related to concussion biomarkers shows a similar gender bias to other fields of research; there is an under-representation of female participants or when females are included there has been no consideration of hormone profile. It is not yet known if hormone profile alters the pattern or expression of concussion biomarkers in the absence of injury or following the injury. It is important to consider this from the outset to ensure accurate interpretation.

Having confirmed that a daily data collection protocol can be done by the participant from home including saliva sampling and storage, this approach was built into Chapter 6 to monitor salivary biomarkers across the menstrual cycle. The Chapter 6 study was intended to be a matched-case controlled design in a women's community football club whereby data were collected by four teams from the start of the season and then if a concussion occurred, that player would be matched with a control player. However, this study was planned to start over the first full season in the post-COVID environment and there were substantial recruitment challenges. Players were either using some form of contraception, which reduced the number of potentially eligible participants, or they were reluctant to engage in the research due to the length of commitment required. As such, this became a single-case time series observational study to monitor salivary concussion biomarkers in a female football (soccer) player for three consecutive menstrual cycles during the season.

Chapter 6. Concussion biomarkers across the menstrual cycle in an un-injured football player: A single-case time series observational study.

This chapter has been submitted and is under review as:

Hardaker, N.J., Hume, P.A., Sims, S.T., King, D.K., Basu, I. Concussion biomarkers across the menstrual cycle in an un-injured football player: A single-case time series observational study. Submitted to *Sports Health.*

6.1 Overview

Purpose: The aim of this exploratory study was to investigate whether concussion biomarkers changed across consecutive menstrual cycles in the absence of injury in a female football (soccer) player. **Method:** A female player self-collected: two daily saliva samples, app-based menstrual cycle and sleep characteristics, and a weekly low energy availability questionnaire (LEAF-Q). Saliva samples measured morning cortisol level and miR-27a-5p/miR-30a-3p. Data from three consecutive menstrual cycles (MC1, MC2, MC3) were analysed. Visual inspection was used to identify patterns, t-tests compared concussion biomarker mean values between menses and pre-menses phases, and Kendall's tau-b examined correlations between variables. **Results:** There was cyclical variation of miR-27a-5p/miR-30a-3p across MC1 (0.83 to 1.03), MC2 (0.83 to 1.09) and MC3 (0.73 to 1.08). MC3 was shorter and showed significantly (p=0.031) lower miR-27a-5p/miR-30a-3p in the pre-menses compared to the menses phase. Morning cortisol stayed within a normal reference range (2-22 nmol/L), MC1 mean 11.6 ±2.9, MC2 mean 11.2 ±5.3, MC3 mean 9.5 ±2.6) and showed a statistically significant (p=0.005) mean difference between menses and pre-menses phases. **Conclusion:** miR-27a-5p/miR-30a-3p may be impacted by sub-clinical menstrual disturbances. Larger studies in injured and un-injured females are needed to elucidate a clinically relevant threshold for miR-27a-5p/miR-30a-3p and must include reliable measures of hormone profile.

6.2 Introduction

Concussion results in a range of physical, vestibulo-ocular, cognitive, and emotional symptoms [371]. Currently there is no definitive marker or test for concussion diagnosis, and symptoms remain the primary predictor and a key measure of recovery over time [371, 417]. Many concussion symptoms are indistinguishable from those associated with cyclical sex hormone perturbations. Research has indicated that even when symptoms subside following concussion, some residual physiological and autonomic deficits may still be present [417-419]. In a sporting context, a reliable objective test for concussion may help convince athletes that they have sustained a brain injury and need appropriate recovery time. An objective test could also

reassure clinicians, athletes, coaches, and parents when it is safe to return to sport. A growing body of research investigating biomarkers to support concussion diagnosis, and monitor recovery includes serum protein-based biomarkers, serum and salivary hormones and micro ribonucleic acids (microRNAs or miRNAs) [415, 416, 420-428]. MicroRNAs (miRNAs) are small non-coding RNA molecules that play crucial roles in regulating gene expression.

A reliable salivary biomarker may offer wider applicability than a blood-based marker; sample collection is non-invasive, can be done outside of the clinical or laboratory settings and does not require any specialist training. Salivary miRNAs and cortisol have both shown potential as concussion biomarkers [416, 423]. MicroRNAs are ~22 nucleotides in length, act at the post-transcriptional level to regulate protein synthesis and are actively involved in neuronal injury and repair [413]. Over 2,000 miRNAs have been identified in humans, with an estimated 70% of these being expressed in the central nervous system (CNS) [429]. Different 'families' of miRNA's may be upregulated or downregulated in response to various conditions (e.g., post-exercise or injury) or disease states [430-432]. MicroRNAs are good candidate biomarkers due to their resistance to degradation, ability to cross the BBB into blood and saliva, [414] and their stability in saliva [413]. Forty-nine miRNAs have shown potential as biomarkers of concussion, 17 of which have been used consistently across multiple studies [433]. A systematic review [423] of concussion specific salivary miRNAs found that the ratio of 2 miRNA's (miR-27a-5p/miR-30a-3p) showed increased sensitivity and specificity in the acute and sub-acute phases of injury. The MiR ratio is important because it considers the relative balance between miRNAs within a cell which can impact gene regulation and cell signalling pathways, therefore providing a more reliable indicator of disruption to normal physiological processes, which can occur after a brain injury like concussion.

Salivary cortisol has been identified as a prognostic concussion biomarker in paediatric sports players [416]. Morning salivary cortisol is lower post injury when compared to pre-injury levels [416] and athletes with abnormally low cortisol following a concussive injury may be more at risk for increased symptom burden [415] and prolonged recovery [415, 416]. A change in cortisol levels following all severities of traumatic brain injury (TBI) has been reported in adult and paediatric populations [415, 416, 434, 435]. Cortisol has not yet been considered as a primary prognostic biomarker for concussion in female athletes. It might still play a role in understanding the broader physiological response to concussion, especially in relation to stress and recovery.

Current concussion biomarker studies report results on the assumption that these biomarkers are stable at baseline and follow the same pattern in females as they do in males. However, there are variables that can influence the presence of biomarkers. Notably, sex differences in miRNA expression are evident in other tissues [367, 436] potentially influenced by ovarian hormones [437]. Salivary morning cortisol levels can also be

affected by hormone profiles [428, 438]. Although previous studies on miRNA and cortisol as concussion biomarkers have included female participants, the numbers have been small, and the methodological approaches did not give any consideration to hormone profile. Understanding how these biomarkers vary with different hormone profiles in the absence of injury is important for establishing baseline patterns in female athletes and ensuring accurate interpretation of data when an injury does occur.

Therefore, this exploratory study aimed to examine the expression and ratio of concussion-specific miRNA (miR-27a-5p/miR-30a-3p) and morning cortisol across consecutive menstrual cycles during a competition season in a community (Tier-3) football player. The results from this research are intended to inform larger studies to advance our understanding of concussion biomarkers in female athletes. Daily collection of salivary concussion biomarkers was the priority in this study and for that reason, to minimise participant burden and maximise adherence to the daily data collection protocol, menstrual cycle phase estimation was conducted only using the backward calculation method [55, 403] and not the 'gold standard' basal body temperature (BBT), urinary testing of luteinizing hormone (LH) surge or serum measures of progesterone for confirmation of ovulation and phase verification [54].

6.3 Materials and methods

This single-case observational study was conducted according to the Declaration of Helsinki, with approval from the Health and Disability Ethics committee (HDEC #2021 EXP11904) and Auckland University of Technology Ethics Committee (AUTEC #21/135). The participant was recruited through a female community football team in Wellington, New Zealand and provided written informed consent. The player participated in the women's Capital Premier League competition administered by Capital Football (regional federation) that ran from 28th April 2023 to 3rd September 2023 (a total of 126 days) and consisted of 17 league and three cup competition matches. Data collection took place from day 24 through to the last game of the competition season and was undertaken to capture a minimum of three full menstrual cycles. Inclusion (>16 years old; natural regular menstrual cycle) and exclusion criteria were established to ensure other exogenous and endogenous factors did not influence the endocrine system during the 12 weeks prior to study participation. Exclusion criteria were: 1) Commenced medication in the last three months that could alter reproductive hormone concentrations (glucocorticoids – e.g., prednisone. Antidepressant or antipsychotic medication); 2) Unresolved musculoskeletal injury; 3) Clinical diagnosis of eating disorder; and 4) Using any form of contraception. There was a 15-minute introduction session with the lead researcher (NH) to provide more detailed instructions on the data collection protocols and to enable the participant to ask further questions.

6.3.1 Participant

The female participant had a natural regular menstrual cycle (self-reported to be between 21 and 35 days in length), was 41 yrs old at time of enrolment in the study (1.64 m, 73 kgs, BMI 27.1 kg/m2). The participant trained and competed up to 10 hours per week specifically in football and was classified as a tier-3 athlete [63]. There was one league or cup match per week, and this involved 90-minutes pregame preparation time including a 30-minute warm-up and then 70 to 90 minutes of competition participation time.

6.3.2 Measures and analysis

The participant received a data collection kit with everything required to complete the study including: sterile cryotubes to collect daily saliva samples; Pens to label cryotubes (1 black and 1 coloured to indicate first day of bleeding); instructions for the passive drool saliva collection method (supplementary material i); and instructions for menstrual cycle tracking in a mobile phone application. Data collection included saliva samples, menstrual cycle data and the low energy availability in females' questionnaire (LEAF_Q) [47]. All data were self-collected by the participant. Participation in the study ended after the last match of the 2023 season at which point data from three full menstrual cycles had been collected.

6.3.2.1 Saliva sample collection and analysis

Each day 2 separate 2 ml saliva samples were collected consecutively using a passive drool method (Supplementary material 5.1). The miRNA measurement sample was prioritised and were collected using SpeciMax stabilised saliva collection kits (Thermo Scientific SpeciMAX stabilised Saliva Collection Kit A50697, Life technologies Corp, Austin, Texas USA) prefilled with 1 ml of virus inactivating and nucleic stabilization solution and labelled with a unique barcode assigned to the collection date. The second sample for cortisol measurement was collected in a cryotube prelabelled with the participants unique identifier number and space to record sample collection time and date. Due to circadian rhythms, cortisol levels peak 10-30 minutes after waking up, typically between 7 and 10 am,[397] therefore saliva samples were collected shortly after waking. Both saliva samples were immediately stored in the participant's home freezer for up to four weeks before being transferred on dry ice to the laboratory for storage at −80°C.

For analysis, samples were taken from the −80°C freezer and left to thaw to room temperature of 22.0 ±.05°C. The miRNA targets from saliva were extracted using the MagMAX mirVana total RNA isolation kit (A27828, Thermo Fisher Scientific, US) following manufacturer's instructions with modification for saliva extraction. After isolation of total RNA (including snc/miRNA), the sample underwent polyadenylation, adapter ligation, reverse-transcription and miR-Amp reactions using the TaqMan™ Advanced miRNA cDNA

Synthesis Kit (A28007, Thermo Fisher Scientific, US) following manufacturer's instructions. Reactions were performed on the standard thermocycler BioRad. The cDNA was diluted, and real-time PCR runs for miRNA analysis were performed using the Taqman™ Fast Advanced Mastermix for qPCR (4444964, Thermo Fisher Scientific, US) on QuantStudio™ 7 Flex Real-Time PCR System (Thermo Fisher Scientific, US). Using TaqMan™ Assays for miR-27a-5p and miR-30a-3p (assays 002445 and 000416, Thermo Fisher Scientific, US) their respective expressions were calculated by noting their cycle threshold value. Endogenous miRNA, mir-16a-5p (Thermo Fisher Scientific, US) was used as the reference gene [439]. Each saliva specimen was run in replicates of 2. The calculation of miR-27a-5p/miR-16-5p divided by miR-30a-5p/miR-16-5p provided the normalized ratio for miR-27a-5p/miR-30a-3p.

For cortisol analysis, saliva samples were processed by aliquoting 500 µL into Eppendorf tubes and centrifuging at 1,500 relative centrifugal force (rcf) for 5 minutes until all the proteins had pelletised and the supernatant was clear. Samples were transferred to prelabelled Assay Cups (Hitachi, Japan). Cortisol levels were measured using electrochemiluminescent immunoassay (ECLIA) in the COBAS e801 analyser (Roche Diagnostics) with the Cortisol II (second-generation, monoclonal antibody) kit (Roche Diagnostics NZ). The assay was standardized with Roche calibration kits before use of every new reagent and an external quality control was run with every batch. The automated assay took 30 minutes. The upper and lower limits of quantification of Cortisol were 0.05 to 63.40 µg/dL. Hormone values were measured in nanomols per litre (nmol/L). The Cobas analyzer automatically calculated the analyte concentration and determined the units of measurement.

6.3.2.2 Menstrual cycle, sleep and symptom tracking

Menstrual cycle, sleep and symptom data (person-centred variables) were collected using a mobile menstrual tracking app (WILD AI Wild Technologies AI Limited, London, UK) designed specifically for physically active females. The user can create their own profile and select which symptoms or metrics to monitor daily. For this study, the training and nutrition advice/notifications were 'turned off' and the daily symptom and lifestyle check-in was standardised from an evidence informed pre-determined list (Supplementary Table 4.1). The participant logged the presence of symptoms and sleep characteristics (quality and duration) daily throughout the study. Symptoms were rated on a four-point Likert scale as "None (0), Low/mild (1); moderate/medium (3); or high/severe (5)" and a daily symptom score was calculated by summing the ratings for each endorsed symptom. Sleep quality was rated on a 3-point scale as "Poor (1); Ok (3); and Great (5)" and the score was calculated by multiplying the number of hours sleep by the quality rating. Adherence to the menstrual tracking protocol was calculated by dividing the number of days completed by the total number of days monitored [40]. The participant downloaded their data as a JSON file and shared with the lead researcher (NH).

6.3.2.3 Low Energy Availability in Females Questionnaire (LEAF_Q)

The Low Energy Availability in Females Questionnaire (LEAF-Q) was designed and validated to evaluate the physiological symptoms of insufficient energy intake [47]. The LEAF-Q consists of 25 items rated on a Likert scale across three categories; (1) Sports injuries; (2) Gastrointestinal problems; and (3) Menstrual function. There are three questions relating to menstrual function including recent menstrual history. The LEAF-Q was administered weekly via the online data management platform REDCap (Research Electronic Data Capture; https://www.project-redcap.org) [377, 440]. A unique link to the LEAF-Q was emailed to the participant on the first day of data collection. A total LEAF-Q score ≥8 in combination with an injury score ≥2 and/or menstrual dysfunction score ≥4 is considered to be having symptoms of Low Energy Availability (LEA). [378] The LEAF-Q score was collected weekly to give a more accurate indication of LEA status of the participant, i.e., presence or absence of LEA over time rather than a single point in time. LEA is known to impact the menstrual cycle [46, 48] and was therefore monitored as a potential confounder.

6.3.3 Data analyses

All statistical analyses were performed in JASP (Version 0.19). Descriptive statistics were used to characterise the menstrual cycle and to summarise the outcome variables. Data are reported as mean ±standard deviation (SD) for miR ratio, cortisol, symptoms and sleep score within each menstrual cycle. Raw outcome data (miR ratio and morning cortisol values) and person-centred data (symptom score and sleep score) were plotted as graphs using Microsoft Excel 2019 (Microsoft Corporation, Washington, USA) and visual inspected to look for patterns across the menstrual cycle. Visual inspection considered the level and variability of any observed patterns in the data [441].

Given the variations in length between each menstrual cycle, the biomarker variables were visualised across cycle progression, with day-1 of menstruation as 0% and the day before menstruation as 100%. Raw outcome data were also plotted with person-centred data and visual inspected for possible relationships.

Data from the WILD AI app were used for menstrual cycle phase estimation using the backward calculation method [55, 403]. Given that ovulation was not confirmed in this study, cycle phasing for data analysis was restricted to the menses and pre-menses phase; pre-menses was the five days before the onset of menses and menses was days one to five where day one is the first reported day of bleeding. Any missing data were assessed using statistical tests, visual patterns or domain knowledge as appropriate to diagnose the missingness mechanism and determine how to handle the missing data. Concussion biomarker variables were assessed for normality and homogeneity of variance with a Shapiro-Wilks test and the Levene's test respectively. A series of independent samples Welch's t-tests of combined data from all three menstrual cycles and for each unique menstrual cycle were conducted to determine whether there was a significant

difference in mean miR ratio or mean morning cortisol value between the menses and pre-menses phases of the menstrual cycle. Alpha level was set at $p<0.05$ for all statistical tests. Due to the ordinal nature of the person-centred data, a Kendall's tau (τ) correlation was conducted to examine the relationships between the biomarker variables and the person-centred variables.

6.4 Results

The player had 95% adherence to the menstrual tracking protocol. Fifteen saliva samples were not collected due to illness or travel. Data were collected over 102 days, starting, and ending partway through a menstrual cycle. Consequently, only data from three complete menstrual cycles (MC1, MC2, MC3), a total of 84 days, were included in the analysis.

6.4.1 LEAF_Q

The player recorded a LEAF_Q score of 2 and this remained unchanged across the season. The player did not report any history of amenorrhoea or other menstrual irregularities. Although the player had previously used hormonal contraception, it had not been used for at least three years prior to starting the study.

6.4.2 Concussion biomarkers across the menstrual cycle

Across the three menstrual cycles included in the analysis, 73 miRNA samples were collected. Of those 73 samples, 43 (59%) amplified miR-27a-5p, miR-30a-3p, and miR-16-5p, 16 (22%) amplified miR-27a-5p and miR-30a-3p, and 6 (8%) amplified miR-16-5p plus either miR-27a-5p or miR-30a-3p. Seven (9%) samples amplified miR-27a-5p only and 1 (1.4%) sample did not amplify any of the miRNAs. The samples that amplified all three miRNAs (n=43) were used to calculate the normalized value for miR-27a-5p/miR-30a-3p. Given that the normalised values were the same as the value of miR-27a-5p/miR-30a-3p those samples that did not amplify miR-16-5p but did amplify both miR-27a-5p and miR-30a-3p (n=16) were also included in the analysis, resulting in 59 miRNA samples included in the final visual analysis. Only two of the missing miRNA values fell within the pre-menses and menses phases of MC1 and MC2 respectively. These data were considered 'missing completely at random' (MCAR) therefore given the time series nature of the data, prior to running the Welch's t-tests the linear interpolation method was used to determine the missing values. All 73 samples collected for cortisol measurement were included in the final analysis.

The length of the menstrual cycle varied from 26 days (MC3) to 30 days (MC2) with 28 days for MC1. The range of miR-27a-5p/miR-30a-3p ratio was consistent across MC1 (0.83 to 1.03) and MC2 (0.83 to 1.09) and was wider in MC3 (0.73 to 1.08) (Table 6.1).

Table 6.1: Menstrual cycle characteristics and range of salivary miRNA ratio, cortisol values, symptoms and sleep score within each cycle.

Menstrual cycle			Salivary measures		App based measures	
number	Length (days)	Phase	miR-27a-5p/miR-30a-3p. Mean ratio ±SD (range) Cohen's *d* n^1	Cortisol (nmol/L) Mean ±SD (range) Cohen's *d* n^1	Symptom score. Mean ±SD (range) n^2	Sleep score. Mean ±SD (range) n^2
1	28	**Menses**	0.91 ±0.08 (0.83 to 1.03)$^{0.25}$ n=6	12.15 ±8.97 (6.64 to 13.9)$^{0.88}$ n=7		
		Pre-menses	0.89 ±0.09 (0.83 to 0.99) n=5	8.97 ±4.34 (3.04 to 14.3) n=5		
		Full cycle	0.90 ±0.05 (0.83 to 1.03) n=19	11.60 ±2.90 (3.04 to 15.90) n=28	3.7 ±2.5 (0 to 10) n=28	21.6 ±3.9 (18 to 32.5) n=28
2	30	**Menses**	0.92 ±0.03 (0.87 to 0.94)$^{-0.71}$ n=5	14.42 ±6.78 (7.47 to 15.2)$^{1.60}$ n=5		
		Pre-menses	0.96 ±0.08 (0.9 to 1.09) n=5	6.46 ±1.84 (4.7 to 8.83) n=5		
		Full cycle	0.93 ±0.06 (0.83 to 1.09) n=21	11.60 ±5.30 (2.75 to 25.00) n=27	2.2 ±1.5 (0 to 5) n=30	19.6 ±6.2 (0 to 30) n=30
3	26	**Menses**	0.94 ±0.11 (0.82 to 1.08)$^{1.88}$ n=5	11.30 ±2.81 (6.84 to 14.6)$^{0.41}$ n=5		
		Pre-menses	0.79 ±0.04 (0.73 to 0.82) n=5	9.84 ±3.00 (6.52 to 14.4) n=5		
		Full cycle	0.87 ±0.09 (0.73 to 1.08) n=16	9.50 ±2.60 (6.00 to 14.60) n=18	3.1 ±1.7 (0 to 7) n=25	19.7 ±4.3 (15 to 25.5) n=25
1+2+3	84	**Menses**	0.92 ±0.07 (0.82 to 1.08) $^{0.51}$ n=16	12.49 ±4.26 (6.64 to 15.50) $^{1.06}$ n=17		
		Pre-menses	0.88 ±0.09 (0.73 to 1.09) n=15	8.42 ±3.33 (3.04 to 14.40) n=15		
		3 full cycles	0.90 ±0.07 (0.73 to 1.09) n=56	10.9 ±3.96 (2.75 to 25.00) n=73	3.0 ±2.0 (0 to 10) n=83	20.3 ±5.0 (0 to 32.5) n=83

n^1= number of samples included (note the number of samples does not total the number of days in each cycle due to missing samples or samples that did not amplify miRNA); n2=number of days reported; Cohen's d=effect size

6.4.3 Visual inspection analysis

MiR ratio consistently showed some cyclical variation, with a downward tendency towards the end of MC3 (Figure 6.1a). Morning cortisol (nmol/L) showed a wider range of values that were within 'normal' reference ranges (Table 6.1) (MC1: 3.04 to 15.90; MC2: 2.75 to 25.00; MC3: 6.00 to 14.60). Visual inspection analysis showed no indication of a pattern throughout the menstrual cycle (Figure 6.1b).

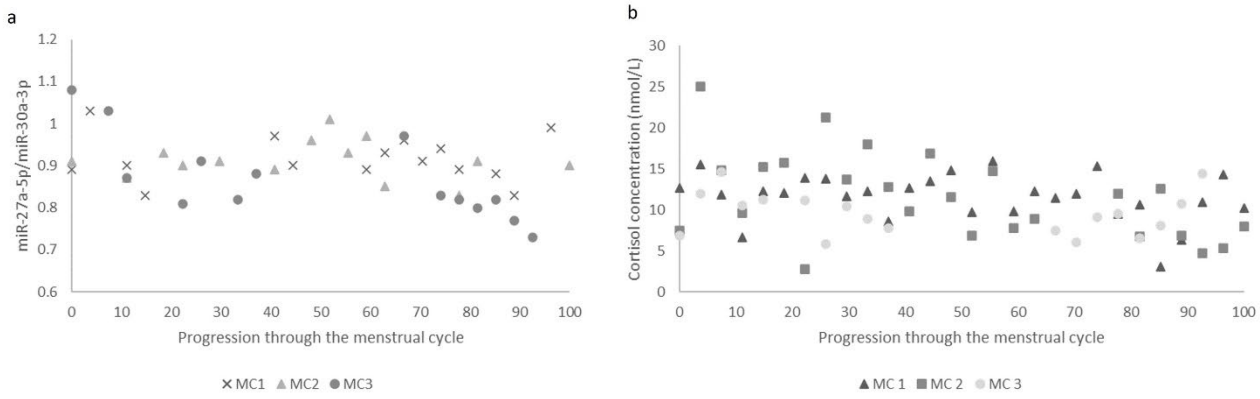

Figure 6.1: a) Visual scatter plot of miRNA ratio across 3 time normalized (0-100%) menstrual cycles (MC1=menstrual cycle 1, MC2=menstrual cycle 2, MC3=menstrual cycle 3). b) Visual scatter plot of cortisol across 3 time normalized (0-100%) menstrual cycles. (MC1=menstrual cycle 1, MC2=menstrual cycle 2, MC3=menstrual cycle 3).

Self-report symptom score showed some variation across the menstrual cycle in a consistent range within each menstrual cycle (Table 6.1 and Figure 6.2a). Self-report sleep score did not indicate a pattern related to the menstrual cycle but showed wider variation in MC 2 (Table 6.1 and Figure 6.2b).

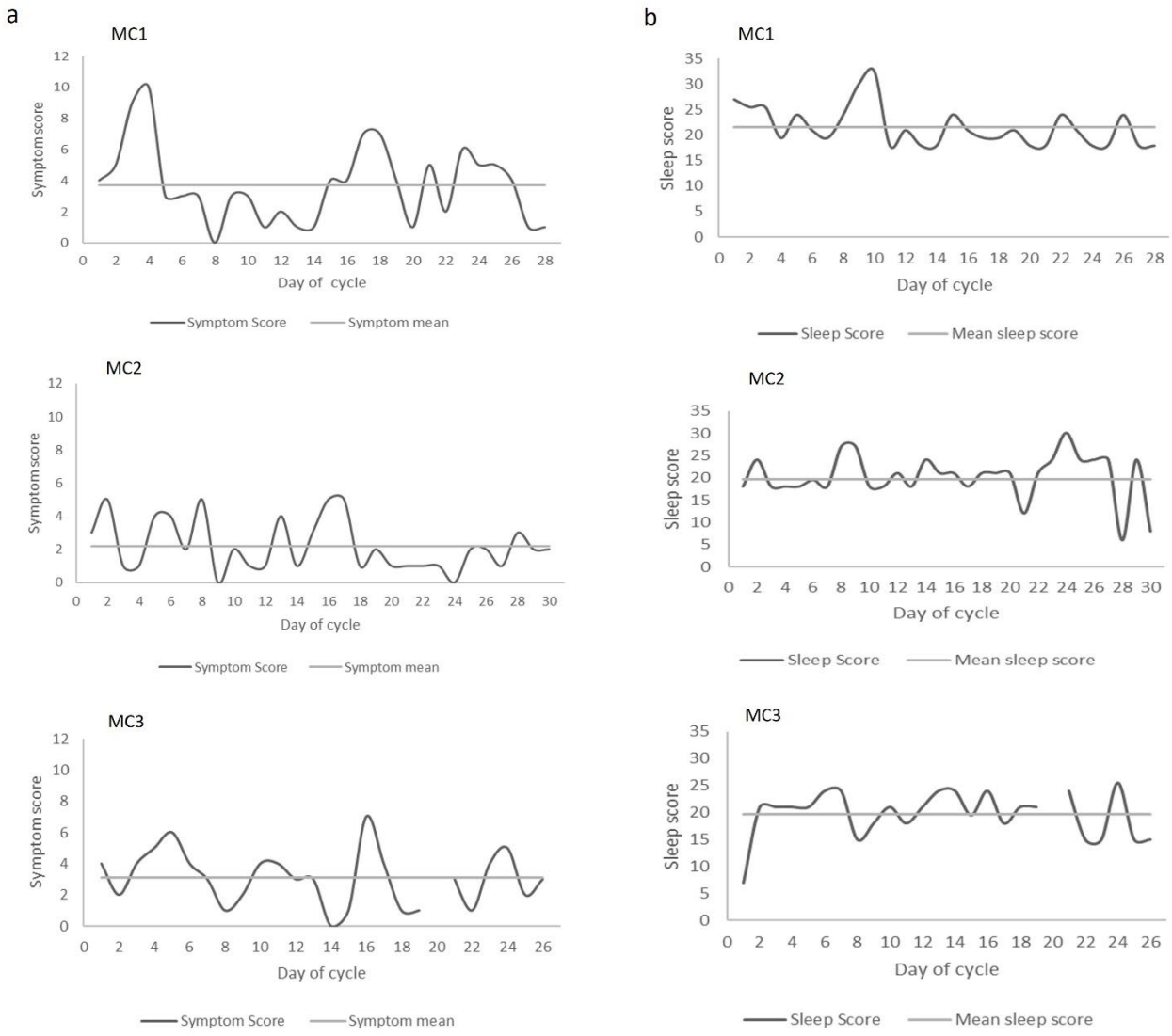

Figure 6.2: a) Self report symptom score across the days of the menstrual cycle for 3 menstrual cycles (MC1=menstrual cycle 1, MC2=menstrual cycle 2, MC3=menstrual cycle 3). b) Self report sleep score across days of the menstrual cycle for 3 menstrual cycles (MC1=menstrual cycle 1, MC2=menstrual cycle 2, MC3=menstrual cycle 3).

6.4.4 Differences between phases

Preliminary data screening showed that miR ratio values in both groups (phases) met the assumptions of normality with a Shapiro-Wilks test ($p>0.01$). The Levene's test was not significant, ($F(29)=0.56$, $p=0.46$); indicating that the assumption of homogeneity of variance had been met. Similarly, values of morning cortisol in both groups (phases) met the assumptions of normality (Shapiro-Wilks test, $p>0.01$) and homogeneity of variance (Levene's test, $F(30)=0.05$, $p=0.83$).

6.4.4.1 miR ratio

The groups did not differ significantly, t(27)=1.40, p=0.17, d=0.51. The mean miR ratio in the menses phase (M=0.92, SD=0.07) was not significantly different (p=0.17) than the mean miR ratio in the pre-menses phase (M=0.88, SD=0.09) (95% CI [-0.02 – 0.10]), despite the medium Cohen's d effect size (d=0.51, 95% CI [-0.22 – 1.22]). Within individual menstrual cycles, MC3 showed a significant difference for miR ratio between phases (t(5)=2.97, p=0.03, d=1.88), mean miR ratio in the menses phase (M=0.94, SD=0.11) was significantly (p=0.03) higher than the mean miR ratio in the pre-menses phase (M=0.79, SD=0.04) (95% CI [0.02 – 0.29]), with a large effect (d=1.88, 95% CI [0.15 – 3.51]) size (Table 1).

6.4.4.2 Cortisol

There was a significant difference in morning cortisol value between the menses and pre-menses phase, t(30)=3.02, p=0.01, d=1.06. The mean morning cortisol value in the menses phase (M=12.49, SD=4.26) was significantly (p=0.01) higher than the mean morning cortisol value in the pre-menses phase (M=8.42, SD=3.34) (95% CI [1.32 – 6.82]), with a large Cohen's d effect size (d=1.06, 95% CI [0.31 – 1.79]) (Table 6.1).

6.4.5 Relationships between variables

MC2 showed greater variability in both cortisol (Figure 1b) and sleep (Figure 2b), therefore these variables were visual inspected together. There was no observable trend between sleep score from the Wild AI app and morning salivary cortisol value, this was further confirmed in the correlational analysis (Figure 3a-d and Supplementary table 6.1).

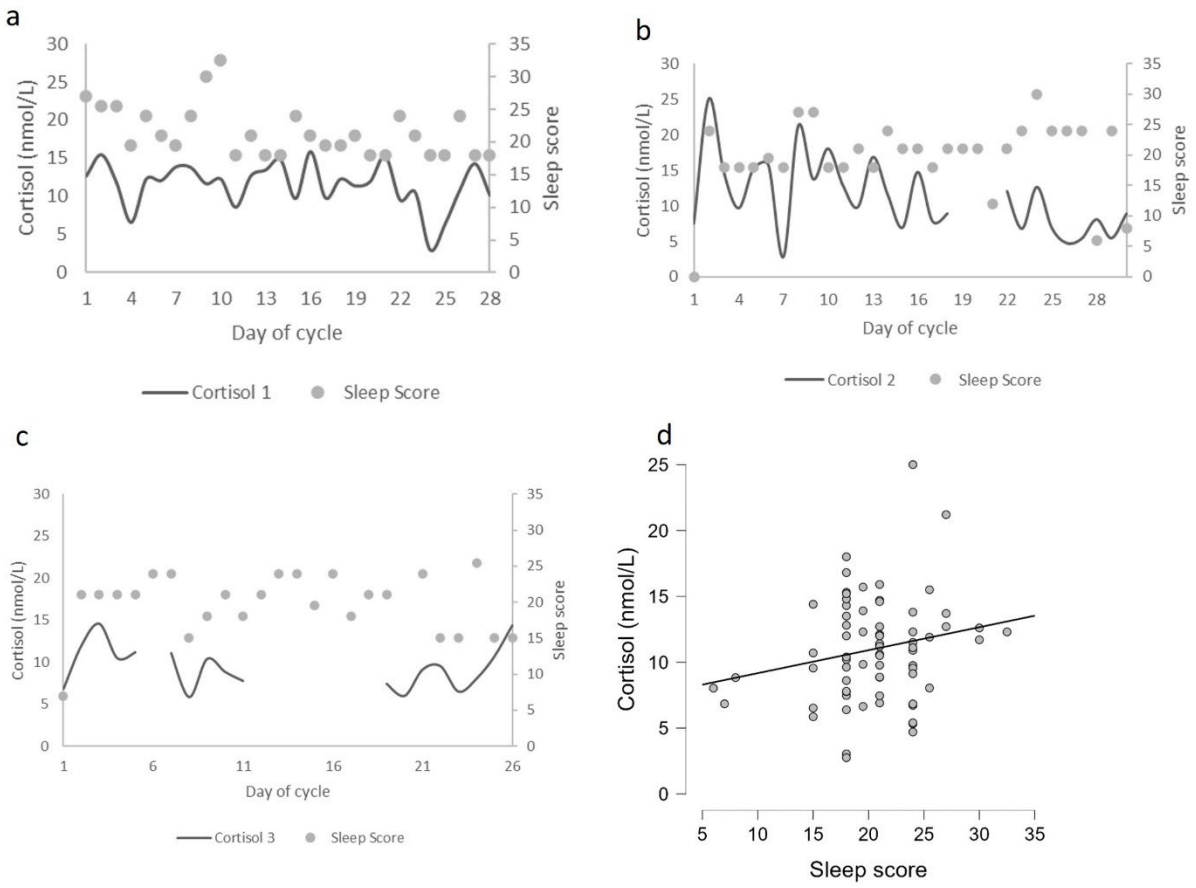

Figure 6.3: Self report sleep score and cortisol across days of the menstrual cycle a) Menstrual Cycle1, b) Menstrual Cycle2, c) Menstrual Cycle3, d) Kendall's tau correlation plot for Self-report sleep score and cortisol across 3 consecutive menstrual cycles (τb=0.10; p=0.27).

6.5 Discussion

The primary aim of this exploratory study was to investigate whether the ratio of concussion specific salivary miR-27a-5p/miR-30a-3p (miR ratio) changed across the menstrual cycle in the absence of concussion. The findings for this player did demonstrate some cyclical variability throughout the menstrual cycle. In 2 of the three menstrual cycles the miR ratio was stable overall and ranged between 0.8 and 1.1. Previous research[423] suggested that the miR ratio in concussed individuals is lower (below 1.0) than in those without concussion. Although the miR ratio in this player went below 1.0, given the frequency (daily) of measures and that there was no statistically significant change across 2 different analysed phases of the menstrual cycle this may just reflect the normal within-person variation of the miR ratio. It remains unclear what a reliable clinically relevant threshold value of miR ratio would be and this is an important question for future studies. A key finding of this study came from the third menstrual cycle that was shorter in length than the previous cycles and the miR

ratio range was wider (0.73 to 1.1) with the lower values observed towards the end (pre-menses phase) of the cycle and resulting in a significantly (p=0.03) lower miR ratio in the pre-menses phase when compared to the menses phase. It must be noted that this result is from one menstrual cycle in one female and the relatively wide confidence intervals highlight the uncertainty around this result.

Menstrual cycles that are shorter than would be considered 'typical' for a particular woman could be indicative of a luteal phase defect (LPD) or anovulatory cycle [31, 442]. Both conditions are considered sub-clinical menstrual disturbances [31] that impact the luteal phase of the menstrual cycle but in different ways. LPD occurs when the ovaries do not produce sufficient progesterone after ovulation, an anovulatory cycle occurs when the ovaries do not release an egg and there is no mid-cycle surge of gonadotropins, as a result there is no increase in progesterone in the luteal phase [31]. These subclinical menstrual disturbances result in a lower level of hormones in the luteal phase than would be expected in a regular ovulatory cycle, meaning that the luteal and follicular phases would be more closely matched in terms of absolute hormone concentrations. This suggests that the notable impact on miR ratio is likely due to an altered activity of the hypothalamic pituitary ovarian (HPO) axis, and a disruption to the normal feedback loops and signalling pathways rather than the absolute concentrations of hormones. This is an important consideration for concussion research given that the injury can also disrupt the HPO [296, 367]. Menstrual disturbances may be a confounder of miR ratio. Although this finding must be interpreted with caution due to the single-case design and the method of phase estimation. This also highlights the importance of confirmation of ovulation (or not) and phase verification as part of the methodological design of future concussion biomarker studies.

Morning salivary cortisol has been used as a prognostic biomarker in concussion, the measure is lower than baseline post-injury and those with abnormally low morning cortisol reportedly take longer to recover [415, 416]. A secondary aim of this study was to track morning salivary cortisol against the menstrual cycle in the absence of injury to further understand baseline patterns in females. Recent meta-analytic findings indicated higher cortisol levels during the follicular phase when compared to the luteal phase of the menstrual cycle [428 443]. The follicular phase and luteal phase utilised by researchers [428 443], would most closely relate to menses and pre-menses phases used in this single-subject design respectively. As a result, the player in the current study consistently showed the opposite pattern in the three consecutive menstrual cycles analysed, as cortisol levels were significantly (p=0.01) higher in the menses than in the pre-menses phase. Hamidovic et al. [428], also suggested a gradual reduction in cortisol levels of ~20 nmol/L between these phases that would follow a linear pattern as circulating cortisol levels adjust dynamically to support adaptive physiological processes when both estradiol and progesterone are low.

Visual inspection of morning salivary cortisol in this player did not reveal a linear or cyclical pattern throughout the menstrual cycle. The contradictory findings between these studies are likely due to the different methodological approaches to phase verification or estimation and the number of data points included in the analyses. This a common challenge across menstrual cycle related research [444]. Further studies adopting the methodological approaches previously outlined [42, 44, 54] are needed to confidently understand any impact of hormone profile on morning cortisol measures. Despite the wide variability in morning cortisol shown in this player (2.75 to 25.00 nmol/L), nearly all values fell within the 'normal' reference range (1.50 to 22.00 nmol/L) [396]. There was 1 outlier value of 25 nmol/L and this only occurred during the second menstrual cycle throughout the data collection period. Given this intra-individual variation, baseline measures would be required to rule out any pre-existing cortisol dysregulation and to determine normal patterns and ranges for individual athletes if cortisol were to be utilised as a biomarker to support the diagnosis and management of concussion. Another consideration is that low or abnormally low morning salivary cortisol levels may be caused by adrenal insufficiency or dysregulation [396, 436] or from the accumulation of chronic stress, [436] highlighting that this measure lacks specificity to concussion.

There was no evidence of an association between any of the physiologic and person-centred variables in this player. Given that there was a degree of variability in sleep score, it could have been expected that there would be some correlation between sleep and morning cortisol that has been shown in other research [397]. This may be due to a lack of sensitivity in the self-reported sleep measures utilised in this study. Similarly, symptom score showed no association to any of the other measures, and this was anticipated given that there was no occurrence of injury or illness that could reasonably be expected to impact trait level symptoms reported in the app. It would be important to include symptom scores in future studies investigating concussion biomarkers in injured athletes.

It was also observed from this exploratory study that the player was 95% compliant with the daily data collection protocol and was able to self-collect salivary miRNA samples, record menstrual cycle and sleep characteristics using the Wild AI app and complete the LEAF questionnaire (weekly) over 102 days. Therefore, the protocol could be used in further studies.

6.5.1 Limitations and future research

As a single-case design, the results lack generalisability but do indicate that larger studies of female athletes with, and without, concussion are needed to elucidate a clinically relevant miR-27a-5p/miR-30a-3p threshold ratio for concussion and further understand the relevance and relationship to hormone profile. This study used phase estimation and did not include phase verification or confirmation of ovulation to enable detection of anovulatory cycles and categorisation of ovulatory

cycles into follicular, peri-ovulatory, ovulatory, and luteal phases for fuller analysis of the data across the entire menstrual cycle and more accurate data interpretation. It is recommended that as a minimum, future studies include urinary detection of luteinizing hormone (LH) surge and confirmation of ovulation with serum progesterone measurement of >16nmol/L. To manage participant burden, this aspect of the protocol should be prioritised and based on the findings of this study, biomarker sampling could be reduced to three samples in each of the phases as defined by Schmalenberger [403].

6.6 Conclusion

This exploratory study indicated that the ratio of miR-27a-5p/miR-30a-3p shows a consistent cyclical pattern across the menstrual cycle and this likely reflects natural day to day individual variation but is stable overall in a normal menstrual cycle. Threshold miR-27a-5p/miR-30a-3p ratio values need to be established for injured and un-injured females. Sub-clinical menstrual disturbance may alter miR ratio and this needs to be more fully investigated. A high frequency data collection protocol is a feasible approach for studies investigating the interaction of hormone profile with concussion in female athletes. Given the intra-variability of morning cortisol, the current lack of understanding of any impact of hormone profile on morning cortisol, and the non-specific nature of cortisol dysregulation, cortisol may not be an efficient or accurate prognostic biomarker for concussion in female athletes.

Chapters 6 and 7 linking section

A saliva-based biomarker for concussion would not only have application on the sports field, but it would also be ideal in the emergency department (ED) environment due to the non-invasive nature and the lack of need for specialist trained personnel to collect the sample. In addition, using the ratio of two miRNA eliminates the need for a baseline sample to compare against for each person. Chapter 6 indicated that more data are needed to confirm whether hormone profiles interact with the expression of concussion specific miRNA or not. The work done in chapter 6 also required developing the quantitative real-time polymerase chain reaction (qPCR) technique to amplify the miRNA in the saliva samples. There was sufficient volume of saliva samples to allow repeat testing and to adjust the dilution of the samples to optimise the amplification of the miRNA. It became evident that further work is needed to identify a consistent endogenous control.

Chapters 7 and 8 continue this exploratory work on the use of the same salivary miRNA in the ED environment in females with concussion. These chapters also investigate whether different hormone profiles are associated with different recovery times in females with concussion. Development of protocol to test salivary biomarkers alongside standard clinical care was needed.

Chapter 7. Female RNA Concussion (FeRNAC) study protocol: Assessing Hormone Profiles and Salivary RNA in Females with Concussion by Emergency Departments in New Zealand; a prospective cohort study.

This chapter has been published as:

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7.1 Overview

Background: Females of reproductive age with concussion report a greater number of symptoms that can be more severe and continue for longer than age matched males. Underlying mechanisms for sex differences are not well understood. Short non-coding Ribonucleic Acids (sncRNAs) are candidate salivary biomarkers for concussion and have been studied primarily in male athletes. Female sex hormones influence expression of these biomarkers, and it remains unclear whether a similar pattern of sncRNA expression would be observed in females following concussion. This study aims to evaluate recovery time, the ratio of salivary sncRNAs and symptom severity across different hormone profiles in females presenting to emergency departments (ED) with concussion and, to investigate the presence of low energy availability (LEA) as a potential modifier of concussion symptoms. **Methods:** This prospective cohort study recruits participants from New Zealand EDs who are biologically female, of reproductive age (16-50 years) and with a confirmed diagnosis of concussion from an ED healthcare professional. Participants are excluded by ED healthcare professionals from study recruitment as part of initial routine assessment if they have a pre-diagnosed psychiatric condition, neurological condition (i.e., epilepsy, cerebral palsy) or more than three previously diagnosed concussions. Participants provide a saliva sample for measurement of sncRNA's, and online survey responses relating to hormone profile and symptom recovery at 7-day intervals after injury until they report a full return to work/study. The study is being performed in accordance with ethical standards of the Declaration of Helsinki with ethics approval obtained from the Health and Disability Ethics Committee (HDEC #2021 EXP 11655), Auckland University of Technology Ethics Committee (AUTEC #22/110) and locality consent through Wellington hospital research office. **Discussion:** If saliva samples confirm presence of sncRNAs in females with concussion, it will provide evidence of the potential of saliva sampling as an objective tool to aid in diagnosis of, and confirmation of recovery from, concussion. Findings will determine whether expression of sncRNAs is influenced by steroid hormones in females and may

outline the need for sex specific application and interpretation of sncRNAs as a clinical and/or research tool.

Trial Registration: Australian New Zealand Clinical Trials Registry (ANZCTR) registration number ACTRN12623001129673.

7.2 Introduction

Concussion, as a subset of mild traumatic brain injury, is a brain injury induced by direct or indirect biomechanical force being transmitted to the head via a blow to the head or the body. Disturbance in cellular homeostasis in the brain initiates complex biochemical and neurometabolic changes [445] resulting in a transient 'energy crisis' in the brain. Concussion is typically considered a functional rather than structural injury and presents as a variable cluster of physical, cognitive, emotional, and sleep related symptoms. Sports Related Concussion (SRC) has become almost synonymous with male collision sport and has in recent years received increased profile and greater awareness generated through traditional and social media. However, concussion occurs in many activities and environments with data showing that sports collectively account for only 20–40% of all concussions [323, 446]. Motor vehicle accidents and military combat are other common mechanisms of injury in males [447], whereas females have tended to sustain concussion from falls or incidents of intimate partner violence [447]. As greater numbers of females enter the armed forces and play collision sports, the incidence of concussion in these environments will likely increase. There is a need to ensure that both sport and non-sports related concussion are addressed in research and clinical settings, and in public awareness and media campaigns.

Sex differences are evident in concussion [145, 287, 288, 293, 294, 316, 317, 386, 448, 449]. Females often have more severe symptoms that are longer in duration [328, 382, 450, 451]. Studies in female athletes also show that symptom endorsement at baseline (i.e., when there is no injury) is more prevalent than that seen in males [383]. Underlying mechanisms for these observed sex differences are not yet well understood and sex as a biological variable in injury recovery remains understudied [15]. Limited data show that sex differences in concussion outcomes (i.e., severity and duration of symptoms) first appear during adolescence, are evident throughout the reproductive years, but that postmenopausal women have similar or better outcomes than men [294]. Women have a considerable physiologic change when transitioning between these life stages with altered levels of estrogen and progesterone. Bi-directional relationships between female sex hormones and concussion could influence symptoms and recovery outcomes [294-296, 447]. Primary female sex hormones, estrogen and progesterone are typically associated with reproduction, but these hormones are powerful biochemical messengers that influence every body system including, temperature regulation, macronutrient metabolism, hydration, central nervous system fatigue, and brain bioenergetics [362, 452-454]. During reproductive years, estrogen and progesterone fluctuate across the menstrual cycle, which typically

lasts 21–35 days [31, 455]. Hormone related symptoms have significant overlap with those symptoms associated with concussion and are similarly considered across physical, cognitive, emotional and sleep related domains. Low energy availability (LEA) occurs when dietary energy intake is insufficient to support energy expended in exercise and results in inadequate energy to support the functions required by the body to maintain optimal health; prolonged LEA is a clinical concern that disproportionately affects females, can disrupt endocrine function [46, 48] and presents with a similar set of symptoms to concussion. The prevalence of LEA ranges from 33.5% in recreationally active (tier 2 [63]) females [53] to 59% in sub-elite [51] (tier 3 [63]) and 88% in professional female football players [456] and could be a potential modifier in concussion. Female specific research is therefore needed to understand how sex hormones interact with concussion and to what extent concussion may impact hormone regulation and symptoms.

Diagnosis of concussion may not always be clear; assessment typically includes the use of neurocognitive and physical tests and subjective symptom report. It is also important to identify a plausible mechanism of injury, which can also be difficult to recognise in cases where there are multiple injuries. There are currently no validated tools or tests that can confirm a diagnosis of concussion, this relies on clinical judgement. Recent research in clinical settings and on sports field sidelines [415, 416, 421-424, 426, 427, 457-459] indicated potential blood biomarkers that may be specific to concussion, including beta-amyloid protein, ubiquitin carboxy-terminal hydro-lase L1 (UCH-L1), glial fibrillar acidic protein (GFAP), S100β, Tau, neurofilament light protein (NFL) and brain derived neurotrophic factor (BDNF) [415, 416, 421-424, 426, 427, 457-459]. However, due to relatively small heterogenous sample sizes and varying methodological approaches there have been inconsistent research findings and a lack of clarity on the time course or presence of these biomarkers in relation to injury. More recent advances in technologies have enabled investigation into ribonucleic acid (RNA) species as potential biomarkers of disease [422, 423, 426, 457, 460]. MicroRNAs (miRNAs, miRs) act at the post-transcriptional level to regulate protein synthesis and belong to the family of small non-coding RNAs (sncRNAs) (20 to 200 nucleotides in length). MiRNA signatures specific to traumatic brain injury (TBI) including concussion have been reported in blood, cerebrospinal fluid and saliva, and these signatures appear to vary according to TBI severity. Different ‘families’ of miRNA’s may be upregulated or downregulated in response to various conditions (e.g., post-exercise or injury) or disease states.

Non-invasive saliva sampling enables rapid collection of data immediately after injury and at specified time points thereafter, with the participant able to collect the sample independently. In a recent study [457] utilising a saliva collection protocol, a panel of 14 different sncRNAs present in saliva accurately discriminated between clinical diagnosis or absence of concussion in 156 professional male rugby union players. These sncRNAs have potential to provide objective insight into pathophysiological responses following a concussive injury and may be a useful adjunct as a sideline test and in the clinical environment to aid diagnosis. Findings of studies in male athletes cannot be extrapolated to female populations without

further investigation, as expression of sncRNAs in other diseases of the central nervous system is sex dependent [461-464].

7.3 Methods

7.3.1 Aim

The aims of this study were to:

1) determine if the time to recovery (in days) differs among groups categorised by their hormone profiles at the point of concussion injury,
2) examine whether the ratio of sncRNAs in saliva differ across groups categorised by their hormone profiles at the point of concussion injury,
3) assess whether self-reported symptoms differ among groups categorised by their hormone profiles at the point of concussion injury, and
4) investigate whether low energy availability changes across the recovery period, and if this is associated with symptoms experienced.

7.3.2 Study design

The prospective cohort study design involves recruitment of participants from Emergency Departments (ED) in Wellington, New Zealand.

7.3.3 Patient and public involvement

Patients and members of the public were involved in the choice of outcome measures and the recruitment strategy in development of this research protocol. During the study design women with and without experience of a brain injury provided input on the feasibility and participant burden associated with each outcome measure through early experimental work and through informal discussion. The recruitment strategy and dissemination plan were refined based on discussions with patients presenting to ED with suspected concussion.

7.3.4 Participants and recruitment

Females presenting to ED within three days of injury with suspected concussion are recruited by ED healthcare professionals. Participants are invited to participate if they are biologically female (self-report), of reproductive age (16–50 years) and have a confirmed diagnosis of concussion from the consulting healthcare professional. The diagnostic criteria for concussion are those outlined in the most recent international consensus statement from the concussion in sport group [370]. Participants will be excluded if they have Polycystic Ovarian Syndrome (PCOS), a pre-diagnosed psychiatric disorder or neurological

condition (e.g. epilepsy, cerebral palsy) or if they have had more than three previously diagnosed concussions; these are known modifiers for prolonged recovery [371, 465]. These specific exclusion criteria will be ascertained by the consulting ED healthcare professional as part of the initial routine assessment for concussion. Full inclusion/exclusion criteria are shown in Table 7.1. The standard concussion assessment is detailed in Figure 7.1. Study recruitment commenced February 2022, however there was interruption due to COVID related sickness and changes in the ED through November 2022. The study recommenced in January 2023 and is in progress.

Table 7.1: Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion criteria
• Females of reproductive age: 16–50 years (with a menstrual cycle for a minimum of 2 years if naturally cycling) • Natural regular menstrual cycle 28–35 days long, or • Currently taking Oral Contraceptive Pill (OCP), or • Currently have Intra Uterine Device (IUD), or • Confirmed diagnosis of concussion by a medical doctor (within 3 days of injury)	• Had three or more previously diagnosed concussions • Current concussion was more than 3 days ago • Post-menopause • Started taking medication that would alter reproductive hormone concentrations (Corticosteroids, e.g., Prednisone. Antidepressant or Antipsychotic medication) within the last 3 months • Current clinical diagnosis of an eating disorder • Pre-diagnosed psychiatric disorder • Pre-diagnosed neurological condition • Polycystic Ovarian Syndrome (PCOS) • Oligomenorrheic (irregular periods), or • Amenorrheic (loss of periods for 3 months or longer) • GCS* score less than 15 at 4 hours after initial assessment. These patients may be referred for a CT scan and will not be eligible for the study.

*GCS = Glasgow Coma Scale is used to objectively describe the extent of impaired consciousness in all types of acute medical and trauma patients. In the context of acute brain injury scores of <8=Severe, 9–12=moderate, 13–15=mild.

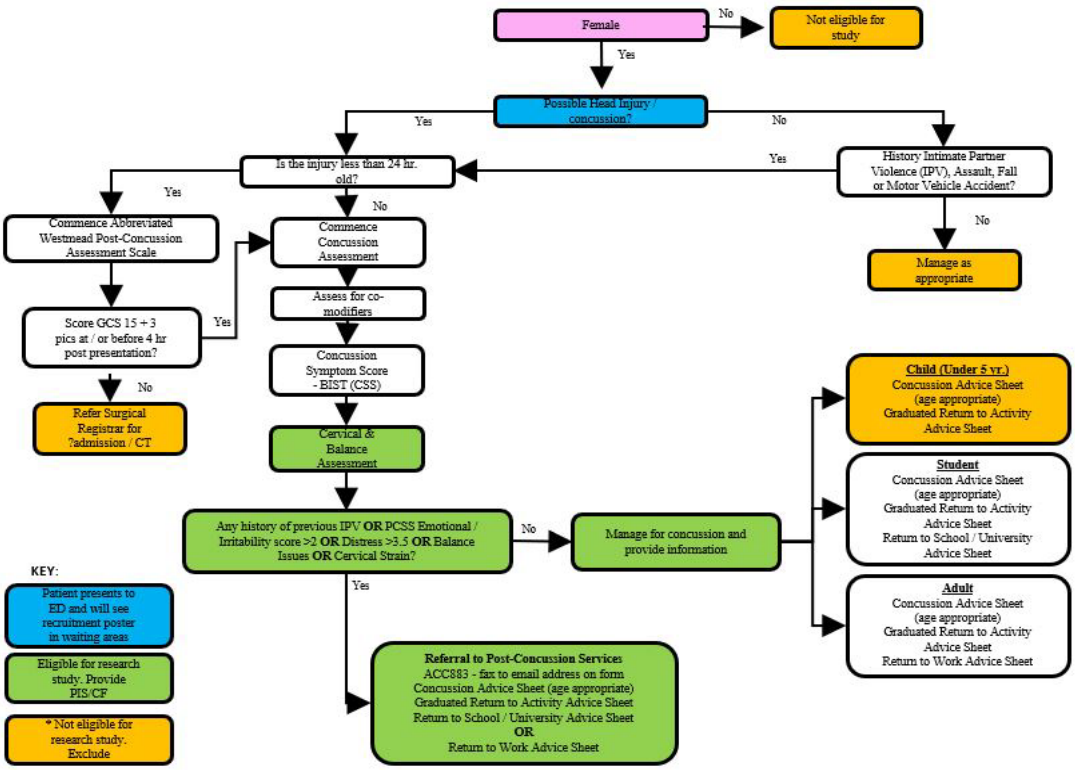

Figure 7.1: Routine clinical assessment and study flow.

All participants will be over the age of 16 years and can therefore provide their own consent after reading a participant information sheet about the project. Participants will have the opportunity to ask questions of the research team prior to providing consent. Upon providing written consent, participants will be assigned a unique identifier number (e.g., CH001). If a healthcare professional advises that a patient may have temporary incapacity to provide consent due to the brain injury being treated at the ED, consent will be re-confirmed via follow up with the participant within three days using the contact information already provided on the consent form. Interpersonal violence (IPV) disproportionately affects females, to ensure safeguarding of any participants in this study that may be vulnerable the Te Whatu Ora (Health NZ) Family Violence Guidelines are implemented [466]. It is important that patients presenting to ED with injuries from IPV, assault or motor vehicle accidents are included as potential participants; concussion may often go unidentified due to polytrauma or an unrecognised mechanism of concussive injury. A specific concussion biomarker may be of most value in this cohort.

7.3.5 Sample size calculation

Using PASS15 software, and a Cox Proportional-Hazards regression model it was estimated that the number of participants needed to examine the primary aim (recovery time in days) is 30. This will allow the detection a hazard ratio of 1.5 (log hazard ratio coefficient = 0.4055), with 80% power, and a type I error rate of 0.05. This was calculated assuming a covariate SD of 1.5, a multiple regression R^2 (variable of

interest regressed on covariates) of 0.2, and an expected event (clinical recovery) rate of 0.9 within the follow-up period [295, 328]. Allowing for 20% drop out during follow-up, 38 participants will be recruited. As of 18 August 2024, 18 eligible women have started the study.

7.3.6 Procedures and measures

Data collected from participants includes one saliva sample and two different surveys administered online weekly until the participant reports a full return to work/study.

7.3.6.1 Saliva samples

Participants will be asked to provide a 2 ml saliva sample. The sample will be used to measure sncRNAs and will be collected in SpeciMax stabilised saliva collection kits (Thermo Scientific SpeciMAX stabilised Saliva Collection Kit A50697, Life technologies Corp, Austin, Texas USA) which are pre-filled with 1ml of virus inactivating and nucleic stabilization solution. Tubes are pre-labelled with a unique barcode linked with each participant's unique identifier number. All samples are collected using a passive drool method [392]. Before providing each sample, participants take a small sip of water, swish the water around in their mouth and then swallow the water. This stimulates saliva release and clears any food debris that may be in the mouth. The participant's approximate last mealtime is noted as this may influence saliva sample quality. The saliva sample is immediately stored in the freezer at −80°C until all samples are collected. Samples are then couriered on dry ice to laboratories in Auckland for processing and analyses.

7.3.6.2 Saliva sample analyses

On the day of analysis all samples will be brought to room temperature. Processing of saliva samples will involve 500 µL of each sample being aliquoted into an Eppendorf tube and centrifuged at 2,500 rpm for 5 min until all the proteins pelletise and the supernatant is clear.

7.3.6.4 sncRNA (miRNA) expression analysis

The sncRNA targets from saliva will be extracted using the MagMAX mirVana total RNA isolation kit (A27828, Thermo Fisher Scientific, Waltham, Massachusetts, US) using manufacturer's instruction with modification for saliva extraction. After isolation of total RNA (including snc/miRNA), these will undergo polyadenylation, adapter ligation, reverse-transcription and miR-Amp reactions using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (A28007, Thermo Fisher Scientific, Waltham, Massachusetts, US) following manufacturer's instructions. Reactions will be performed on the standard thermocycler BioRad. The cDNA will be diluted, and real-time PCR runs for miRNA analysis will be performed using the Taqman Fast Advanced Mastermix for qPCR (4444964, Thermo Fisher Scientific, Waltham, Massachusetts, US) on QuantStudio™ 7 Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts, US). Using TaqMan® Assays for miR-27a-5p and miR-30a-3p (assays 002445 and 000416 respectively, Thermo Fisher Scientific, Waltham, Massachusetts, US) their respective expressions will be calculated by the $2^{-\Delta\Delta Ct}$ method by using endogenous miRNA, one of RNU48, RNU44, U47, or RNU6B (Thermo Fisher Scientific, Waltham,

Massachusetts, US) will be used as the reference genes. The ratio of miR-27a-5p/miR-30a-3p expression will be calculated. The ratio of these two miRNAs has previously been reported to differentiate concussed and non-concussed individuals; concentrations of miR-27a-5p and miR-30a-3p differentiated 75 concussed individuals from 97 non-concussed individuals with 82.4% sensitivity and 73.3% specificity [433]. The levels of miR-27a-5p/miR-30a-3p were lower among concussed individuals and importantly showed no effect of acute or chronic exercise. These miRNAs showed similar accuracy for both sport and non-sport related concussion.

7.3.6.5 Surveys and questions

Survey and question data are collected and managed using REDCap (Research Electronic Data Capture) which is a secure, web-based software platform designed to support data capture for research studies [377, 440]. Participants are provided with an online link to REDCap via email. A new link is sent every seven days after initial appointment until a full return to work/study is reported. It takes approximately 10 minutes to complete all questions each week.

Two concussion specific questions are asked only once, seven days after the initial appointment. The first question relates to concussion history and is utilised to capture the number of previous concussions the participant has had. This information is important as prior concussion can impact recovery from subsequent concussions [371]. The second question is used to capture whether the participant's concussion is sport related or not.

Participants are also asked to complete the Low Energy Availability in Females Questionnaire (LEAF–Q) online seven days after initial assessment and then at seven-day time intervals throughout recovery. The LEAF-Q [47] is designed and validated to evaluate physiological symptoms of insufficient energy intake or low energy availability (LEA). The LEAF-Q contains questions regarding injuries, gastrointestinal and reproductive function. In section 3.2 three questions (19 items) relate to menstrual function including recent menstrual history. This questionnaire section is used to determine hormonal profile of participants and has been modified as outlined below.

Part C of section 3.2 includes the following question:

C: Do you have normal menstruation? Yes ¨ No (go to question C6) I don't know (go to question C6)

C1: If yes, when was your last period? 0-4 weeks ago ¨ 1-2 months ago ¨ 3-4 months ago ¨ 5 months ago or more ¨

This question has been modified to capture more accurate detail about the last menstrual cycle as follows:

C: Do you have normal menstruation? "Yes" "No" (go to question C6) "I don't know" (go to question C6)

C1: If yes, when was your last period? 1 week ago “2 weeks ago” “3 weeks ago” “4 weeks ago” “1-2 months ago” ¨ “3-4 months ago” “5 months ago or more”

Participants are categorised into one of five hormone profile groups as follows;
1) Follicular phase if question C1 is answered as “1 week ago”,

2) Mid-cycle if question C1 is answered “2 weeks ago”,

3) Luteal phase if question C1 is answered “3 weeks ago” or “4 weeks ago”,

4) Oligomenorrheic if C1 was answered “1-2 months ago” , “3-4 months ago” or “5 months ago or more”,

5) Using contraceptive if question 3.1a is answered “yes”

Scoring of question 3.2 remains the same, i.e., if a participant tick any of the boxes within the 0–4 weeks’ timeframe the same score is applied as the original question. In eumenorrheic participants this will help estimate menstrual cycle phase at point of injury. In addition, if question 3.1a is answered yes, the participant will also be able to indicate what type of contraception is used for further sub-categorisation for data analysis. This information is used to understand any association between hormonal profile and or LEAF_Q score with symptom severity and duration of concussion.

The Brain Injury Screening Tool (BIST) is a brief screening tool for use on initial presentation after injury to guide health care pathway decision making and to monitor symptoms and recovery over time [467]. The BIST was developed in the NZ context to fit the clinical environment and to provide consistency across multiple environments and referral pathways (e.g., GP clinic, ED, Urgent Care Centres), the tool has also been psychometrically tested. There are two sections in the BIST; the first section is used to determine if there are any clinical ‘red flags’ which require referral to hospital, therefore this component is not included in this study. The second section of the BIST includes a 16-item symptom report scale for patients to rate how much they now experience the symptoms listed on a scale of 0 (not at all) to 10 (severe). Higher symptom scores are indicative of higher symptom burden. Participants are asked to complete this part of the BIST at point of presentation with injury and at seven-day time intervals throughout the study to monitor symptoms and recovery over time.

The final question asks if the participant has made a full return to work/study and how many follow-up appointments they had. The number of days between injury and full return to work/study will be used as a proxy for recovery. This question is included in the weblink sent at all timepoints (i.e., 7, 14, 21, 28 days etc.) until the participant reports they have achieved a full return to work/study, at which point participation in the study will be complete. Detail of information collected each week is included in Table 7.2.

Table 7.2: Research measures and collection points.

Measure	How	Who	When		
			Initial assessment	During recovery (7-day time intervals)	Full return to work/study
Saliva Samples	Passive drool into cryovial	Consulting clinician, research assistant or Lead Researcher	X		
Number of prior concussions#	Online*	Participant to complete		X	
Sport or non-sport related concussion#	Online*	Participant to complete		X	
LEAF-Q	Online*	Participant to complete		X	
BIST	Online*	Participant to complete	X	X	
Number of days from injury to recovery (full return to work/study)	Online*	Participant to complete			X

7.4 Data analysis

In this study a range of statistical tests will be used to analyse the data corresponding to the four aims. Aim 1 will use Cox proportional hazards regression to determine if the time to recovery from concussion (measured in days), differs among groups categorised by hormone profile at the point of injury (five profiles: Follicular phase, Midcycle, Luteal phase, oral contraception, IUD). Aim 2 will apply an ANOVA to compare the ratios of miR-27a-5p/miR-30a-3p expression, across these five groups using the follicular phase as the reference group. Model-estimated means and pairwise contrasts between groups will also be computed. For Aim 3, the focus is on whether self-reported symptoms, quantified both as the number of symptoms present (range 0–16) and as a total symptom burden (sum of severity scores; range 0–160), differ among hormone profile categories. These dependent variables will be analysed using ANOVA, with the five hormone profiles specified as the independent variable. Aim 4 will employ a linear mixed model to investigate changes in low energy availability across the recovery period. This model will incorporate the day of recovery and total symptom burden as fixed effects and will account for repeated measures over time by specifying participant as a random effect. The interaction between symptom burden and recovery time will also be evaluated to understand if these variables are collectively associated with low energy availability.

7.4.1 Dissemination plan

A study fact sheet will be provided on our website https://sprinz.aut.ac.nz/areas-of-expertise/sports-kinesiology-injury-prevention-and-performance/female-athlete-performance-and-health-research-programme. Data will be available upon request from the primary author. Study findings will be published

in peer-reviewed scientific journals and presented at relevant conferences. Participants will receive a summary of their individual results, written for a non-clinical audience. Participants will be informed of the full study results through the university website (https://sprinz.aut.ac.nz/areas-of-expertise/sports-kinesiology-injury-prevention-and-performance/female-athlete-performance-and-health-research-programme).

7.5. Discussion

There is a lack of female specific sports science and biomedical research [11-13, 15, 16, 468, 469]. Previous studies investigating candidate biomarkers for concussion have either been conducted in exclusively male cohorts or have not considered the influence that fluctuations in circulating sex hormones may have on the expression of salivary biomarkers. This protocol is important as it will help to determine whether females with a suspected concussion have similar salivary sncRNA expression to those reported in the literature for male athletes [457] and highlight any potential for false negative or positive results when considering salivary biomarkers as a diagnostic tool for females with suspected concussion. If this study confirms presence of sncRNAs in females with concussion, it will provide evidence of the potential of saliva sampling as an objective tool to aid in diagnosis of and confirmation of recovery from concussion. Findings will highlight whether expression and ratio of two sncRNAs may be associated with sex hormones in females and whether this tool could be further investigated for routine clinical use in this cohort. Findings will also highlight how different hormone profiles may be associated with concussion symptom severity and duration in females.

7.5.1 Strengths and limitations of the study

This is the first study to consider the impact of fluctuating female sex hormones on salivary sncRNA expression following concussion. Limitations of this study are that it relies on self-report hormone profiles and does not include verification using the 'gold standard' approach [42]. Menstrual cycle information is based on recall which can be subject to bias and inaccuracy. Return to work is being used as a proxy for recovery from concussion which does not necessarily reflect full physiological recovery. Information regarding treatment interventions and follow up appointments for each participant will not be collected, it will therefore not be known how the ongoing management may influence the recovery time. One further limitation could the relatively small sample size compared the number of variables under investigation. The power calculation is based on the primary aim of the study.

Chapters 7 and 8 linking section

The protocol was accepted and implemented in the emergency department (ED) environment in the Wellington region. The study protocol was designed with input from key ED staff however, data collection was slower than anticipated due research activity becoming lowest priority when the ED was under-resourced.

Since the inception of data collection there was a minor change to the protocol to simplify the participant information sheet (PIS) and consent form. Initially the PIS was multiple pages long and included very detailed information (as a requirement for the ethics application). However, this became a barrier for some potential participants struggling with the impact of concussion, and it was also too time consuming in a busy ED environment. A more practical summary sheet was developed in collaboration with ED staff supporting study recruitment so that patients could quickly read and understand what was involved in the study and decide if they wanted to participate. The more comprehensive PIS is still provided for participants to take away and read in their own time.

The study has been well received by senior ED staff and seen as a valuable way to raise awareness of concussion and provide the opportunity for all staff to be involved in research.

The original protocol documented using the backward count method to determine phase of the menstrual cycle at point of injury in those females with a natural menstrual cycle (NMC). However, when all data were collected, it became evident that this approach would be inaccurate due to the cross-sectional nature of the information. Date of injury was captured, and each participant self-reported the number of weeks since the start of their last menstrual cycle (MC). Due to not having information about the length of each participant's previous MC and not collecting follow up information of the next MC it was not possible to estimate the phase of the MC at point of injury. For example, a participant injured on 12.05.24 reporting a menstrual cycle start date '3 weeks ago' could have been mid-cycle or could have been in the follicular phase, dependent on how long the MC was. In addition, the a priori calculation assumed that an equal number from each phase would be present. When looking at the complete datasets it was more logical and accurate to group the data as oral contraceptive (OCP), Progestin only (PROG) which included the Jadelle implant and the Depo-Provera injection and then to have a natural menstrual cycle group. This provided more certainty about comparing two different delivery methods of exogenous hormones with endogenous hormones.

Chapter 8. The female RNA in concussion (FeRNAC) study: Assessing Hormone Profiles and Salivary RNA in Females with Concussion by Emergency Departments in New Zealand: A prospective cohort study

This chapter has been submitted and is under review as:

Hardaker, N.J., King, D.K., Hume, P.A., Sims, S.T., Basu, I. The Female RNA in Concussion (FeRNAC) study. Article submitted to: *Journal of Head Trauma Rehabilitation.*

8.1 Overview

Objective: To evaluate the association between hormone profiles and time to return to learn/work (RTL/W), symptom severity and salivary miR-27a-5p/miR-30a-3p following concussion. **Setting:** Emergency Department (ED). **Participants:** Based on an a priori power calculation, 36 females aged 28.8±7.5 (17-44 years) with a confirmed diagnosis of concussion presenting within 72 hours of injury. **Design:** Prospective cohort study. Participants were grouped according to one of three hormone profiles: n=20 natural menstrual cycle (NMC); n=8 progestin only hormonal contraception (PROG); and n=8 oral contraception (OCP). **Main measures:** Participants provided a saliva sample for measurement of miR-27a-5p/miR-30a-3p, and online survey responses for symptom scores at 7-day intervals after injury until they re-ported RTL/W. **Results:** The overall mean symptom score at time of presentation was 47.0±23.7 (8-100), and mean time to recovery was 27.3±33.1 (2-179 days). Cox hazard regression revealed a statistically significant association of hormone profile on time to RTL/W. PROG (HR 2.5, 95%CI: 1.0-6.1, p=0.048) and OCP (HR 2.7, 95%CI: 1.1-6.4, p=0.027) were significantly associated with increased likelihood of RTL/W. Initial symptom score was not significantly associated with time to RTL/W (p=0.628). There were no statistically significant mean differences between groups for initial symptom score ($F_{2, 33} = 1.755$, p=0.189). Fourteen saliva samples provided a full miR-27a-5p/miR-30a-3p measure and were included in sub-group analysis. The mean miR-27a-5p/miR-30a-3p was 0.84±0.06 (0.75-0.92). There were no statistically significant mean differences between groups for expression of miR-27/miR-30 ($F_{2, 11} = 0.519$, p=0.609). **Conclusion:** Females using either form of contraception were 1.0-6.7 times more likely to achieve earlier RTL/W than NMC at any given time. Hormone profile, but not initial symptom score, was a predictor of time to RTL/W. Salivary miR-27a-5p/miR-30a-3p may be a useful biomarker in females with concussion and warrants further research.

8.2 Introduction

Concussion is a brain injury induced by direct or indirect biomechanical force being transmitted to the head via a blow to the head or the body. Disturbance in brain cellular homeostasis initiates complex biochemical

and neurometabolic changes[370] resulting in a transient 'energy crisis' in the brain. Concussion is a functional rather than structural injury and presents as a variable cluster of physical, cognitive, emotional, and sleep related symptoms. Sex differences are evident in concussion; females often having more severe symptoms that are longer in duration when compared with males.[447] Underlying mechanisms for these observed sex differences are not yet well understood. Limited data show that female hormone profile may be associated with recovery outcomes.[295, 470] Primary female sex hormones, estrogen and progesterone have a modulatory effect on multiple body systems including temperature regulation,[452, 454] macronutrient metabolism,[362, 453, 454] central nervous system fatigue,[454] and brain bioenergetics.[362, 453, 454] During reproductive years, estrogen and progesterone fluctuate across the menstrual cycle, which typically lasts 21–35 days.[31]

Diagnosis of concussion may not always be clear; assessment includes use of neurocognitive and physical tests and subjective symptom report. Immediate and early postinjury symptoms are reported as best predictors of recovery.[370] However, athletes or those that are subject to domestic violence may hide their injury and symptoms. Research investigating potential biomarkers of concussion, have identified salivary miRNAs that can accurately discriminate between clinical diagnosis or absence of concussion,[423, 433, 457] more specifically the ratio of two miRNA has shown greater accuracy[433] and is advantageous because it does not require baseline measurement. These miRNAs could provide a useful objective adjunct as a sideline test and in the clinical environment to aid concussion diagnosis. However, these studies have focused on male athletic populations. Given miRNA expression in other central nervous system diseases is sex dependent [463] and there is a potential modulatory effect of sex hormones on concussion outcomes, findings of studies in male athletes cannot be extrapolated to female populations without further investigation.

This paper presents findings from a previously published study protocol,[471] the aims of which were to: 1) Determine if time to recovery (in days) differs among females categorised by their hormone profiles at the point of concussion injury; 2) Examine whether salivary miRNA ratio differs across females categorised by their hormone profiles at the point of concussion injury; and 3) Assess whether early post-injury self-reported symptoms differ among females categorised by their hormone profiles at the point of concussion injury.

8.3 Methods

The study was performed in accordance with the ethical standards of the Declaration of Helsinki given ethics approval was obtained from the Health and Disability Ethics Committee (HDEC #2021 EXP 11655), Auckland University of Technology Ethics Committee (AUTEC #22/110) and locality consent through Hutt Valley hospital research office on 24th November 2021. Written informed consent was obtained from all participants.

8.3.1 Study design

The prospective cohort study recruited female participants from an Emergency Department (ED) in Lower Hutt, New Zealand. This study used a previously published protocol[471] and included an a priori sample size calculation that estimated 30 participants were needed to have adequate power to examine the primary study aim and detect a hazard ratio of 1.5 (log hazard ratio coefficient = 0.4055), with 80% power, and type I error rate of 0.05. This was calculated assuming a covariate standard deviation (SD) of 1.5, multiple regression R^2 (variable of interest regressed on covariates) of 0.2, and expected event (clinical recovery) rate of 0.9 within the six months follow-up.[295, 328] To allow for 20% drop-out rate during follow-up, 38 participants were recruited.

8.3.2 Participants

Thirty-eight females of reproductive age (16–44 years) presenting to ED within three days of injury and receiving a confirmed concussion diagnosis were recruited by ED healthcare practitioners. Diagnostic criteria for concussion were those outlined in the most recent international consensus statement from the concussion in sport group[370] Full study inclusion/exclusion criteria are shown in Table 8.1. The routine concussion assessment and study flow is detailed in Figure 8.1.

Table 8.1: Study inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Females of reproductive age: 16–42 years (with a menstrual cycle for a minimum of 2 years if naturally cycling)	Had three or more previously diagnosed concussions
Natural regular menstrual cycle 28–35 days long, or	Current concussion was more than 3 days ago
Currently using hormonal contraception^	Post-menopause
	Started taking medication that would alter reproductive hormone concentrations (Corticosteroids, e.g., Prednisone. Antidepressant or Antipsychotic medication) within the last 3 months
	Current clinical diagnosis of an eating disorder
	Pre-diagnosed psychiatric disorder
	Pre-diagnosed neurological condition
	Polycystic Ovarian Syndrome (PCOS)
	Oligomenorrheic (irregular periods), or

	Amenorrheic (loss of periods for 3 months or longer)
Confirmed diagnosis of concussion by a medical doctor (within 3 days of injury)	GCS* score less than 15 at 4 hours after initial assessment. These patients may be referred for a CT scan and will not be eligible for the study.

*GCS = Glasgow Coma Scale is used to objectively describe the extent of impaired consciousness in all types of acute medical and trauma patients. In the context of acute brain injury scores of <8=Severe, 9–12=moderate, 13–15=mild. ^The published protocol stated Oral Contraceptive Pill, this was broadened to include all forms of hormonal contraception.

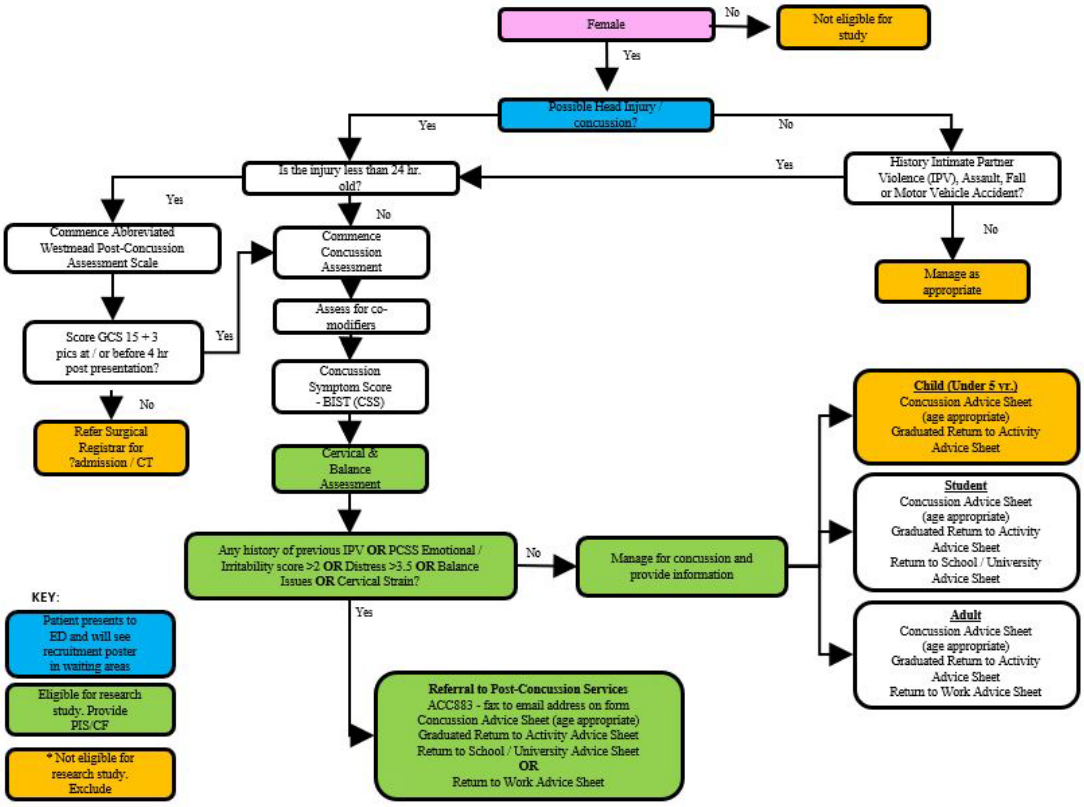

Figure 8.1: Routine clinical assessment and study flow.

Given that interpersonal violence (IPV) disproportionately affects females, to ensure safeguarding of any participants in this study that may have been vulnerable, the Ministry of Health Family Violence Assessment and Intervention Guideline were utilised.[466]

8.3.3 Procedures and measures

Due to logistics, adopting best practice research methodologies and refinement of the technique for evaluation of miRNA it was necessary to make modifications to the published protocol including; storage of saliva samples, recategorization of hormone profile, the endogenous control gene used in miRNA analyses.

Data collected from participants included a 2 ml saliva sample to measure miRNAs and two different follow-up surveys administered online weekly until the participant reported full return to learn/work (RTL/W). Saliva samples were stored in the ED freezer at −20°C and then transferred to the −80°C freezer within three months of initial collection and were stored until the point of processing and analyses.

8.3.3.1 Hormone profile

Hormone profile groups examined were: 1) natural menstrual cycle (NMC); 2) Progestin only contraception which included the Jadelle implant and the Depo-Provera injection, (PROG); and 3) oral contraceptive pill (OCP). The PROG and OCP groups were examined separately (rather than as one contraceptive group) due the different delivery mode and pharmacokinetic properties of each method. The NMC group was examined as one hormone profile group rather than three separate phases outlined in the original protocol due to the lack of certainty of phase estimation when applying the backward calculation method particularly when estimating 'mid-cycle'. For example, a participant with a date of injury 13.11, completed the LEAF_Q 17.11 and reported her last period as '2weeks' ago. Therefore, she could have been anywhere from day 4-10 of her cycle on the day of injury.

8.3.3.2 MicroRNA expression analysis

For consistency with the previous study[423, 439] utilising miR-27a-5p/miR-30a-3p ratio for differentiating concussed from non-concussed individuals, miR-16-5p was used as the reference gene. As miR-16-5p only amplified in 34% samples, a second suitable reference gene,[439] miR-25-3p was used and amplified in 69% samples. In samples where both reference genes were amplified, the ratio values were identical. Therefore, miR-25-3p was used as the endogenous control for all samples included in final analyses. Details of information collected each week is included in Table 8.2.

Table 8.2: Research measures and collection points.

Measure	How	Who	When		
			Initial assessment	During recovery (7-day time intervals)	Full return to work/study
Saliva Samples	Passive drool into cryovial	Consulting clinician, research assistant or Lead Researcher	X		
Number of prior concussions#	Online*	Participant to complete		X	
Sport or non-sport related concussion#	Online*	Participant to complete		X	
LEAF-Q	Online*	Participant to complete		X	
BIST	Online*	Participant to complete	X	X	
Number of days from injury to recovery (full return to work/study)	Online*	Participant to complete			X

* Weblink emailed to participant; # question only asked once, at first (7-day) timepoint.

8.3.4 Statistical Analyses

All data were captured, cleaned and coded for analysis in Microsoft Excel 2024 (version 16.0.18324.20092). Data were imported into JASP (Version 0.19.3; JASP Team, 2020) statistical software for analysis. To examine associations between hormone profile or initial symptom score, with time to return to learn/work (RTL/W), a Cox proportional hazards regression was conducted. The model assumed that the hazard ratios were constant over time (proportional hazards assumption). The time-to-event variable was defined as the duration from point of injury to RTL/W in days. The proportional hazards assumption was assessed using Scaled Schoenfeld residuals and verified model adequacy through log-minus-log plots and concordance statistics. Hazard ratios (HRs) with 95% confidence intervals (CIs) were reported. Two separate one-way ANOVA were used to determine whether there were significant mean differences between hormone profile groups for initial symptom score and miR ratio. The statistical significance level was set at $p<0.05$ for all analyses.

8.4 Results

Of 38 participants recruited, one was excluded due to using the hormonal contraceptive coil and one had one set of data excluded due to having already participated in the study with a prior concussion. Table 8.3 outlines the demographic information of the remaining 36 participants. The mean LEAF_Q score was 4.8

±2.6 (2-10). A LEAF_Q total score ≥8 (in combination with an injury score≥2 and/or menstruation dysfunction score≥4) was used to identify those having symptoms of LEA.[378] Based on these scores, no participants were considered at risk of LEA. Twenty-seven percent of participants reported history of amenorrhea. One participant was a full-time mum, therefore her RTL/W was taken as the number of days until her symptoms no longer impacted on her ability to achieve all daily tasks and in doing so there was no provocation of her symptoms.

Table 8.3: Demographic information.

	Age (yrs) M±SD (range)	Hx Conc*	Symptom Score M±SD (range)	RTL/W (days) M±SD (range)	miR ratio M±SD (range)
NMC (n=20)	30.5±8.4 (17-44)	10	53.2±21.6 (14-100)	37.8±40.9 (7-179)	0.85±0.06 (0.75-0.92)
PROG (n=8)	29.4±6.5 (19-35)	4	36.0±24.0 (8-77)	14.0±10.6 (2-35)	0.81±0.02 (0.79-0.82)
OCP (n=8)	24.1±4.3 (20-33)	4	42.4±26.4 (11-92)	14.4±10.9 (6-39)	0.85±0.08 (0.79-0.90)
Overall (n=36)	28.8±7.5 (17-44)	18	47.0±23.7 (8-100)	27.3±33.1 (2-179)	0.84±0.06 (0.75-0.92)

All data are presented by group count unless otherwise stated. NMC, natural menstrual cycle; PROG, progestin only contraception; OCP, oral contraceptive pill; M(±SD), mean (standard deviation); Hx Conc, history of concussion; RTL/W, return to learn/work.*only reported in 34 participants.

8.4.1 Hormone profile and time to RTL/W

Analysis of 36 participants showed RTL/W was achieved within mean follow-up of 27.3 ±33.1 (2 to 179) days. Proportional hazards assumptions were tested using Scaled Schoenfeld residuals and found to be met. There was a statistically significant association of hormone profile on time to RTL/W. Both PROG hormone profile (HR = 2.5; 95% CI: 1.0 to 6.1, *p*=0.048) and OCP hormone profile (HR = 2.7; 95% CI: 1.1 to 6.4, *p*=0.027) were significantly associated with increased likelihood of RTL/W (figure 8.2). Initial symptom score was not significantly associated with time to RTL/W (*p*=0.628) indicating that females using either form of contraception were 1.0 to 6.4 times more likely to have achieved RTL/W than those in the NMC group at any given time and that hormone profile, but not initial symptom score was a predictor of time to RTL/W. Given the wide confidence intervals and that the lower value was 1.0, there was uncertainty about the magnitude of the effect.

Outlier Assessment:

There was one participant in the NMC group with an extreme time-to-event value (179 days). There was no evidence from the Schoenfeld residuals and log-minus-log plots that proportional hazards assumptions had been violated. However for transparency a “leave-one-out” check or exclusion analysis was undertaken and

showed that there was still a statistically significant association of hormone profile on time to RTL/W, but only for OCP hormone profile (HR = 2.4; 95% CI: 1.1 to 5.8, *p*=0.049) which was significantly associated with increased likelihood of RTL/W when compared to NMC. PROG hormone profile was not significantly different to NMC (HR = 2.3; 95% CI: 0.95 to 5.7, *p*=0.064). Given that the observation was valid, it was not considered a data error and did not materially affect model fit or assumptions and HR changed by <2%, it was retained in all analyses.

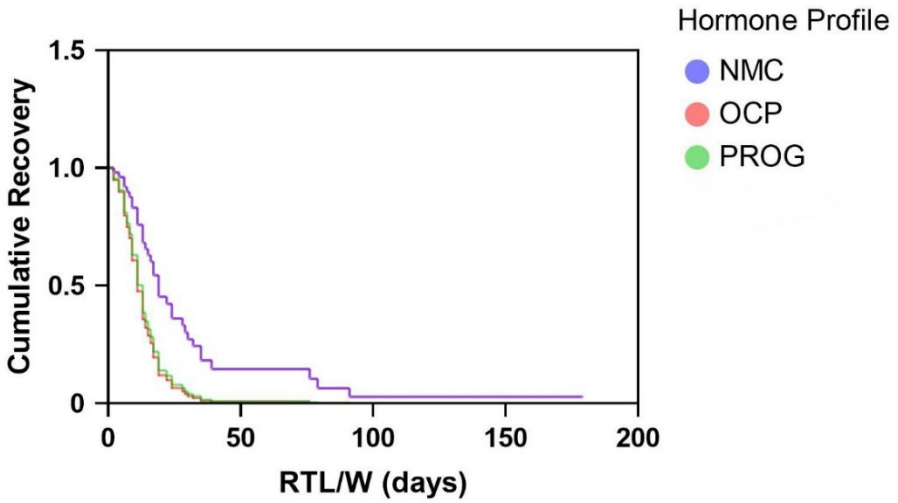

Figure 8.2: Recovery probability by hormone profile. NMC, natural menstrual cycle; OCP, oral contraceptive pill; PROG, progestin only contraception; RTL/W, return to learn/work.

8.4.2 Expression of miRNA ratio

Of 36 samples collected, 29 (81%) amplified miR-27a-5p, 14 (39%) amplified miR-30a-3p, therefore only 14 samples that amplified both miRNA and could provide a ratio were included in data analyses. Levene's test was non-significant (*p*=0.223), indicating that the assumption of homogeneity was not violated. No deviations were noted in normality checked with a Q-Q plot. There was no statistically significant difference between groups for expression of miR-27/miR-30 ($F_{(2, 11)}$=0.519, p=0.609).

8.4.3 Symptoms

Levene's test was non-significant (*p*=0.732), indicating homogeneity was not violated. No deviations were noted in normality checked with a Q-Q plot. There was no statistically significant difference between groups for initial symptom score ($F_{(2, 33)}$=1.755, *p*=0.189).

8.4 Discussion

8.4.1 How hormonal contraceptives downregulate endogenous hormones

Hormonal contraceptives downregulate endogenous hormones through inhibition of the hypothalamic-pituitary-ovarian axis.[472] The implant is a long-acting reversible contraceptive (LARC) administered subcutaneously that delivers low-dose, steady-state concentration of progestin. The contraceptive injection is administered intramuscularly, works in the same way as the implant and is effective for between eight and 13 weeks.[473] Both methods are parenteral[473] therefore enter the blood stream directly and do not undergo first pass metabolism in the liver.[474] The OCP is ingested, undergoes first-pass metabolism prior to being released into systemic circulation, and needs to be administered daily to maintain serum concentrations of exogenous hormones at levels sufficient to provide contraceptive efficacy.[474] The OCP primarily interrupts the feedback loops between the hypothalamus, pituitary and the ovaries due to the synthetic hormones in the blood stream whereas the progestins in the implant/injection can also bind to progesterone receptors in the hypothalamus. In addition, contraceptive progestins appear to alter the serotonergic system, and modulate inhibitory GABA-ergic signalling in the brain.[475] Importantly, brain interaction and effect on mood and cognition may be different between the two contraceptive methods and still not fully understood.[476]

8.4.2 Associations of hormone profile and symptom severity with time taken to return to learn/work

Females in this study using either form of contraception (OCP or PROG) were at least two times more likely to have achieved RTL/W than those in the NMC group at any given time. There was no statistically significant association between initial symptom score and RTL/W, indicating that hormone profile and not symptom score was a predictor of time to RTL/W. There was a non-significant trend towards a higher early post-concussion symptom score in those participants in the NMC group. These findings were somewhat consistent with other studies[295, 367, 470] investigating whether HCs or menstrual cycle phase at point of injury affect post-concussion outcomes, all of which reported that females using HC or in the follicular (low hormone) phase of the menstrual cycle had better QoL scores,[295] lower symptom scores[295, 367, 470] and a trend towards shorter length of recovery.[367, 470] This may not necessarily reflect that HC offers a neuroprotective effect or that females using HC physiologically recover faster, rather that HC may modulate symptoms that these females experience. It is also possible that hormone related symptoms are misattributed to concussion during recovery form the injury.

Given that some females use hormonal contraception to reduce pre-menstrual symptoms,[473] that females respond differently to various forms of hormonal contraception available, and some experience side-affects and others don't, it is plausible that females in this in study self-selected the form of hormonal contraception that was optimal for them, with minimal side-affects and general reduction in symptoms. This participant bias would have been reflected in these study results. In addition, two different studies[295,

[367] reported that females in the menstrual cycle follicular phase (FP) at point of injury also had better outcomes when compared to those in the luteal phase (LP) and that there were no differences between FP and HC.[295] Collectively these studies suggested that the low endogenous hormone state may be associated with lower symptom severity and shorter length of recovery. However, given that length of recovery is typically measured by RTL/W or return to sport and that this is informed by the subjective symptom report, it remains unclear whether this reflects a true physiological recovery or only lessening of symptoms. Early postinjury symptoms are cited as the best predictor of recovery[370, 371] and that females are at greater risk for having symptoms that persist for more than one month.[371] Yet, 80% of data informing consensus statements is still based on males[13] and a seemingly overlooked data point from one study with 90 participants[470] showed symptom severity was a strong predictor of recovery time in males ($r=0.051$, $p<0.01$) but not females ($r=-0.003$, $p>0.05$). The current study builds on this finding showing that symptoms were not a significant predictor of time to RTL/W, but hormone profile was; in those females using contraception time to RTL/W was shorter.

8.4.3 Salivary miR ratio

Sex differences in post-concussion symptoms have been hypothesised to stem, in part, from reporting bias from females. A different perspective is that both males and females report their concussion experiences, which differ in nature and response. Both may be equally susceptible to deliberate non-disclosure or hiding of symptoms. Being focused on symptoms as a predictor or marker of recovery is probably insufficient and the ambiguity around symptom reporting, and experience highlights the need for more objective measures of concussion and recovery. A novel aspect of the current study was inclusion of sampling and measurement of the ratio of concussion specific salivary miRNA (miR-27a-5p/miR-30a-3p) in a female cohort presenting to ED. Of 36 samples collected only 14 (39%) amplified both miRNA (miR-27a-5p and miR-30a-3p) of interest and the endogenous control gene. In those 14 samples, all miR-27a-5p/miR-30a-3p ratios were less than 1; consistent with findings of Hicks[423] for being indicative of concussion. Comparing mean ratio values across hormone profile groups revealed no meaningful differences; this suggested that miR ratio could be a useful objective measure of concussion in females and that it was not affected by circulating exogenous hormones. Further research examining different menstrual cycle phases would be needed to confirm whether fluctuating endogenous hormones affect this measure. In addition, given that 76% of samples amplified miR-27a-5p but not miR-30a-3p, it may be there are other factors that can affect regulation and abundance of miR-30a-3p and whether it is amplified or not. Differences in experimental design affect miRNA expression. From technical or methodological perspectives, saliva collection method and storage, miRNA extraction and isolation, and miRNA expression profiling and aligner technique (in next generation sequencing) must be considered. Use of RNA stabiliser and the saliva collection method are two technical factors that account for most variance.[439] In the current study, RNA stabilizer was used however

the method of sample collection was passive drool which may have affected the variance in whether the target miRNA was expressed and could be amplified. In addition, miRNA expression may be dependent on participant factors including age, sex, prandial status, exercise and BMI[439] and there is also evidence of diurnal variation in salivary miRNAs.[477] Further research is needed to achieve consistency in the methodological approach to salivary miRNA measurement, the exclusion of miRNAs confounded by numerous biologic factors, and to identify appropriate miRNA controls.

8.4.4 Strengths and limitations of the study

This study considered whether hormone profile (menstrual cycle, contraceptive use) affected recovery time and salivary miRNA expression following concussion and contributed to addressing gaps in understanding female-specific responses to concussion. However, there were some study limitations that must be considered when interpreting the results. Return to learn/work (RTL/W) was a proxy for recovery from concussion and did not necessarily reflect full physiological healing of the brain. Information regarding treatment interventions and follow up appointments for each participant was not collected, therefore it was unknown if or how ongoing management may have affected overall outcome. Hormone profile categorisation was based on self-report responses to the LEAF_Q and did not include verification with any objective measurement. Data from those participants with natural menstrual cycles were analysed as one group and did not examine whether a specific menstrual cycle phase affected any outcome variables. The hormonal contraception included in analyses were the oral contraceptive pill (OCP) and the progestin only implant or injection, which means the findings may not apply to females using IUD, IUS or vaginal ring. Those using oral contraception were analysed as one group and therefore the different types of OCP were not examined. Despite use of self-report information in this study, the LEAF_Q is a valid questionnaire that provides a degree of standardisation to the answers and although there is no quantification of hormone concentrations, what is known is that circulating levels of endogenous hormones would be lower and more stable in both contraceptive groups when compared to the NMC group. Only 14% of saliva samples amplified all miRNA, therefore the findings from the data analysis of these 14 samples were limited due to the reduced sample size. The study strengths were the prospective data collection following a previously registered protocol[471] that included a sample size based on an a priori power calculation and the study recruited enough participants to reach statistical power.

8.4.5 Recommendations, future direction and call to action

Earlier studies[367, 478] investigating hormones and concussion have made recommendations that clinicians include hormone profile as part of clinical assessment, but this is still not considered or mentioned in concussion consensus statements,[370, 387] which may be due to data from these studies being of limited quality combined with the recommendation not being clearly actionable. There is risk of repeating the

similar studies and making the same recommendations that are not adopted due to methodological flaws compromising the data quality. With improved consistency and coordination in generating data, research can more effectively inform how clinicians can better manage females with concussion. Ongoing research into how hormone profiles influence concussion experience and recovery has practical value from improving early patient education and developing hormone-informed treatment protocols and recovery timelines, to tailoring return-to-learn/work and return-to-play decisions. This may also involve collaboration with healthcare disciplines not typically included in concussion management, such as endocrinology. Additionally, given the role of endogenous estrogen and progesterone in the function, bioenergetics and control of inflammatory processes of the brain,[362, 479, 480] it may also be possible to develop nutritional interventions to support the brain's ability to recover in varied hormonal environments. Prophylactic and therapeutic nutritional intervention for concussion is an emerging area and compounds under investigation include creatine monohydrate, omega-3 fatty acids, BCAAs, riboflavin, choline, magnesium, berry anthocyanins, Boswellia serrata, enzogenol, N-Acetylcysteine and melatonin.[481] Creatine may be particularly beneficial for brain health in females.[482]

To accelerate understanding of hormonally driven responses to brain injury in females and generate higher quality data there needs to be consistency across future research in definitions, measurement approaches and recovery endpoints beyond symptom resolution. Critically, this study combined with earlier research confirmed that hormone measurement needs to be included in female-specific concussion research, which adds a layer of biological complexity and can be difficult when conducting research in clinical environments, particularly a busy ED. Collecting reliable, objective hormone data is a key component missing from this study and from earlier work and presented one of the bigger challenges. The endocrinology and sport science performance literature clearly articulates gold standard approaches to hormone profile verification, with methods that include calendar counting, urinary measurement of luteinizing hormone (LH) and blood serum measurement of progesterone.[54, 403] This three-step method requires measurement at multiple time points and often before 'testing' or 'intervention' phases to support the predetermined scheduling of the session. In longitudinal studies with multiple data collection timepoints this approach is recommended and should provide data for at least two menstrual cycles before the risk for injury occurring to capture baseline information and intraindividual variability. However, this is not feasible for research investigating effects of hormone profile on incidence and recovery from injury when pre-injury information cannot be collected. In cross sectional or prospective studies with limited follow-up opportunity, as a minimum, a blood-based hormone measurement at point of injury should be taken and it will be necessary to include in the consent information that participants will be contacted within the following 30-35 days to confirm the onset of the next menstrual cycle. While saliva has utility for monitoring hormone changes overtime it is not yet reliable enough for phase verification.[376, 483] The current study also demonstrated that saliva sampling is a feasible non-invasive biomarker sampling method that can be used by clinicians in an ED environment. However,

further work needs to be done to validate the technique and use of a miRNA ratio as a concussion biomarker and continue to understand how that correlates with hormone profile and symptom severity over time. This would be valuable to improve diagnostic accuracy and confirmation of recovery.

There are single and multicentre programmes of concussion research but there is lack of consistency in the approach to female specific concussion research questions. Development of a consensus on methodological considerations for different clinical and research scenarios specific to concussion research in females is needed.

Chapter 9. Summary and conclusions

It has been observed and widely accepted that female athletes have a 4-10 times greater incidence of ACL injuries[25]. Chapter 2 from highlights that there are other differences between the sexes in injury profiles; male athletes tend to have a higher incidence of hip/groin, hamstring injury and upper limb injury, whereas females are more at risk for foot/ankle injury, bone stress injury and sports related concussion. The data also suggest that female athletes experience greater symptom severity and take longer to recover and return to sport following concussion. This difference in concussion is particularly evident in football. This PhD exclusively studied females and aimed to understand how sex hormones may be associated with concussion outcomes. To achieve this aim evidence was provided towards answering the specific questions: 1) How much does baseline symptomology change across the menstrual cycle?; 2) Do salivary concussion biomarkers change across the menstrual cycle in the absence of injury?; 3) Does hormone profile at point of injury predict prolonged recovery from a concussion?; 4) Does hormone profile at point of injury affect the expression of salivary concussion biomarkers? Protocols to support data collection were developed and tested in the absence of injury to inform the design of the final study. The findings of each individual study within this PhD have been discussed at length in the respective chapter. To avoid repetition, a general summary discussing the key themes that are common across multiple chapters of this thesis is provided below combined with a discussion of the practical implications of the thesis overall.

9.1 Key findings

The common themes emerging across the chapters of this thesis were: (1) the need for the approach to concussion to acknowledge the distinct baseline characteristics and lived experiences of females are different and should not be measured against males; and (2) the methodological challenges associated with collecting high-quality data when applying menstrual cycle-related research protocols in free-living populations.

9.1.1. Theme 1 – Key aspects of females (with concussion) – Attitudes towards the injury, symptomology, reporting behaviour, biomarkers, hormone profile and experience.

This PhD uniquely investigated how different hormonal profiles affect recovery time and concussion biomarker expression. This approach introduced a layer of biological complexity that has been overlooked in prior research. Most concussion research has historically focused on male populations or has not stratified by hormone profile.

Concussion research, education, and management for females needs to recognise their unique baseline in areas such as knowledge and attitudes for education delivery, symptom monitoring and reporting, and careful interpretation of salivary biomarker data.

The increased incidence of concussion in female athletes has been attributed to:

1. Females are more honest at reporting the injury and the symptoms than males [259].
2. Female sex hormones may affect outcomes following concussion [294, 295].
3. Weaker neck muscles in females may result in decreased absorption capability [318].
4. Differences in Cerebral Blood Flow (CBF) pre- and post-injury [319].
5. Females have smaller neuroanatomy more susceptible to damage and dysfunction [293].

The above hypotheses assume males as the reference group or the 'norm'. However, it may be more appropriate to recognize that males and females experience and respond to the injury differently. Neither sex should serve as the default reference point. Instead, each should be considered as starting from—and returning to—a distinct physiological and experiential baseline.

Chapter 3 evaluated differences in concussion knowledge and attitudes (including intention to report) between male and female football players and found that male players had a notably higher overall concussion knowledge index (CKI) than females. Females with a history of concussion had higher CKI than those with no history of the injury but this trend was not observed in males. In addition, when examining the sub-sections of the questionnaire, only 30 % of men knew that concussion could impact *'long-term health and well-being*' versus 95 % of women and; only 32 % of men knew that playing with concussion would impact performance, whereas 95 % of women recognised that this would be detrimental yet over a third of both men and women players indicated that they would play on with symptoms of concussion. Although this study had a small sample size, this did highlight a possible area for future research to further investigate if the barriers to translating knowledge into the desired behaviours are different between men and women. A study [348] investigating the experiences of 365 women and 247 men with not reporting concussions that occurred during sport found that overall, 78% of men and 69% of women were likely to continue playing through a suspected concussion without reporting it, and that, when considering why these athletes did not report concussion, it was evident that females were more likely to not report based on the "pain principle," an ideological belief that athletes must play through pain to be valued. Whereas for males the primary reason was due to team allegiance. These subtle differences are important to understand when developing and delivering concussion education. Gendered environmental factors and cultural norms may play a substantial role in reporting behaviour. One of five key themes identified in a study of retired female athletes [484] was that gender stereotypes embedded in the sport system contribute to minimising injury concerns, creating barriers to early diagnosis and treatment (e.g., "They didn't say it's in your head, but essentially that was the diagnosis.") highlighting that females don't feel that their concerns or injuries are taken seriously. To a degree this is illustrated by the hypothesis that the greater incidence of concussion in female athletes is due to 'more honest' reporting, this assumption does put females at risk of not having the injury or their symptoms taken seriously enough and consequently not seeking or receiving care. Symptoms are also subjective and cannot easily be quantified, concussion

symptoms are non-specific and many are congruent with those experienced across the menstrual cycle. Chapter 4 shows that in the absence of injury, the number of symptoms reported and the magnitude of change in day-to-day symptom score is similar to that which would meet one of two diagnostic criteria for concussion. The clinical interpretation and management of symptom change in the ongoing monitoring of recovery from concussion in females may therefore need to be different to that of males. Symptoms are often cited as the strongest predictor of prolonged recovery [370, 371] but this is based on predominantly male data [13] and a seemingly overlooked data point is that in collegiate athletes, symptom severity was strongly related to recovery time for males ($r = 0.513$, $p < 0.01$) but *not* females ($r=-0.003$, $p > 0.05$) [470]. The preliminary findings of the FeRNAC study (Chapters 7 and 8) also highlighted that in an all-female cohort, hormone profile may be a predictor of time to recover more so than initial symptom score. Collectively this this research highlights that symptom score may a be a less useful predictive metric in females and also that their symptom reporting shouldn't be judged based on what has been measured and observed in males. Further reinforced by a recent narrative review [485] articulating that the dominant understandings of concussion, construct the male experience as the 'norm' and socially determined and female experience as being "other". This "othering" results in an exclusive focus on physiological explanations for female experiences; however, these experiences are likely a complex interplay of social, morphological, biomechanical, physiological, and psychological factors. Furthermore, "othering" may overlook the fact that there is variation within female cohorts, as demonstrated by the range in symptom scores and time to return to learn/work in chapter 8.

A more objective measure of concussion diagnosis could alleviate some of the outlined challenges. The application of salivary miRNA as a diagnostic and prognostic tool in concussion is an emerging area and the non-invasive nature of saliva sampling makes it more accessible than serum-based biomarkers [486]. The FeRNAC study (chapters 7 and 8) examined salivary miRNA in acutely injured females presenting to ED with concussion and builds on a previous body of research [423] that utilised next generation sequencing (NGS) to identify that the ratio of two miRNA, 27a-5p/miR-30a-3p, was the most accurate for differentiating concussed from non-concussed patients. Measuring the ratio of two miRNA is ideal for clinical environments like the ED where a pre-injury baseline measurement for comparison to the injury sample is not possible. The miRNA analysis in this PhD employed quantitative Polymerase Chain Reaction (qPCR) which is the method used to validate findings from NGS for clinical use. Over 60% samples did not return a full result, primarily because miR-30a-3p could not be amplified from the saliva samples as discussed in chapter 8. A further technical finding of the studies in chapters 6 and 8 was that the intended endogenous reference gene for normalization, miR-16a-5p selected due to it's abundance, high expression and stability [439], also only amplified in 55% of samples collected across the two studies. Recent research [487, 488] has shown that the COVID-19 virus may be associated with the dysregulation of miR-16a-5p. Although the data are limited this highlights a further unexpected impact of COVD-19 that needs to be considered. An

alternative reference gene, miR-25-3p, and was amplified in 69% of samples and therefore used in the final analysis. Other technical and individual factors that can affect the expression abundance and amplification of miRNA in saliva are discussed in chapter 8 and have been previously outlined [439]. These factors are similar to the challenges with blood-based protein biomarkers [486]. The results of the FeRNAC study were consistent with the earlier work [423], all participants had a miR ratio is less than 1.0, which was the threshold for concussion diagnosis. However, the exploratory work done in *chapter 6* of this thesis showed that in the absence of injury, the ratio of 27a-5p/miR-30a-3p in saliva demonstrated a cyclical pattern across consecutive menstrual cycles in a range between 0.73-1.3. It was unexpected that this participant had miR ratio values below 1.0 as the participant did not have a recent concussion, but did have a history of concussion that occurred and resolved more than 12 months prior to the start of the study. The lowest miR ratio values were observed in the shorter menstrual cycle which *may* suggest that sub-clinical menstrual disturbances impact miR ratio or that the stressor causing the menstrual disturbance also affects miR ratio. Given that the role of miRNA is to regulate cellular processes including modulating inflammatory pathways, and that a change in miRNA expression can be indicative of disease or dysfunction [477], it could also be hypothesised that the values below 1 were due to the previous concussion and that they will take longer to return to +1.0. That doesn't necessarily account for the cyclical variation observed and may potentially be reflective of neuroinflammatory and neuroregenerative processes that are ongoing. This highlights that 1) further work is needed to refine the qPCR technique, 2) miR ratio references ranges need to be confirmed for age, sex, ethnicity, 3) the time course and dynamics of salivary miRNA expression beyond the acute and sub-acute period post injury period need to be investigated, 4) other miRNA specific to concussion may still need to be explored through NGS. These points could also apply to other concussion biomarkers. Recently a new TBI classification framework [489] has been proposed and includes four pillars; the clinical pillar (full GCS and pupillary reactivity), a biomarker pillar (blood-based measures), an imaging pillar (pathoanatomical measures) and a modifier pillar (features influencing clinical presentation and outcome; CBI-M). The biomarker pillar recommends acute (<24 h) post-TBI measurement of one or more of the following three biomarkers: glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase L1 (UCH-L1), or S100 calcium-binding protein B (S100B). This recommendation was based on the diagnostic and prognostic utility of these biomarkers in acute care settings. The research on these biomarkers is predominantly male data, does not account for hormone profile in female participants. A study in trauma patients with mTBI [490] did evaluate sex differences in patterns of GFAP and UCH-L1 release over the seven days post-injury, although the pattern was similar between the sexes, there were significantly higher concentrations of UCH-L1 in males at several timepoints post-injury. Despite this, it was concluded that the overall diagnostic accuracies of both GFAP and UCH-L1 over time for detecting mTBI and CT lesions were not significantly different between male and female trauma patients. However, this study did not account for hormone profile in female patients, females were considered as one homogenous group, yet the age range was 18-79,

therefore the hormone profile would have varied considerably within that sample. The studies in chapter 6-8 introduce this layer of biological complexity for salivary miRNA that has been largely overlooked in concussion and biomarker research. GFAP, UCH-L1 and S100B have gained the most attention and advancement recently being included in TBI frameworks and guidelines [489] however a recent systematic review [420] evaluating fluid biomarker data supports the use and continued research into salivary miRNA due to their potential diagnostic, prognostic and monitoring utility in concussion. In addition, a systematic review and meta-analysis [486] has shown that there are still some technical aspects that need to be resolved with GFAP, UCH-L1, S100B, some of which are similar to those already outlined for salivary miRNA [439]. This continued research into both blood-based protein biomarkers *and* salivary miRNA may lead to a 'biomarker toolkit' that can be used in different settings and for different cohorts of patients. The approach taken by the American College of Rehabilitation Medicine [387] better accommodates for this:

> *The ACRM diagnostic criteria do not name specific tests, neuroimaging sequences, or blood-based biomarkers to avoid the criteria becoming obsolete with emerging research evidence or advances in technology. This approach has been used in diagnostic criteria for other health conditions.25 We found some limited evidence for the blood-based biomarker glial fibrillary acidic protein (see Evidence Statement #9, online supplemental material, available online only at http://www.archives-pmr.org/), but optimal cut-off scores and timing of blood collection have not yet been established. (Silverberg et al., 2023, p.1351)*

To summarise, over 80% of data informing consensus statements and guiding clinical care still comes from predominantly male cohorts [13]. To address this, it is essential to continue developing a robust female-specific dataset to answer questions related to concussion. In studies involving both sexes, comprehensive sex-based analysis must be conducted to identify where differences are most pronounced. This information could guide strategies for injury prevention, clinical management, and return-to-learn/work, and return-to-sport protocols. To date, few studies investigating the link between hormone profile and concussion incidence and outcome have been undertaken. The link has also not been disproven; it is therefore important to continue research in this area.

9.1.2 Theme 2 – Female research methodologies

There is a well-documented gender gap in sports science and biomedical research [11, 12, 491]. This is due to fewer female participants being recruited into studies and, when females are included, the methodologies and data analysis techniques not adequately accounting for sex as a biological variable. As a result, there aren't enough data to support evidence informed practices across many disciplines including sport and exercise science and medicine. Fluctuating ovarian hormones, estrogen and progesterone, create

physiological variability not apparent in men. There are also different hormone profiles within and between females that change across the lifespan from puberty to menopause and these profiles need to be accounted for in research design [492]. The best practice methodological approach for research focused on menstrual cycle and sex difference related questions is well documented [44, 54, 403] and designed to *objectively* confirm the hormone profile of females. This approach includes three steps: 1) calendar-based counting combined with, 2) measurement of the luteinizing hormone surge via serum or urine, and 3) serum measurement of progesterone with a strict luteal phase verification limit of >16 nmol·L−1 of [54, 403]. This enables researchers to accurately assess any association between hormone profiles and health or performance outcomes, as well as responses to specific interventions. Dependent on the research question, the measurement of serum estrogen may also be required. Confirmation of ovulation is often carried out retrospectively to the participant starting the study, therefore those participants with anovulatory cycles or luteal phase defects (LPD) subsequently need to be excluded from the study or considered as a different group in the final data analysis. This decision will be based on the research question and the final number of participants completing the study. Although the 'gold standard' approach to menstrual cycle research is very important for scientific rigour and accurate interpretation of data, there are some practical implications of its application. In addition, there are other challenges with other aspects female specific or menstrual cycle research.

9.1.3 Recruitment and retention for well powered studies

Across the studies within this thesis there was difficulty in achieving a full sample size. Recruitment and retention of 'free-living' participants is challenging across many fields of research [493]. Study inclusion criteria inherently reduce the number of eligible participants. This thesis aimed to investigate females with a regular, natural menstrual cycle which further limits the pool of potential participants. It is estimated that 50% of females in New Zealand are using some form of contraception [494], 10-15% have endometriosis [495] and 5-10% have PCOS [496]. It is also evident in the literature that 14-25% of women of childbearing age have an irregular cycle. Those females that do fit study inclusion criteria then need to be willing to participate.

Data reported in chapters 4 and 5 are from the same participants recruited for a ~~feasibility~~ study to trial the overall data collection protocol and to test the validity of salivary hormone measurements. The target 'n' was 20 females. Although 30 females were recruited, the study had a low retention rate, of those 30 participants, four were excluded due to having a prior head injury, two exited the study after contracting long COVID, two exited the study due to the time commitment, one exited the study due to falling pregnant, five were excluded due to being on contraceptives and eight attended the introduction session but did not collect any data. As a result, only eight eligible females completed the study.

Of note, are the five participants excluded due to using some form of contraception despite the recruitment information clearly stating that the study was of women with a regular menstrual cycle. It

became evident when these women attended their respective introductory sessions that they were unaware that the withdrawal bleed that occurs with contraceptive use is not menstruation. Similarly in chapter 8, the FeRNAC study, one participant reporting using oral contraception also recorded the start of her menstrual cycle. In the context of the FeRNAC study this just required re-categorisation of that participant rather than exclusion. These examples perhaps demonstrate a lack of 'menstrual health literacy' (MHL). MHL has been highlighted as problematic in other research [497-500]. Recent studies in New Zealand have shown that the overall knowledge score for MHL of physically active women aged 16-40 is considered low (51%) particularly in how the menstrual cycle relates to health [497] and over 60% of those aged 13-25 received little to no information or education about menstruation prior to menarche [499]. An earlier study [501] in New Zealand highlighted a low level of knowledge in young women aged 14-18 around contraception and it's different forms and actions. Since data collection was undertaken for this thesis, there have been two [502, 503] frameworks developed to help athletes, coaches and support staff with consistent terminology and understanding of methods for female-specific health monitoring that can be used in both sports and research [502, 503]. Part of this includes a decision tree that, if stepped through, would ensure a female athlete or research participant self-classified their hormone profiles more accurately as part of a two-step process. These frameworks would be valuable to adopt in recruitment process and participant information packs of future studies. This also provides an education opportunity for female athletes to understand more about their own hormone profile and what it means for them; this education could also serve as a useful retention strategy to encourage ongoing participation in the research process.

There were similar recruitment challenges in chapter 6 which was intended to be a matched-case controlled design in a women's community football team, the players were either using some form of contraception, which reduced the number of potentially eligible participants, or they were reluctant to engage in the research due to the length of commitment required. As such, this became a single-case time series observational study. The participant provided data for three consecutive menstrual cycles, the third cycle was shorter in length (days) than the previous two cycles and could be considered irregular for that person. A shorter cycle may be due to a luteal phase defect (LPD) or anovulation [31]. Given the exploratory single-case nature of the study, the irregular cycle was an important finding and could be factored into the analysis however, in a larger study the data from this menstrual cycle or from this participant would likely have needed to be excluded. An estimated 3-19% of cycles are anovulatory [504] and the 3-month prevalence of LPD in recreationally active women has been estimated to be as high as 48% [505]. In a research context this would be discovered retrospectively and data from these cycles or participants would need to be excluded from or re-categorised in the final data analysis. In menstrual cycle research the above statistics need to be considered when determining the target 'n' to allow for a higher drop-out rate.

9.1.4 Data collection

Given the identified recruitment and retention challenges, it becomes even more important that the data collection protocol is adhered to and completed to optimise the amount of data that can be included in the final analysis and maintain the overall quality of the study. There were two common data collection challenges across the studies in this thesis were i) missing data points, and ii) data collection procedures.

Missing data can compromise the statistical power of a study. Understanding the mechanism behind the missingness is important so that it can been handled fairly in the data analysis. Missing values typically follow one of three main mechanisms:

- Missing Completely at Random (MCAR): The missingness is unrelated to both the observed and unobserved data. It occurs randomly with no discernible pattern. It is most likely inadvertent.
- Missing at Random (MAR): The missingness is related to observed data but not to the unobserved data. It is missing at random but can be related to some features of the observed dataset. For example, it may be related to age or gender.
- Missing Not at Random (MNAR): The missingness is related to the unobserved data itself. The missing data is related to specific patterns or reasons. For example, respondents in a survey may choose not to respond to some questions that are of a sensitive nature.

Chapters 4-6 employed a protocol that required daily use of a menstrual tracking app and self-collection of a saliva sample for three consecutive menstrual cycles. The app-based data included symptom scores, sleep hours and self-rated sleep quality. In chapter 4, there were days where participants did not record their sleep and symptom data in the app. Missing data are a challenge of app-based data collection in research [506]; previous studies utilising menstrual cycle tracking apps [40, 41] have further highlighted this challenge. Li [41] proposed a method to quantify engagement with cycle tracking and although this method was developed to try and separate true menstrual patterns from tracking anomalies, it could also be useful to determine missingness mechanism to decide whether data from a menstrual cycle could be reliably included in the final analysis with the missing values appropriately handled or whether all of the data from that cycle should be excluded. In addition, Dupuit [40] calculated players' adherence (completion rate) to daily menstrual tracking by dividing the number of completion days by the total number of monitoring days; this was also analysed for each month in the study to monitor any changes in adherence with time.

In the context of chapter 4, given the daily collection protocol and the inconsequential nature of the data (i.e it does not determine an outcome or affect social standing for the participant) it is likely that the mechanism of missingness was MCAR and participant's forgot to enter their data on some days. Similarly in chapters 5 and 6 there were missing saliva samples. Participants reported that they had forgotten to collect the sample upon waking or were unable to collect the sample due to travel and being away from home

which meant the sample could not immediately be stored in a freezer, therefore the missingness mechanism was MCAR. Given that menstrual cycle research will often require high frequency data collection and / or self-collection by the participant, to prevent missing data it is recommended that pilot testing clear communication strategies with participants, data monitoring and immediate follow up queries are considered in the study design. A method for handling missing values should also be part of the data analysis plan.

The second challenge was the data collection procedures including scheduling laboratory (lab) visits and collecting the blood sample. In chapter 5, blood samples were collected at two different time points in the participant's third menstrual cycle, this was calculated using the backward calculation method [55] and intended to provide the opportunity to measure two different hormonal concentrations. It can be difficult to co-ordinate the lab visits as the scheduled day needs to be convenient to the participant combined with lab availability (not accessible on weekends). This meant that for some females, participation in the study continued for an additional one to two menstrual cycles to accommodate this co-ordination. A further related challenge was the actual collection of the blood sample, one participant in the study experienced some anxiety around the venipuncture procedure and required an extra visit to overcome this. The pain and discomfort of the venipuncture procedure can also be a barrier to research participation and often induces a stress response [507]. In cross sectional research like the FeRNAC study (chapters 7 and 8) where pre-injury data collection isn't possible and the time point of interest (i.e the injury) cannot be predicted or planned, the most reliable way to collect data pertaining to hormone profile would be a blood-based progesterone measurement combined with asking the participant the start date of their last menstrual cycle, and then following up to ask the start date of the next cycle; this would facilitate the backward-count method to confirm the phase of the cycle at the point of injury. It is therefore recommended that participant information and consent should include that they may be contacted upto 30 days after their study participation to report the start date of their next menstrual cycle. Given the additional complexities of concussion and brain injury, a method that does not rely on recall is preferrable. Although in the absence of physiological measurements, a phase estimation approach has been documented, this is not a valid way of grouping the data [54]. There have been recent calls to action to increase the quality and transparency of reporting, including a statement of why physiological measurements were not used and the implications on the findings [444, 508]. Where blood measurement is not feasible or is not tolerated by the participant, an alternative less invasive and more easily accessible sampling method of hormone measurement would alleviate these challenges for participants and researchers.

The Low Energy Availability in Females Questionnaire (LEAF-Q) was included in three studies in this thesis (Chapter 5, Chapter 6 and Chapter 8). The LEAF_Q is a screening tool developed to detect symptoms of low energy availability (LEA) and was included in these studies because; 1) LEA is known to affect hormone

profile, 2) many of the symptoms of LEA are congruent with concussion symptoms, and 3) a common response to injury is to restrict caloric intake due the decreased exercise energy expenditure and may therefore be used to further exclude participants or to use the data as a confounding variable. The tool was originally validated in dancers and endurance athletes and includes three sub-scales (injury, gastrointestinal symptoms and menstrual dysfunction). A score of ≥8 (with sub-scale cut-offs: injury ≥ 2, gastrointestinal symptoms ≥ 2 and menstrual dysfunction ≥ 4) is considered to be at risk of problematic LEA [47]. Based on these cut-off scores, none of the participants in Chapter 5 and Chapter 6 of this thesis were identified as being at risk of LEA. Two participants in the FeRNAC study (Chapter 8) did have an overall score of ≥8 (one score of 9 and one score of 10). Importantly though, neither of them had a menstrual dysfunction score ≥ 4 and the elevated score overall was due to either a high GI symptom score or a high injury score that was due to the current concussion. Despite being used in a wide range of athlete populations, the LEAF_Q has not yet been validated outside of dancing and endurance sports. Since the studies in this thesis were planned it has become evident that the LEAF-Q score has low specificity and perhaps shouldn't be used to classify athletes from non-endurance sports as "high risk" of conditions related to LEA, or as a surrogate diagnostic tool for LEA [509]. It can however be used to 'rule out' risk of LEA-related conditions or to create selective low-risk groups that do not need management [510] and is very good at reliably identifying those with amenorrhea [509]. Further, due to the prevalence of acute and impact injuries in team sports like football, compared to endurance sports, may lead to a higher total LEAF_Q score [509] that overestimates the risk of LEA. Similarly, the gastrointestinal GI sub-scale aims to detect reduced resting metabolic rate indicative of LEA included in the LEAF_Q due to the prevalence of GI symptoms in female athletes suffering from disordered eating. However, these GI symptoms can exist in the absence of disordered eating and may be overstated in some females, leading to a misleading higher overall score. It has been recommended that the LEAF_Q cut-off scores could be increased for injury to ≥ 5) and an overall LEAF_Q cut off score of ≥10). Applying these higher cut-off scores, none of the participants in chapter 8 would be identified as at risk for LEA. Although the LEAF_Q data collected in this thesis was not included in any of the data analyses, it was useful in confirming that none of the participants had symptoms of LEA and therefore did not need to be excluded. It was also useful in collecting the information relating to hormone profile. The LEAF-Q demonstrates good utility due to its quick and cost-effective administration. However, further validation is required across female athletes from different sports/activities to assess the accuracy of cut-off scores in identifying females at risk of low energy availability (LEA). Additionally, research is needed to determine whether the tool is sufficiently sensitive to detect changes over time. The LEAF_Q may be valuable in future concussion research involving female athletes, given the overlap between symptoms of concussion and those associated with LEA.

9.2 Practical implications and recommendations

The studies in this thesis go some way to addressing the sex disparity in concussion research and start to fill a critical gap in understanding sex-specific responses to brain injury, which is essential for equitable healthcare. This also has ethical and social implications as the findings could be used to raise awareness of the need for sex-specific concussion research and influence funding priorities. This thesis also highlights the potential for personalized concussion care that could include hormone-informed treatment protocols or recovery timelines for females and may help clinicians tailor return-to-learn/work and return-to-play decisions based on individual hormonal and molecular profiles. These studies have also helped to inform a methodological approach to future concussion research in females which needs to include reliable hormone profile data as discussed in detail in chapter 8; other aspects that need to be considered are outlined below. Females have their own symptomatic and experiential 'baseline' that should not be measured against males.

In addition to hormone profile data, a consensus methodological approach to female only or mixed gender cohorts concussion research needs to include:

- An a priori power calculation to ensure fair statistical analyses that reflect the effect of the findings.
- A consistent assessment and diagnosis for concussion. The sports concussion consensus statement[370] and the American College of Rehabilitation Medicine (ACRM) position statement[387] are recommended and should be clearly articulated in the methodology.
- A consistent symptom assessment tool. Many have been modified for the local context. In NZ, the Brain Injury Screening Tool (BIST)[511-513] has been developed and is recommended due to the consistency across different clinical environments.
- Two or more outcome measures to reflect the subjective experience and any functional or cognitive deficit and recovery.
- If resources allow, a blood based or salivary biomarker.

It is also recommended that:

- Biomarker research includes measurement beyond the acute phase to inform time course and dynamics of each biomarker in relation to concussion recovery.
- Sampling frequency for the measurement of concussion biomarkers could be every second day if there is pre-injury phase collection, and this is increased to daily during the injury and recovery phase.

- When managing females with concussion, it is recommended that health practitioners ask about hormone profile, reproductive history and typical hormone related symptoms that may be experienced.
- Where possible, monitoring of the menstrual cycle in female athletes with the provision of supporting education should be promoted in youth and community sport so that regardless of whether the athlete pursues a high-performance pathway or not, participation is sustainable, and the athlete understands what is 'normal' for them and when to engage a health practitioner. In the context of concussion, information about baseline symptomology may be particularly valuable.

More generally, study planning requires a specific strategy to maximise participant recruitment and retention. There are menstrual health tools that can be used to help participants understand their eligibility for a study based on their hormone profile and could be included in participant information and consent packs. Data collection protocols need to balance ease of adherence, participant burden and quality data collection. Mobile-phone applications are widely used and easily accessible to support calendar-based counting and may be useful for tracking within-person trends and changes. Saliva may be useful for monitoring daily changes in hormones and with further validation work it may also offer a non-invasive sampling method for phase verification in menstrual cycle research. It is recommended that:

- The MHL tools are used as part of the recruitment and retention strategy for studies and a way to educate participants about their menstrual health and about the immediate benefit (to them and the wider impact of the research.
- If time and resource are constrained and the three-step method [54, 403] for collecting hormone profile data is not feasible, the two-step method may be a fair valid alternative [514].
- There is a planned over-recruitment of participants in hormone related research.
- In injury related research, there is a 30-35 day follow up question about menstrual cycle start date included in the consent process.
- Monitoring of data collection and immediate issuing of a data query to minimise missing data.
- Handling missing values should be factored into data analysis plans.
- Where possible, researchers organise access to laboratories on weekend days.
- Mobile phone based menstrual tracking apps can be used as a practical method of tracking sleep characteristics, the menstrual cycle and any associated symptoms.

- Research can be used as an education opportunity, for example; in an applied setting where menstrual cycle tracking is required as part of a research study, this also becomes as ideal time to provide education to the participant on why tracking is important ongoing, and how menstrual cycle tracking supports overall health.
- Frequent salivary hormone measures may be useful to monitor patterns and changes over time within an individual but not for phase verification.
- When using the LEAF_Q with athletes outside of endurance sports or dancing, it is recommended to examine individual subscale scores to better understand the factors contributing to an elevated overall score.

9.3 Strength and Limitations of the work

The limitations of each study are discussed in more detail in the relevant chapters. The main limitation across this thesis is the small sample sizes within each of the studies, which somewhat limits the application of findings and limits the amount of statistical analysis that can be done. With the exception of Chapter 5, the lack of objective measurement to determine hormone profile in the studies of this thesis was a major limitation. For that reason, conclusions cannot be drawn about specific phases of the menstrual cycle. In chapter 4 and 6, patterns and trends can be observed in the days between menses phases and in chapter 8 data could only be analysed and reported according to whether there was a down regulation of endogenous hormones due to hormonal contraceptive use or not. In chapters 4 to 6, the use of a mobile phone application for tracking self-report menstrual cycle and sleep characteristics could be subject to recall bias, this risk is reduced due to the daily collection protocol. A further limitation of using the app is that the symptoms may be interpreted differently between users, however within participant monitoring overtime will be consistent. The number of days taken to return to learn/work (RTL/W) was the proxy for recovery from concussion in the FeRNAC study (chapter 8), this does not necessarily reflect that the brain has fully recovered. Further, date of RTL/W was self-reported by participants through the weekly online surveys. This is potentially inaccurate for two reasons; 1) a participant may forget or report an inaccurate date, and 2) if a participant went back to learning or work too soon and then required further time off, this was not captured in the data. The unsupervised self-collection of saliva samples in chapters 5 and 6 could introduce some measurement error as participants may not have fully adhered to the collection protocol, affecting the minimum required sample volume, food/drink residue or collection at the wrong time of day. The biomarker studies in chapter 6 and 8 are lacking a control group and although the use of a miR ratio aims to remove the need for a baseline or a control, there is a need for more reference data from multiple

cohorts to define miR ratio references ranges. There are however data from previous studies [423] that utilise a similar measurement and can be used for comparison.

The strengths of this thesis are that it contributes to addressing sex disparities in concussion research and starts to fill a critical gap in understanding female responses to brain injury, which is essential for equitable healthcare. This work also goes some way to advancing miRNA research; previous studies have explored miRNAs as concussion biomarkers, but few (if any) have done so in an ED setting specifically in female patients and with consideration for hormone profile. This is also the first study to use qPCR to analyse the candidate miRNA identified using NGS.

9.4 Conclusion

More research is required to further investigate the neuroendocrine response to concussion in females. In addition, baseline symptomology in females is likely to be different to that of males and should be given consideration when managing females with concussion. Although concussion biomarkers do show potential, more work needs to be done before they can be considered scientifically and clinically reliable. Although progress is being made to close the sex data gap in sports science and exercise medicine research there is still a need for more female data from multidisciplinary studies.

9.5 Future direction/research opportunities

Based on the findings of this thesis, future research recommendations include:

- Research to validate the use of saliva sampling for phase verification.
- Further development of salivary qPCR for miRNA.
- Qualitative research to understanding the different underlying motivations of male and female athletes' intention to report a concussion, or not.
- Surveillance studies in New Zealand to understand the true incidence of concussion.
- Female specific case-controlled studies that consider hormone profile (naturally cycling and contraceptive use) and collect pre and post injury data and evaluate the neuroendocrine response to concussion.
- Future studies evaluating any concussion specific biomarker needs to consider hormone profile in the methodology and analysis.
- Further work to refine the optimal technique for salivary sex hormone measurement.
- Prospective studies to evaluate symptom management strategies in female athletes pre- and post-concussion.
- Studies to develop and inform primary and secondary concussion prevention strategies.

- Studies across female athletes from different sports/activities to assess the accuracy of LEAF_Q cut-off scores in identifying females at risk of low energy availability (LEA).
- Research to determine whether the LEAF_Q is sufficiently sensitive to detect changes over time.

9.6 Reflective thinking and learning

Aside from the problems presented by COVID-19, conducting the studies in this thesis provided first hand insight into the reality of what it takes to run a high-quality female specific study. I will be able to apply everything I have learnt and understood to future research. This PhD has provided multiple opportunities to learn and improve as an applied sports scientist and injury prevention practitioner. In parallel with the in-depth subject matter knowledge I have developed, I have also gained technical skills through course work and practice for venepuncture, statistical analysis, and lab-based blood and saliva sample analysis of hormones and miRNA. What I have enjoyed the most is that this work afforded me the opportunity to engage with new disciplines, for example molecular biology and medical diagnostics for the upskilling and analysis of the salivary miRNA.

I completed a phlebotomy course so I could do blood sample collection and have been able to maintain this skill by participating in 'in service' sessions with staff at the medical research laboratory in Wellington. I worked alongside the Professor of biomedicine at AUT to learn how to process and analyse the salivary sex hormones using the Cobas analyser in the Roche lab at AUT. I developed a relationship with Dr Indira Basu at Awanui labs to learn and understand the manual steps for the extraction and analysis of miRNA from saliva using the qPCR technique. Throughout this PhD I have improved my academic skills particularly in scientific reasoning which has grown my confidence in challenging other research and providing honest peer review. For example, I was asked to review a study from a large national sports organisation that included the use of salivary miRNA's and included female players. The methodology did not account for hormone profile; therefore, I was able to advise on the importance of this and provide the recommended approach supported by key research papers. As time allowed, I have also reviewed manuscripts in my field submitted to journals for peer review when invited by the respective editors. Further to this, some of my deepest learning has come from submitting my own manuscripts for consideration by target journals and receiving the critical feedback and a different perspective.

Perhaps what I underestimated at the outset of the PhD was how much my 'soft skills' would be both tested and advanced. I am based in Wellington, however some data collection and analysis occurred in Auckland. The complex logistics of the data collection and analysis improved my ability to co-ordinate individuals and activities across departments, disciplines and cities to ensure that the quality of the blood and saliva samples was maintained. Throughout this PhD I have maintained full-time employment as 'Injury Prevention Partner – Sport and TBI' at the Accident Compensation Corporation (ACC), which required a

substantial amount of time management and resulted in constant adjustment to timeframes and my own expectations. Overtime this has increased my confidence in recognising real opportunities and prioritising what is important over what may be an interesting distraction. In undertaking this PhD I have developed and proven my ability to build effective relationships and co-ordinate complex logistics which I believe will be valuable in approaching my future research studies and will help overcome some of the challenges of applied research and improve study quality.

Given the small population, New Zealand can be 'nimble' in its approach to research. However, I have recognised the need to find ways to better co-ordinate work related to the female athlete as an emerging topic across our universities and internationally. As a start, I worked with the team at AUT to develop and maintain a website dedicated to the research activity around the female athlete, to raise awareness and support recruitment of studies for female specific research. This has now evolved into the AUT Women's Health in Neuroscience programme of research (Women's health and neuroscience research programme - SPRINZ - AUT).

In my role as 'Injury Prevention Partner – Sport and TBI' at ACC I lead partnerships with National Sports Organisations (NSO) to implement injury prevention initiatives. I have applied new knowledge to that work over the course of my PhD. SportSmart is the evidence informed framework for injury prevention that underpins those initiatives that are typically integrated into coach education and delivery in a code-specific format. The systematic review and meta-analysis (Chapter 2) has been included in the recent update to the Sportsmart framework to ensure that coaches and female players receive relevant information around injury risk reduction. As my skills have developed, I have improved at critically considering how to more efficiently ensure that our sport injury prevention programmes remain effective and continue to evolve as new research emerges. This led to the development of a SportSmart monitoring and evaluation framework that also encourages the sports codes to work together and share injury prevention knowledge. Netball remains the highest female participation sport in NZ. In partnership with Netball NZ, ACC supports the development and implementation of NetballSmart (the Netball injury prevention programme). I worked with Netball through to develop and implement 'SmartHealth' – a resource designed to educate community Netball players about preparing well for playing sport and the key considerations for young female athletes navigating puberty and adolescence. SmartHealth is part of the overall NetballSmart strategy. This resource will now be adapted and used across other NSO partnerships. In continuation of this work I am engaging with Sport NZ and High-Performance Sport NZ on a collaborative project based on 'SmartHealth' to support women and girls across all sports and at every level of participation. This work is in the early stages of development with the start of implementation planned for 2026. One of the most substantial pieces of work has been the development of an updated ACC national concussion guideline for youth and community sport which now includes a consistent approach to return to sport (RTS). Although there is currently insufficient evidence to determine and guide whether a female specific approach is

needed, we (ACC) now own the responsibility of updating the document and will be able to ensure the female perspective is included quickly as the research evolves.

As a member of the TBI network (collaborative network of researchers of brain injury in NZ) members can provide peer review and other support to projects within the network. I have been a key member of the TBIN that understands female specific methodology and the need to ensure sex disaggregated data and sex analysis and can now contribute to the new WHN programme of research.

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Glossary and definitions used in this thesis

Concussion – For the purpose of this thesis, the definition of concussion is that outlined in the 6th consensus statement from the concussion in sport group (CISG) [370].

Eumenorrheic – Female with a natural regular ovulatory menstrual cycle evidenced by urinary Luteinizing Hormone (LH) surge plus blood plasma measure of progesterone >16nmol/L in mid-luteal phase.

Gender – this term will be used to refer to a person's self-representation as male or female [515].

Sex - this term will be used to distinguish male or female subjects according to the reproductive organs and functions that derive from the chromosomal complement. This is distinct from the term *gender*, used to refer to a person's self-representation as male or female [515].

Woman and man – these terms will be used to distinguish a person's self-representation as male or female [515].

Female and male – these terms will be used to distinguish subjects according to the reproductive organs and functions that derive from the chromosomal complement. This is distinct from the term *gender*, used to refer to a person's self-representation as male or female [515].

Naturally cycling/Natural regular menstrual cycle – Female that is not using any form of hormonal contraception and has a natural regular (bleeding every 21-35 days) menstrual cycle, but ovulation has not been confirmed.

Sex hormones – The two dominant classes of female sex hormones are Estrogens and Progesterone. There are in fact four major naturally occurring estrogens in women: estrone (E1), estradiol (E2), estriol (E3), and estetrol (E4) (produced exclusively during pregnancy). Estradiol is the predominant estrogen during reproductive years in both absolute serum levels and estrogenic activity. In this thesis estradiol (E2) is the relevant estrogen and will be referred to and used for data collection.

Football – The term football will be used to refer to association football, also known as 'Soccer'.

Level of participation in sport/physical activity – McKay's participant classification framework [63] will be used throughout this thesis to categorise different cohorts. The Participant Classification Framework uses training volume and performance metrics to classify a participant to one of the following: Tier 0: Sedentary; Tier 1: Recreationally Active; Tier 2: Trained/Developmental; Tier 3: Highly Trained/National Level; Tier 4: Elite/International Level; or Tier 5: World Class.

Appendices

Appendix I: Ethics approval for Chapter 3

23 August 2022

Patria Hume

Faculty of Health and Environmental Sciences

Dear Patria

Re Ethics Application: **22/192 Assessing knowledge and attitudes towards concussion in amateur football players and coaches.**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 23 August 2025.

Standard Conditions of Approval

1. The research is to be undertaken in accordance with the Auckland University of Technology Code of Conduct for Research and as approved by AUTEC in this application.
2. A progress report is due annually on the anniversary of the approval date, using the EA2 form.
3. A final report is due at the expiration of the approval period, or, upon completion of project, using the EA3 form.
4. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form.
5. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
6. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.
7. It is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard and that all the dates on the documents are updated.
8. AUTEC grants ethical approval only. You are responsible for obtaining management approval for access for your research from any institution or organisation at which your research is being conducted and you need to meet all ethical, legal, public health, and locality obligations or requirements for the jurisdictions in which the research is being undertaken.

Please quote the application number and title on all future correspondence related to this project.

For any enquiries, please contact ethics@aut.ac.nz. The forms mentioned above are available online through http://www.aut.ac.nz/research/researchethics

(This is a computer-generated letter for which no signature is required)

The AUTEC Secretariat

Auckland University of Technology Ethics Committee

Cc: Natalie.hardaker@aut.ac.nz; Stacy Sims; dking@aut.ac.nz; tom.stewart@aut.ac.nz

Appendix II: Ethics approval for Chapters 4 & 5

9 July 2021

Patria Hume

Faculty of Health and Environmental Sciences

Dear Patria

Re Ethics Application: **21/167 The validation of measures of female sex hormones in saliva and the relationship between salivary sex hormone profiles and symptoms across the menstrual cycle in healthy eumenorrheic females: A feasibility study.**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 9 July 2024.

Non-Standard Conditions of Approval

1. Inclusion on the recruitment poster of the exclusion of the supervisor's students.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by AUTEC before commencing your study.

Standard Conditions of Approval

9. The research is to be undertaken in accordance with the Auckland University of Technology Code of Conduct for Research and as approved by AUTEC in this application.
10. A progress report is due annually on the anniversary of the approval date, using the EA2 form.
11. A final report is due at the expiration of the approval period, or, upon completion of project, using the EA3 form.
12. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form.
13. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
14. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.
15. It is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard and that all the dates on the documents are updated.

AUTEC grants ethical approval only. You are responsible for obtaining management approval for access for your research from any institution or organisation at which your research is being conducted and you need to meet all ethical, legal, public health, and locality obligations or requirements for the jurisdictions in which the research is being undertaken.

Please quote the application number and title on all future correspondence related to this project.

For any enquiries, please contact ethics@aut.ac.nz. The forms mentioned above are available online through http://www.aut.ac.nz/research/researchethics

(This is a computer-generated letter for which no signature is required)

The AUTEC Secretariat

Auckland University of Technology Ethics Committee

Cc: natalie.hardaker@acc.co.nz; Fabrice Merien; Tom Stewart

Appendix III: Ethics approval for Chapters 6 & 7

24 May 2022
Patria Hume

Faculty of Health and Environmental Sciences

Dear Patria

Re Ethics Application: **22/110 The Impact of the female sex hormones on the occurrence of and recovery from concussion**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 24 May 2025.

Non-Standard Conditions of Approval

2. This approval is given on the understanding that no person who lacks the capacity to give consent will be recruited for this study. We refer you to the notes at the end of Chapter 7 of NEAC National Ethical Standards.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by AUTEC before commencing your study.

Standard Conditions of Approval

16. The research is to be undertaken in accordance with the Auckland University of Technology Code of Conduct for Research and as approved by AUTEC in this application.
17. A progress report is due annually on the anniversary of the approval date, using the EA2 form.
18. A final report is due at the expiration of the approval period, or, upon completion of project, using the EA3 form.
19. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form.
20. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
21. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.
22. It is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard and that all the dates on the documents are updated.
23. AUTEC grants ethical approval only. You are responsible for obtaining management approval for access for your research from any institution or organisation at which your research is being conducted and you need to meet all ethical, legal, public health, and locality obligations or requirements for the jurisdictions in which the research is being undertaken.

Please quote the application number and title on all future correspondence related to this project.

For any enquiries, please contact ethics@aut.ac.nz. The forms mentioned above are available online through

http://www.aut.ac.nz/research/researchethics

(This is a computer-generated letter for which no signature is required)

The AUTEC Secretariat

Auckland University of Technology Ethics Committee

Cc: Natalie.hardaker@aut.ac.nz; dking@aut.ac.nz; wee.leon.chang@aut.ac.nz; tom.stewart@aut.ac.nz

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011
hdecs@health.govt.nz

Ethics reference: 2022 EXP 11655

27 April 2022

Miss Natalie Hardaker

AUT Millennium, 17 Antares Place,
Rosedale
Auckland
0632
New Zealand

Tēnā koe Miss Hardaker

APPROVAL OF APPLICATION

Study title: The impact of the female sex hormones on the occurrence of and recovery from concussion

I am pleased to advise that your application was **approved** by the Northern B Health and Disability Ethics Committee (the Committee). This decision was made through the EXP pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern B Health and Disability Ethics Committee is required.

Standard conditions:

- Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.
- Before the study commences at *each given* locality in New Zealand, it must be authorised by that locality in Ethics RM. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the *SOPs* for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 27th April 2023.

Participant access to compensation.

The Northern B Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialed. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation.

Further information and assistance

Please contact the HDECs Secretariat at hdecs@health.govt.nz or visit our website at www.ethics.health.govt.nz for more information, as well as our General FAQ and Ethics RM user manual.

Nāku noa, nā

Ms Kate O'Connor

Chair

Northern B Health and Disability Ethics Committee

Appendix IV: Ethics approval for Chapter 8

12 May 2022

Patria Hume

Faculty of Health and Environmental Sciences

Dear Patria

Re Ethics Application: **22/135 Hormones, biomarkers and concussion in female athletes; A case series study**

Thank you for providing evidence as requested, which satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 12 May 2025.

Standard Conditions of Approval

24. The research is to be undertaken in accordance with the Auckland University of Technology Code of Conduct for Research and as approved by AUTEC in this application.
25. A progress report is due annually on the anniversary of the approval date, using the EA2 form.
26. A final report is due at the expiration of the approval period, or, upon completion of project, using the EA3 form.
27. Any amendments to the project must be approved by AUTEC prior to being implemented. Amendments can be requested using the EA2 form.
28. Any serious or unexpected adverse events must be reported to AUTEC Secretariat as a matter of priority.
29. Any unforeseen events that might affect continued ethical acceptability of the project should also be reported to the AUTEC Secretariat as a matter of priority.
30. It is your responsibility to ensure that the spelling and grammar of documents being provided to participants or external organisations is of a high standard and that all the dates on the documents are updated.
31. AUTEC grants ethical approval only. You are responsible for obtaining management approval for access for your research from any institution or organisation at which your research is being conducted and you need to meet all ethical, legal, public health, and locality obligations or requirements for the jurisdictions in which the research is being undertaken.

Please quote the application number and title on all future correspondence related to this project.

For any enquiries, please contact ethics@aut.ac.nz. The forms mentioned above are available online through http://www.aut.ac.nz/research/researchethics

(This is a computer-generated letter for which no signature is required)

The AUTEC Secretariat

Auckland University of Technology Ethics Committee

Cc: Natalie.hardaker@aut.ac.nz

Health and Disability Ethics Committees
Ministry of Health
133 Molesworth Street
PO Box 5013
Wellington
6011
hdecs@health.govt.nz

Ethics reference: 2022 EXP 11904

3 May 2022

Miss Natalie Hardaker

AUT Millennium, 17 Antares Place,
Rosedale
Auckland
0632
New Zealand

Tēnā koe Miss Hardaker

APPROVAL OF APPLICATION

Study title: The influence of female athlete's sex hormones on Sports Related Concussion

I am pleased to advise that your application was **approved** by the Northern B Health and Disability Ethics Committee (the Committee) with **non-standard conditions**. This decision was made through the EXP pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern B Health and Disability Ethics Committee is required.

Standard conditions:

- Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.
- Before the study commences at *each given* locality in New Zealand, it must be authorised by that locality in Ethics RM. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Non-standard conditions:

1. The Committee requested that items such as the return-to-play protocols which are part of standard of care and not the research itself may be removed from the Information Sheet
2. Please include specific information about any overseas laboratories. Please also include a specific option in the Consent Form regarding sending samples overseas.
3. Please review the PIS for technical terms and ensure a lay explanation is provided (i.e. 'hormone profiles').
4. The Committee noted that as recruitment of participants shall be in Wellington, please remove the information about the AUT Counselling services. Please ensure counselling services in Wellington should be made available.
5. The Committee asked for clarification on ACC Injury Claim eligibility. Please explain whether the eligibility to claim on ACC pertains only to the sustaining of an SRC during play or training. If participation in research ineligibility for ACC applies here, please ensure this is explained in the PIS.

Non-standard conditions must be completed before commencing your study, however, they do not need to be submitted to or reviewed by HDECs.

If you would like an acknowledgement of completion of your non-standard conditions you may submit a post approval form amendment through the Ethics Review Manager. Please clearly identify in the amendment form that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see paragraphs 125 and 126 of the *Standard Operating Procedures for Health and Disability Ethics Committees (SOPs)*.

After HDEC review

Please refer to the *SOPs* for HDEC requirements relating to amendments and other post-approval processes.

Your next progress report is due by 3rd May 2023.

Participant access to compensation

The Northern B Health and Disability Ethics Committee is satisfied that your study is not a clinical trial that is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialed. Participants injured as a result of treatment received as part of your study may therefore be eligible for publicly-funded compensation through the Accident Compensation Corporation.

Further information and assistance

Please contact the HDECs Secretariat at hdecs@health.govt.nz or visit our website at www.ethics.health.govt.nz for more information, as well as our General FAQ and Ethics RM user manual.

Nāku noa, nā

Ms Kate O'Connor

Chair

Northern B Health and Disability Ethics Committee

Appendix V: Supplementary material for Chapter 2

Supplementary table 2.1: Modified Downs & Black Rating Questions for Methodological Quality Index Categorisation

REPORTING x/11
1. Is the hypothesis/aim/objectives of the study clearly described (yes=1, no=0)
2. Are the main outcomes to be measured clearly described in the introduction or methods section? (yes=1, no =0)
3. Are the characteristics of the patients included in the study clearly described? (yes=1, no=0)
4. Are the interventions of interest clearly described? (yes=1, no=0)
5. Are the distributions of principal confounders in each group of subjects to be cf. clearly described? (yes =2, partially=1, no=0)
6. Are the main findings of the study clearly described? (yes=1, no=0)
7. Does the study provide estimates of the random variability in the data for the main outcomes? (yes=1, no=0)
8. Had all important adverse events that may be a consequence of the intervention been reported? (yes=1, no=0)
9. Have the characteristics of patients lost to follow-up been described? (yes=1, no=0)
10. Had actual probability values been reported (e.g., 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001? (yes=1, no=0)
EXTERNAL VALIDITY x/3
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited? (yes=1, no=0, unable to determine=0)
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited? (yes=1, no=0, unable to determine=0)
13. Were the staff, places, & facilities where the patients were treated, representative of the treatment most patients receive? (yes=1, no=0, unable to determine=0)
INTERNAL VALIDITY - BIAS x/7
14. Was an attempt made to blind study subjects to the intervention they have received? (yes=1, no=0, unable to determine=0)
15. Was an attempt made to blind those measuring the main outcomes of the intervention? (yes=1, no=0, unable to determine=0)
16. If any of the results of the study were based on "data dredging", was this made clear? (yes=1, no=0, unable to determine=0)
17. In trials & cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention & outcome the same for cases & controls? (yes=1, no=0, unable to determine=0)
18. Were the statistical tests used to assess the main outcomes appropriate? (yes=1, no=0, unable to determine=0)
19. Was compliance with the intervention/s reliable? (yes=1, no=0, unable to determine=0)
20. Were the main outcome measures used accurate (valid & reliable)? (yes=1, no=0, unable to determine=0)
INTERNAL VALIDITY – CONFOUNDING (SELECTION BIAS) x/6
21. Were the patients in different intervention groups (trials & cohort studies) or were the cases & controls (case-control studies) recruited from the same population? (yes=1, no=0, unable to determine=0)
22. Were study subjects in different intervention groups (trials & cohort studies) or were the cases & controls (case-control studies) recruited over the same period of time? (yes=1, no=0, unable to determine=0)
23. Were study subjects randomised to intervention groups? (yes=1, no=0, unable to determine=0)
24. Was the randomised intervention assignment concealed from both patients & health care staff until recruitment was complete & irrevocable? (yes=1, no=0, unable to determine=0)
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn? (yes=1, no=0, unable to determine=0)
26. Were losses of patients to follow-up taken into account? (yes=1, no=0, unable to determine=0)
POWER x/1
27. Was a power calculation used & described? (yes=1, no=0)

Supplementary table 2.2: Modified Downs & Black methodological quality index categorisation individual criteria scores for studies.

Tier	Study	OVERALL SCORE /28	Quality index %	Quality index category
5	Edouard et al. 2018 [76]	19	68%	Moderate
5	Engebretsen et al 2010 [75]	17	61%	Moderate
5	Junge et al.2006 [71]	14	50%	Moderate
5	Kim et al. 2015 [77]	14	50%	Moderate
5	Kim et al 2020 [73]	20	71%	Moderate
5	Harris et al 2020 [72]	16	57%	Moderate
5	Trease et al 2020 [74]	18	64%	Moderate
5	Willauschus et al 2021 [78]	18	64%	Moderate
Mean quality score ±SD(range)		**17.0 ±2.2(14-20)**	**60.7% ±7.9%**	**Moderate**
4	Abadi et al. 2021 [97]	19	68%	Moderate
4	Chimera et al. 2022 [106]	16	57%	Moderate
4	Frolich et al. 2021 [98]	19	68%	Moderate
4	Kim et al. 2021 [107]	19	68%	Moderate
4	Mattiussi et al. 2021 [117]	19	68%	Moderate
4	Mertz et al. 2022 [95]	14	50%	Moderate
4	Vedung et al. 2020 [87]	18	64%	Moderate
4	Allen et al. 2012 [100]	20	71%	Moderate
4	Augustovicova et al. 2019 [111]	16	57%	Moderate
4	Cierna et al. 2017 [196]	17	61%	Moderate
4	Bere et al. 2015 [85]	16	57%	Moderate
4	Edouard et al. 2014 [101]	18	64%	Moderate
4	Edouard et al. 2015 [105]	17	61%	Moderate
4	Edouard et al. 2016 [99]	16	57%	Moderate
4	Gescheit et al. 2017 [108]	16	57%	Moderate
4	Hagglund et al. 2009 [79]	20	71%	Moderate
4	Hame 2004 [88]	18	64%	Moderate
4	Hamid et al. 2016 [113]	18	64%	Moderate
4	Harringe et al. 2007 [86]	17	61%	Moderate
4	Hill et al. 2019 [91]	13	46%	Limited
4	Hunt et al. 2017 [89]	16	57%	Moderate
4	Johansen et al. 2015 [109]	17	61%	Moderate
4	Kerr et al. 2016 [70]	17	61%	Moderate
4	Kirialanis et al. 2002 [114]	17	61%	Moderate
4	Larruskain et al. 2018 [82]	21	75%	Moderate
4	Melvin 2018 [83]	16	57%	Moderate
4	Major et al. 2014 [92]	16	57%	Moderate
4	McCurdie 2017 [110]	11	39%	Limited
4	Moreno-Perez 2019 [102]	16	57%	Moderate
4	Niedel 2019 [93]	16	57%	Moderate
4	Owoeye et al. 2017 [80]	15	54%	Moderate
4	Park et al. 2017 [104]	17	61%	Moderate
4	Park et al. 2019 [103]	18	64%	Moderate
4	Steenstrup et al. 2014 [94]	16	57%	Moderate
4	Toohey 2019 [81]	19	68%	Moderate
4	Traneus et al. 2016 [84]	17	61%	Moderate
4	Westermann et al. 2015 [115]	16	57%	Moderate
4	Wolf 2009 [116]	17	61%	Moderate
4	Yang et al. 2012 [96]	18	64%	Moderate
4	Zupon et al. 2018 [90]	15	54%	Moderate
Mean quality score ±SD(range)		**16.9±1.9(11-21)**	**60.4% ±6.8%**	**Moderate**

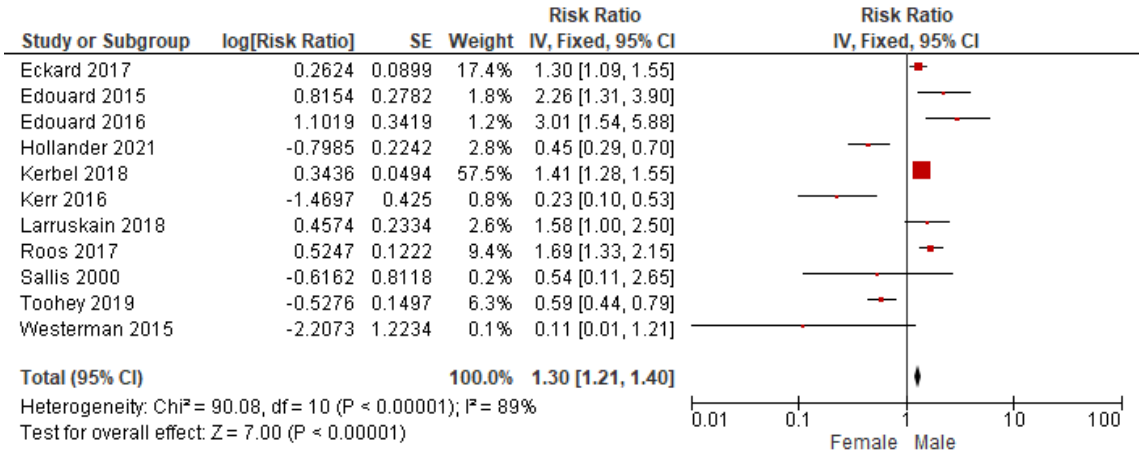
3	Belilos et al. 2023 [201]	17	61%	Moderate
3	Bretzin et al. 2021 [134]	18	64%	Moderate
3	Chan et al. 2021 [161]	17	61%	Moderate
3	Comeau et al. 2023 [139]	20	71%	Moderate
3	Deckey et al. 2020 [162]	18	64%	Moderate
3	Deckey et al. 2020 [126]	15	54%	Moderate
3	Fraser et al. 2021 [202]	18	64%	Moderate
3	Haines et al. 2022 [165]	14	50%	Moderate
3	Hopkins et al. 2022 [163]	18	64%	Moderate
3	Jayanthi et al. 2020 [164]	19	68%	Moderate
3	Lacasse et al. 2021 [132]	11	39%	Limited
3	Lievers et al. 2020 [141]	18	64%	Moderate
3	Marchena-Rodriguez et al. 2020 [211]	18	64%	Moderate
3	Marshall et al. 2020 [209]	16	57%	Moderate
3	O' Connor 2020 [212]	16	57%	Moderate
3	Post et al. 2022 [133]	14	50%	Moderate
3	Schroeder et al. 2022 [140]	15	54%	Moderate

Tier	Study	OVERALL SCORE /28	Quality index %	Quality index category
3	Vaandering et al. 2022 [142]	17	61%	Moderate
3	Beis et al. 2007 [204]	18	64%	Moderate
3	Fares et al. 2019 [210]	18	64%	Moderate
3	Opar et al. 2014 [183]	16	57%	Moderate
3	Opar et al. 2015 [155]	16	57%	Moderate
3	Chandran et al 2016 [122]	15	54%	Moderate
3	Covassin et al. 2003 [145]	17	61%	Moderate
3	Cross et al 2010 [123]	16	57%	Moderate
3	Cross et al. 2013 [124]	17	61%	Moderate
3	Dalton et al. 2015 [146]	17	61%	Moderate
3	Eckard et al. 2017 [143]	16	57%	Moderate
3	Eckard et al. 2017 [143]	16	57%	Moderate
3	Fulstone et al. 2016 [147]	15	54%	Moderate
3	Gessel et al. 2007 [148]	18	64%	Moderate
3	Harmer et al. 2008 [208]	16	57%	Moderate
3	Hassebrock et al. 2019 [125]	17	61%	Moderate
3	Hibberd et al. 2016 [149]	17	61%	Moderate
3	Hurtubise et al. 2015 [150]	16	57%	Moderate
3	Kerbel et al. 2018 [151]	16	57%	Moderate
3	Kerr et al. 2015 [207]	16	57%	Moderate
3	Kronisch et al. 2002 [152]	20	71%	Moderate
3	Mauntel et al. 2017 [153]	16	57%	Moderate
3	Muller at al. 2017 [203]	17	61%	Moderate
3	Owens et al. 2009 [154]	16	57%	Moderate
3	Rizzone et al. 2017 [156]	16	57%	Moderate
3	Roos et al. 2015 [185]	17	61%	Moderate
3	Sallis et al. 2000 [248]	18	64%	Moderate
3	Simmons et al. 2017 [157]	17	61%	Moderate
3	Trojan et al. 2019 [158]	16	57%	Moderate
3	Tummala et al. 2018 [159]	16	57%	Moderate
3	Zuckerman et al. 2015 [160]	18	64%	Moderate
3	Zupon et al. 2018 [90]	15	54%	Moderate
3	Achenbach et al. 2018 [137]	17	61%	Moderate
3	Baugh et al. 2016 [197]	15	54%	Moderate
3	Beynnon et al. 2005 [166]	18	64%	Moderate
3	Borowski et al. 2008 [129]	17	61%	Moderate
3	Bretzin et al. 2018 [167]	18	64%	Moderate
3	Changstrom et al. 2015 [168]	19	68%	Moderate

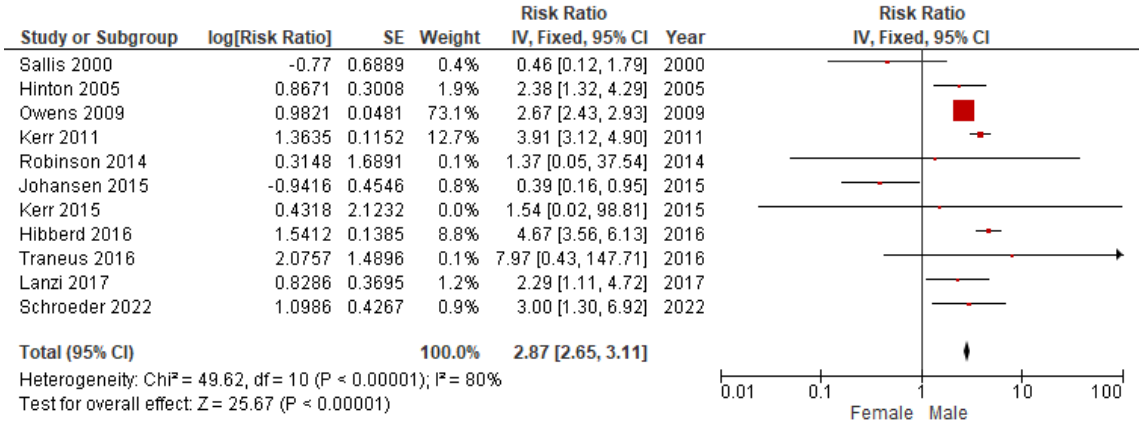
3	Cierna et al. 2017 [196]	19	68%	Moderate
3	Cierna et al. 2018 [195]	18	64%	Moderate
3	Collins et al. 2008 [118]	18	64%	Moderate
3	Covassin et al. 2016 [169]	18	64%	Moderate
3	Darrow et al. 2009 [127]	18	64%	Moderate
3	Fernandez et al. 2007 [170]	17	61%	Moderate
3	Hinton et al. 2005 [119]	16	57%	Moderate
3	Huffman et al. 2008 [171]	15	54%	Moderate
3	Kerr et al. 2011 [172]	16	57%	Moderate
3	Kerr et al. 2011 [173]	19	68%	Moderate
3	Kerr et al. 2017 [120]	16	57%	Moderate
3	Kerr et al. 2018 [174]	18	64%	Moderate
3	Kolstrup et al 2016 [130]	20	71%	Moderate
3	Lanzi et al. 2017 [175]	15	54%	Moderate
3	Lincoln et al. 2011 [176]	17	61%	Moderate
3	Maciejeweski et al. 2016 [205]	19	68%	Moderate
3	Marar et al. 2012 [177]	18	64%	Moderate
3	Messina et al. 1999 [121]	17	61%	Moderate
3	Mitchell et al. 2015 [179]	16	57%	Moderate
3	Mitchell et al. 2016 [178]	16	57%	Moderate
3	Miyake et al. 2016 [199]	19	68%	Moderate
3	Monfort et al. 2015 [180]	16	57%	Moderate
3	Nelson et al. 2007 [181]	17	61%	Moderate
3	O' Connor et al. 2017 [182]	16	57%	Moderate
3	Olsen et al. 2006 [136]	18	64%	Moderate
3	Opar et al. 2014 [183]	18	64%	Moderate
3	Opar et al. 2015 [155]	19	68%	Moderate
3	Pieter et al. 2010 [206]	17	61%	Moderate
3	Pierpont et al. 2016 [198]	16	57%	Moderate
3	Powell et al. 2000 [128]	16	57%	Moderate
Tier	**Study**	**OVERALL SCORE /28**	**Quality index %**	**Quality index category**
3	Pytiak et al. 2018 [184]	18	64%	Moderate
3	Rauh et al. 2000 [200]	17	61%	Moderate
3	Roos et al. 2017 [138]	17	61%	Moderate
3	Schroeder et al 2015 [186]	14	50%	Moderate
3	Stracciolini et al. 2014 [187]	16	57%	Moderate
3	Stracciolini et al. 2015 [188]	18	64%	Moderate
3	Swenson et al. 2009 [193]	16	57%	Moderate
3	Swenson et al. 2010 [192]	20	71%	Moderate
3	Swenson et al. 2012 [191]	19	68%	Moderate
3	Swenson et al. 2013 [190]	16	57%	Moderate
3	Swenson et al. 2013 [189]	16	57%	Moderate
3	Tirabassi et al. 2016 [194]	16	57%	Moderate
3	Yard et al. 2008 [135]	16	57%	Moderate
Mean quality score±SD(range)		**16.9 ±1.4(11-20)**	**60.4% ±5.3%**	**Moderate**
2	Chatha, 2020 [214]	12	43%	Limited
2	Chun et al. 2021 [218]	18	64%	Moderate
2	Dane 2004 [219]	11	39%	Limited
2	Gill et al. 2021 [232]	13	46%	Limited
2	Hoge et al. 2020 [230]	17	61%	Moderate
2	Hollander et al 2018 [213]	16	57%	Moderate
2	Hollander et al. 2020 [233]	19	68%	Moderate
2	Honrado et al. 2021 [234]	14	50%	Moderate
2	Khodaee et al. 2020 [235]	16	57%	Moderate
2	Delaney et al. 2014 [220]	19	68%	Moderate

2	Deloes 1995 [221]	15	54%	Moderate
2	Forward et al. 2014 [222]	19	68%	Moderate
2	Hosea et al. 2000 [223]	16	57%	Moderate
2	Leppanen et al. 2017 [224]	17	61%	Moderate
2	Mintz et al. 2021 [242]	14	50%	Moderate
2	Morrissey et al. 2020 [236]	15	54%	Moderate
2	Owoeye et al. 2020 [215]	20	71%	Moderate
2	Pasanen et al. 2018 [225]	18	64%	Moderate
2	Polites et al. 2014 [226]	18	64%	Moderate
2	Quatman et al. 2009 [239]	16	57%	Moderate
2	Reid et al. 2012 [240]	17	61%	Moderate
2	Robinson et al. 2014 [227]	16	57%	Moderate
2	Rugg et al. 2021 [231]	18	64%	Moderate
2	Schallmo et al. 2017 [228]	19	68%	Moderate
2	Sokka et al. 2020 [216]	19	68%	Moderate
2	Sugimoto et al. 2020 [237]	19	68%	Moderate
2	Willick et al. 2021 [238]	16	57%	Moderate
2	Tsushima et al. 2019 [229]	20	71%	Moderate
2	Ursej et al. 2019 [241]	17	61%	Moderate
2	Zynda et al. 2022 [217]	15	54%	Moderate
Mean quality score ±SD(range)		**16.3±2.2(11-20)**	**67.9% ±8.2%**	**Moderate**
1	Aman et al. 2016 [243]	19	68%	Moderate
1	Aman et al. 2019 [246]	15	54%	Moderate
1	Meixner et al. 2020 [244]	15	54%	Moderate
1	Stogner et al. 2020 [245]	17	61%	Moderate
1	Xiao et al. 2022 [247]	14	50%	Moderate
Mean quality score ±SD(range)		**15.6±1.3(14-17)**	**57.4% ±7.1%**	**Moderate**
Combined age & tier				
	Frey et al. 2019 [250]	16	57%	Moderate
	Lopez 2016 [298]	18	64%	Moderate
Mean quality score ±SD(range)		**17.0 ±1.4(16-18)**	**60.7% ±5.1%**	**Moderate**

Total score out of 28 as one Q in the reporting section has 2 points available. Ratings were poor (<7/28 with <25%), limited (7-13; 25-49%), moderate (14-20; 50-74%), or strong (21+; 75%+)



Supplementary Figure 2.1: **Hip Groin meta-analysis.**



Supplementary Figure 2.2: **Shoulder meta-analysis.**

Appendix VI: Supplementary material for Chapter 3

Supplementary table 3.1: RoCKAS scoring key.

Section														
1			2			3			4			5		
Item	Correct response	Index	Item	Correct response	Index	Item	Safe response	Index	Item	Safe response	Index	Symptom	Distractor / Legitimate	Index
1	TRUE	CKI	1	FALSE	CKI	1	SD/D	CAI	1	SA/A	CAI	Hives	D	NI
2	FALSE	NI	2	TRUE	CKI	2	SA/A	CAI	2	SA/A	CAI	**Headache**	L	CKI
3	TRUE	CKI	3	FALSE	CKI	3	SA/A	NI	3	SD/D	CAI	Difficulty Speaking	D	NI
4	TRUE	VS				4	SA/A	NI	4	SD/D	CAI	Arthritis	D	NI
5	FALSE	CKI				5	SD/D	CAI	5	SD/D	CAI	**Sensitivity to Light**	L	CKI
6	FALSE	CKI				6	SD/D	CAI	6	SD/D	CAI	**Difficulty Rememberi ng**	L	CKI
7	FALSE	CKI				7	SA/A	CAI	7	SA/A	CAI	Panic Attacks	D	NI
8	TRUE	CKI				8	SD/D	NI	8	SA/A	CAI	**Drowsiness**	L	CKI
9	FALSE	CKI							9	SA/A	CAI	**Feeling in a "Fog"**	L	CKI
10	TRUE	VS							10	SA/A	CAI	Weight Gain	D	NI
11	FALSE	CKI										**Feeling Slowed Down**	L	CKI
12	FALSE	CKI										Reduced Breathing Rate	D	NI
13	TRUE	CKI										Excessive Studying	D	NI
14	FALSE	CKI										**Difficulty Concentrati ng**	L	CKI
15	FALSE	VS										**Dizziness**	L	CKI
16	TRUE	CKI										Hair Loss	D	NI
17	TRUE	CKI												
18	FALSE	CKI												

CKI = Concussion Knowledge Index; CAI: = Concussion Attitude Index; NI = No index (item not part of any index); VS = Validity Scale; SD/D = Strongly Disagree / Disagree; SA/A = Strongly Agree / Agree; L = Legitimate symptom; D = Distractor Symptom

Supplementary table 3.2: Modified RoCKAS questions.

Section 1		
Q	**Original Question**	**Modified**
15	High-school freshman and college freshman tend to be the same age.	High school students and university students tend to be the same age.
Section 4		
Q	**Original Question**	**Modified**
Scenario 3	Athlete R suffered a concussion. Athlete R's team has an athletic trainer on the staff.	Player R suffered a concussion. Player's R's team has a medic on the sideline
7	I feel that the athletic trainer, rather than Athlete R, should make the decision about returning Athlete R to play.	I feel that the medic, rather than Player R, should make the decision about returning Player R to play.
8	Most athletes would feel that the athletic trainer, rather than Athlete R, should make the decision about returning Athlete R to play.	Most athletes would feel that the medic, rather than Athlete Player R, should make the decision about returning Player R to play.

Supplementary table 3.3: Concussion Knowledge Index (CKI) Section 1 and 2 responses of male and female players.

Section 1 - DIRECTIONS: Please read the following statements and circle TRUE or FALSE for each question.			
	Frequency (%) Correct		
Statement (correct answer)	Female (n=41)	Male (n=33)	Overall (n=74)
1 There is a possible risk of death if a second concussion occurs before the first one has healed. (TRUE)	35 (85)	31 (94)	66 (89)
2 Running everyday does little to improve cardiovascular health. (FALSE)	38 (93)	31 (94)	69 (93)
3 People who have had one concussion are more likely to have another concussion. (TRUE)	27 (66)	25 (76)	52 (70)
4 Cleats help athletes' feet grip the playing surface. (TRUE)	40 (98)	29 (88)	69 (93)
5 In order to be diagnosed with a concussion, you have to be knocked out. (FALSE)	40 (98)	33 (100)	73 (99)
6 A concussion can only occur if there is a direct hit to the head. (FALSE)	30 (73)	27 (82)	57 (77)
7 Being knocked unconscious always causes permanent damage to the brain. (FALSE)	28 (68)	23 (70)	51 (69)
8 Symptoms of a concussion can last for several weeks. (TRUE)	38 (93)	33 (100)	71 (96)
9 Sometimes a second concussion can help a person remember things that were forgotten after the first concussion. (FALSE)	37 (90)	27 (82)	64 (86)
10 Weightlifting helps to tone and/or build muscle. (TRUE)	41 (100)	31 (94)	72 (97)
11 After a concussion occurs, brain imaging (e.g., CAT Scan, MRI, X-Ray, etc.) typically shows visible physical damage (e.g., bruise, blood clot) to the brain. (FALSE)	10 (24)	16 (48)	26 (35)
12 If you receive one concussion and you have never had a concussion before, you will become less intelligent. (FALSE)	40 (98)	32 (97)	72 (97)
13 After 10 days, symptoms of a concussion are usually completely gone. (TRUE)	10 (24)	13 (39)	23 (31)
14 After a concussion, people can forget who they are and not recognize others but be perfect in every other way. (FALSE)	10 (24)	8 (24)	18 (24)
15 High-school freshmen and college freshmen tend to be the same age. (FALSE)	40 (98)	31 (94)	71 (96)

16 Concussions can sometimes lead to emotional disruptions. (TRUE)	41 (100)	33 (100)	74 (100)
17 An athlete who gets knocked out after getting a concussion is experiencing a coma. (TRUE)	6 (14)	10 (30)	16 (22)
18 There is rarely a risk to long-term health and well-being from multiple concussions. (FALSE)	29 (70)	30 (90)	59 (80)

Supplementary table 3.3: Continued.

Section 2 - DIRECTIONS: Please read the following statements and circle TRUE or FALSE for each question.			
	Frequency (%) Correct		
Statement (correct answer)	Female (n=41)	Male (n=33)	Overall (n=74)
Scenario 1:			
While playing in a game, Player Q and Player X collide with each other and each suffers a concussion. Player Q has never had a concussion in the past. Player X has had 4 concussions in the past.			
1 It is likely that Player Q's concussion will affect his long-term health and well-being. (FALSE)	23 (56)	8 (24)	31 (42)
2 It is likely that Player X's concussion will affect his long-term health and well-being. (TRUE)	39 (95)	10 (30)	49 (66)
Scenario 2:			
Player F suffered a concussion in a game. She continued to play in the same game despite the fact that she continued to feel the effects of the concussion.			
3 Even though Player F is still experiencing the effects of the concussion, her performance will be the same as it would be had she not suffered a concussion. (FALSE)	29 (95)	32 (97)	61 (82)

Supplementary table 3.4: Concussion Attitude Index (CAI) Section 3 and 4 responses of male and female players.

Section 3 - DIRECTIONS: For each question circle the number that best describes how you feel about each statement.			
	Frequency (%) Safe/desirable responses		
Statement (safe/desirable response)	Female (n=41)	Male (n=33)	Overall (n=74)
1 I would continue playing a sport while also having a headache that resulted from a minor concussion. (CRI) (STRONGLY DISAGREE/DISAGREE)	28 (68)	20 (61)	48 (65)
2 I feel that coaches need to be extremely cautious when determining whether an athlete should return to play. (STRONGLY AGREE/AGREE)	38 (93)	31 (94)	69 (93)
3 I feel that mouthguards protect teeth from being damaged or knocked out. (STRONGLY AGREE/AGREE)	37 (90)	27 (82)	64 (86)
4 I feel that professional athletes are more skilled at their sport than high-school athletes.			
5 I feel that concussions are less important than other injuries. (STRONGLY (DISAGREE/DISAGREE)	38 (93)	32 (97)	70 (95)
6 I feel that an athlete has a responsibility to return to a game even if it means playing while still experiencing symptoms of a concussion. (STRONGLY DISAGREE/DISAGREE)	38 (93)	31 (94)	69 (93)
7 I feel that an athlete who is knocked unconscious should be taken to the emergency room. (STRONGLY AGREE/AGREE)	29 (95)	29 (88)	58 (78)
8 I feel that most high-school athletes will play professional sports in the future.			

Supplementary table 3.4: Continued.

Section 4 - DIRECTIONS: For each question read the scenarios and circle the number that best describes your view. (For the questions that ask you what *most athletes* feel, base your answers on how you think MOST athletes would feel.)			
	Frequency (%) Safe/desirable responses		
Statement (safe/desirable response)	Female (n=41)	Male (n=33)	Overall (n=74)
Scenario 1:			
Player R suffers a concussion during a game. Coach A decides to keep Player R out of the game. Player R's team loses the game.			
1 I feel that Coach A made the right decision to keep Player R out of the game. (STRONGLY AGREE/AGREE)	40 (98)	33 (100)	73 (99)
2 Most athletes would feel that Coach A made the right decision to keep Player R out of the game. (STRONGLY AGREE/AGREE)	35 (85)	25 (76)	50 (66)
Scenario 2:			
Athlete M suffered a concussion during the first game of the season. Athlete O suffered a concussion of the same severity during the semi-final playoff game. Both athletes had persisting symptoms.			
3 I feel that Athlete M should have returned to play during the first game of the season. (STRONGLY DISAGREE/DISAGREE)	38 (92)	32 (97)	70 (95)
4 Most athletes would feel that Athlete M should have returned to play during the first game of the season. (STRONGLY DISAGREE/DISAGREE)	33 (80)	28 (85)	61 (82)
5 I feel that Athlete O should have returned to play during the semi-final playoff game. (STRONGLY DISAGREE/DISAGREE)	37 (90)	31 (94)	68 (92)
6 Most athletes feel that Athlete O should have returned to play during the semi-final playoff game. (STRONGLY DISAGREE/DISAGREE)	32 (78)	26 (79)	58 (78)
Scenario 3:			
Athlete R suffered a concussion. Athlete R's team has an athletic trainer on the staff.			
7 I feel that the athletic trainer, rather than Athlete R, should make the decision about returning Athlete R to play. (STRONGLY AGREE/AGREE)	31 (75)	30 (91)	61 (82)
8 Most athletes would feel that the athletic trainer, rather than Athlete R, should make the decision about returning Athlete R to play. (STRONGLY AGREE/AGREE)	33 (80)	27 (82)	60 (81)
Scenario 4:			
Athlete H suffered a concussion and he has a game in two hours. He is still experiencing symptoms of concussion. However, Athlete H knows that if he tells his coach about the symptoms, his coach will keep him out of the game.			
9 I feel that Athlete H should tell his coach about the symptoms. (STRONGLY AGREE/AGREE)	39 (95)	32 (97)	71 (96)
10 Most athletes would feel that Athlete H should tell his coach about the symptoms. (STRONGLY AGREE/AGREE)	34 (83)	26 (79)	60 (81)

Supplementary table 3.5: CKI and CAI score comparison to other studies modified from Shafik study [339].

	Sport	Total Participants (female%)	Mean CKI score (0-25)	Mean CAI score (15-75)	
Williams et al (2016)	English men's professional football players	26 (0)	16.4 ±2.9	59.6 ±8.5	
Gallagher and Falvey (2017)	Irish footballer players	70 (100)	18.7 ±2.2	60.3 ±6.9	
Kraak et al (2018)	University hostel rugby players (SA)	180 (0)	18.8 ±2.4	60.98 ±6.32	
Salmon et al 2020	Community Rugby Union Players (NZ)	533 (38.5%)	Overall 18.6 ±2.4 Women 18.9 ±2.1 Men 18.4 ±2.6	Overall 59.3 ±6.8 Women 60.2 ±6.5 Men 58.8 ±7.0	
Shafik et al (2023)	Elite female football players	111 (100)	20.53 ±2.31	63.28 ±6.33	
Olanrewaju et al (2023)	Nigerian footballer players	331 (29.6)	14.0 ±3.0	54.5 ±9.4	CKI and CAI not reported by gender. But sex was considered as a covariate in further analysis
Travis et al 2024	British American Football players	151 (28)	21.0 ±2.1	55.6 ±6.1	CKI and CAI not reported by gender
Current study	Community football players (NZ)	74 (55)	Overall 19.88 Women 19 ±3.7 (76%) Men 20.76 ±1.4 (80%)	Overall 64.66 (85%) Women 63.74 (85%) Men 65.56 (87%)	

Appendix VII: Supplementary material for Chapter 4

Supplementary table 4.1: WILD AI symptom data collected in the app.

Check-ins	Type	For Study	Type of answer	Scoring	Scoring guide	Symptom cluster	Total Number (max)	Total rating (max)	Total Score (max)
Period	cycle	T	Y/N			Physical	28	5	140
Spotting	cycle	T	Rating	0-1-3-5	none, light, medium, heavy	Cognitive	1	5	5
Withdrawal bleeding	cycle	T	Rating	0-1-3-5		Emotional	4	5	20
Alcohol	lifestyle	T	Rating	0-1-3-5		Sleep related	1	5	5
Appetite	lifestyle	T	Rating	0-1-3-5		**Total**	**34**		**170**
Body image	lifestyle	T	Rating	1-3-5	poor, ok, great				
Hydration	lifestyle	T	Rating	1-3-5	poor, ok, great	Sleep score	Hours	5	
Nutrition	lifestyle	T	Rating	1-3-5	poor, ok, great				
Sex drive	lifestyle	T	Rating	1-3-5	low, med, high				
Insomnia	symptoms	T	Rating	0-1-3-5					
Sleep hours	morning	T	Value						
Sleep quality	morning	T	Rating	1-3-5	poor, ok, great				
Any notes	notes	T - INJURY							
Acne	symptoms	T	Rating	0-1-3-5	none, slight, some, a lot				
Anxiety	symptoms	T	Rating	0-1-3-5					
Bloating	symptoms	T	Rating	0-1-3-5	none, mild, moderate, severe				
Brain fog	symptoms	T	Rating	0-1-3-5					
Breast tenderness	symptoms	T	Rating	0-1-3-5					
Cold flush	symptoms	T	Rating	0-1-3-5					
Constipation	symptoms	T	Rating	0-1-3-5					

Supplementary table 4.1 Continued.

Check-ins	Type	For Study	Type of answer	Scoring	Scoring guide	Symptom cluster	Total Number (max)	Total rating (max)	Total Score (max)
Cravings	symptoms	T	Rating	0-1-3-5					
Depression	symptoms	T	Rating	0-1-3-5					
Diarrhoea	symptoms	T	Rating	0-1-3-5					
Faintness	symptoms	T	Rating	0-1-3-5					
Fatigue	symptoms	T	Rating	0-1-3-5					
Gas	symptoms	T	Rating	0-1-3-5					
Head injury	symptoms	T	Rating	0-1-3-5					
Headache	symptoms	T	Rating	0-1-3-5					
Histamine-related symptoms	symptoms	T	Rating	0-1-3-5					
Hot flush	symptoms	T	Rating	0-1-3-5					
IBS	symptoms	T	Rating	0-1-3-5					
Illness	symptoms	T	Rating	0-1-3-5					
Injury	symptoms	T	Rating	0-1-3-5					
Joint pain	symptoms	T	Rating	0-1-3-5					
Lower back pain	symptoms	T	Rating	0-1-3-5					
Migraine	symptoms	T	Rating	0-1-3-5					
Mood	factor	T	Rating	1-3-5	low, ok, great				
Mood	factor	T	Rating	1-3-5	low, ok, great				
Mood swings	symptoms	T	Rating	0-1-3-5	none, mild, moderate, severe				

Supplementary table 4.1 Continued.

Check-ins	Type	For Study	Type of answer	Scoring	Scoring guide	Symptom cluster	Total Number (max)	Total rating (max)	Total Score (max)
Motivation	factor	T	Rating	0-1-3-5					
Nausea	symptoms	T	Rating	0-1-3-5	none, slight, mod, high				
Night sweats	symptoms	T	Rating	0-1-3-5					
Soreness	symptoms	T	Rating	0-1-3-5					
Stomach pain	symptoms	T	Rating	0-1-3-5					
Stress	symptoms	T	Rating	0-1-3-5	none, low, medium, high				
Uterine cramps	symptoms	T	Rating	0-1-3-5					
Water retention	symptoms	T	Rating	0-1-3-5					
Coordination	training	T	Rating						
Eagerness	training	T	Rating						
Muscle strength	training	T	Rating						
Perceived heart rate	training	T	Rating						
Rate of perceived exertion	training	T	Rating						
Repetitions in reserve	training	T	Rating						
Stamina	training	T	Rating						
Training modifications	training	T	Y/N						
Workout readiness	training	T	Rating						

Supplementary material 4.1: LEAF_Q questions and instructions.

The low energy availability in females questionnaire (LEAF –Q), focuses on physiological symptoms of insufficient energy intake. The following pages contain questions regarding injuries, gastrointestinal and reproductive function. We appreciate you taking the time to fill out the LEAF-Q and the reply will be treated as confidential.

Unique Study Code:
Address:
E-mail:
Mobile:
Occupation:
Education:
Age: (years)
Height: (cm)
Weight: (kg)

Your highest weight with your present height: (kg) (excluding pregnancy)?
Your lowest weight with your present height: (kg)?
What is your desired weight (kg)?
Do you smoke?

- Yes
- No

Do you use any medication (excluding oral contraceptives)?

- Yes
- No

If yes, what kind of medication?
Your normal amount of training (average) – number of hours per week and what kind of exercise, such as running, swimming, cycling, strength training, technique training etc. ('training' is any kind of physical activity)

Comments or further information regarding exercise:

1. Injuries

A: Have you had absences from your training, or participation in competitions during the last year due to injuries?

- No, not at all
- Yes, once or twice
- Yes, three or four times
- Yes, five times or more

A1: If yes, for how many days absence from training or participation in competition due to injuries have you had in the last year?

- 1-7 days
- 8-14 days
- 15-21 days
- 22 days or more

A2: If yes, what kind of injuries have you had in the last year?

Comments or further information regarding injuries:

2. Gastrointestinal function

- A: Do you feel gaseous or bloated when you do not have your period?
- Yes, several times a day

- Yes, several times a week
- Yes, once or twice a week or more seldom
- Rarely or never

B: Do you get cramps or stomach aches that are not related to your menstruation?
- Yes, several times a day
- Yes, several times a week
- Yes, once or twice a week or more seldom
- Rarely
- Never

C: On average how often do you have bowel movements?
- Several times a day
- Once a day
- Every second day
- Twice a week
- Once a week or more rarely

D: How would you describe your normal stool?
- Normal (soft)
- Diarrhoea-like (watery)
- Hard and dry

Comments regarding gastrointestinal function:

3.1 Use of contraceptives
A: Do you use oral contraceptives?
- Yes
- No

A1: If yes, why do you use oral contraceptives?
- Contraception
- Reduction of menstruation pains
- Reduction of bleeding
- To regulate the menstrual cycle in relation to performances etc.
- Otherwise, menstruation stops
- Other

A2: If no, have you used oral contraceptives in the past?
- Yes
- No

A2:1: If yes, when and for how long?

B: Do you use any other kind of hormonal contraceptives? (e.g., hormonal implant or coil)
- Yes
- No

B1: If yes, what kind?
- Hormonal patches
- Hormonal ring
- Hormonal coil

- Hormonal implant
- Other

3.2 Menstrual function

A: How old were when you had your first period?

- 11 years or younger
- 12-14 years
- 15 years or older
- I don't remember
- I have never menstruated (If you have answered "I have never menstruated" there are no further questions to answer)

B: Did your first menstruation come naturally (by itself)?

- Yes
- No
- I don't remember

B1: If no, what kind of treatment was used to start your menstrual cycle?

- Hormonal treatment
- Weight gain
- Reduced amount of exercise
- Other

C: Do you have normal menstruation)

- Yes
- No (go to question C6)
- I don't know (go to question C6)

C1: If yes, when was your last period?

- 0-4 weeks ago
- 1-2 months ago
- 3-4 months ago
- 5 months ago or more

C2: If yes, are your periods regular? (Every 28th to 34th day)

- Yes, most of the time
- No, mostly not

C3: If yes, for how many days do you normally bleed?

- 1-2 days
- 3-4 days
- 5-6 days
- 7-8 days
- 9 days or more

C4: If yes, have you ever had problems with heavy menstrual bleeding?

- Yes
- No

C5: If yes, how many periods have you had during the last year?

12 or more

- 9-11

- 6-8
- 3-5
- 0-2

C6: If no or “I don’t remember”, when did you have your last period?

- 2-3 months ago
- 4-5 months ago
- 6 months ago or more
- I am pregnant therefore I do not menstruate

D: Have your periods ever stopped for 3 consecutive months or longer (besides pregnancy)?

- No, never
- Yes, it has happened before
- Yes, that’s the situation now

E: Do you experience that your menstruation changes when you increase your exercise intensity, frequency or duration?

- Yes
- No

E1: If yes, how? (Check one or more options)

- I bleed less
- I bleed fewer days
- My menstruations stops
- I bleed more
- I bleed more days

Supplementary table 4:2: Sleep hours across the menstrual cycle.

Parameter	Coefficient	SE	CI	CI_low	CI_high	T=	df_error	p	Effects	Group
(Intercept)	6.904613	0.498319	0.95	5.926357	7.88287	13.85582	754	4.66E-39	fixed	
poly(day, 3)1	0.705853	1.2018	0.95	-1.65342	3.065124	0.58733	754	5.57E-01	fixed	
poly(day, 3)2	-0.77421	1.194701	0.95	-3.11954	1.571128	-0.64803	754	5.17E-01	fixed	
poly(day, 3)3	1.607492	1.167292	0.95	-0.68404	3.89902	1.377112	754	1.69E-01	fixed	
SD (Intercept)	1.404352	NA	0.95	NA	NA	NA	NA	NA	random	participant_id
SD (Observations)	1.131322	NA	0.95	NA	NA	NA	NA	NA	random	Residual

Supplementary table 4.3: Sleep quality across the menstrual cycle.

Parameter	Coefficient	SE	CI	CI_low	CI_high	t	df_error	p	Effects	Group
(Intercept)	3.634504	0.564952	0.95	2.525415	4.743594	6.433301	744	2.23E-10	fixed	
poly(day, 3)1	-3.56644	1.196409	0.95	-5.91518	-1.2177	-2.98095	744	0.002967	fixed	
poly(day, 3)2	0.425494	1.195991	0.95	-1.92243	2.773414	0.355767	744	0.722116	fixed	
poly(day, 3)3	2.560373	1.160664	0.95	0.281806	4.83894	2.205955	744	0.027692	fixed	
SD (Intercept)	1.593442	NA	0.95	NA	NA	NA	NA	NA	random	participant_id
SD (Observations)	1.121912	NA	0.95	NA	NA	NA	NA	NA	random	Residual

Supplementary table 4.4: Sleep quality and symptoms across the menstrual cycle

Parameter	Coefficient	SE	CI	CI_low	CI_high	t	df_error	p	Effects	Group
(Intercept)	6.402232	1.162844	0.95	4.118741	8.685723	5.505669	634	5.35E-08	fixed	
sleep_quality	-0.6546	0.115579	0.95	-0.88156	-0.42763	-5.66362	634	2.25E-08	fixed	
poly(day, 3)1	-1.97109	8.34631	0.95	-18.3608	14.41866	-0.23616	634	0.813382	fixed	
poly(day, 3)2	33.60271	8.066608	0.95	17.76221	49.44321	4.165655	634	3.54E-05	fixed	
poly(day, 3)3	-25.2207	7.348441	0.95	-39.6509	-10.7905	-3.43212	634	6.38E-04	fixed	
sleep_quality:poly(day, 3)1	1.004722	2.050868	0.95	-3.02259	5.032038	0.489901	634	0.624373	fixed	
sleep_quality:poly(day, 3)2	-2.82617	2.043237	0.95	-6.8385	1.186165	-1.38318	634	0.167096	fixed	
sleep_quality:poly(day, 3)3	2.765597	1.91687	0.95	-0.99859	6.529779	1.442767	634	0.14958	fixed	
SD (Intercept)	3.032992	NA	0.95	NA	NA	NA	NA	NA	random	participant_id
SD (Observations)	3.375841	NA	0.95	NA	NA	NA	NA	NA	random	Residual

Appendix VIII: Supplementary material for Chapter 5

Supplementary material 5.1: Saliva sample collection instructions

AUT
TE WĀNANGA ARONUI O TĀMAKI MAKAU RAU

Saliva Sample Collection Guide

Why do I have to collect saliva samples?
The purpose of the saliva samples is to measure estrogen, progesterone and cortisol, these hormones are a key part of this study. As a participant in this study you will need to adhere to a number of instructions to ensure sample collection is standardised, these instructions are detailed below.

When do I collect the samples?
You will be collecting saliva samples every morning upon waking, ideally between the hours of 06.00 and 09.00. You will collect a single sample each day for upto 120 days dependent on the length of your menstrual cycle. Approximately 2ml (1/2tsp) will be collected in each sample.
It's very important that you collect the sample as close to the same time of day as possible each day.

How do I collect a saliva sample?
In your sample collection kit, you will be provided with collection tubes called cryovials.

Please collect all saliva samples with these tubes. When it is time to take a sample, follow the steps below:

1. Take a small sip of water.
2. Swish the water around in your mouth for a few seconds, and then swallow the water. This will stimulate saliva release and clear any food debris that may be in your mouth.
3. Allow saliva to collect in your mouth – relaxing your tongue from the roof of your mouth will help this process.
4. Lean forward and drool into the cryovial directing the flow of saliva from your mouth into the tube – a teaspoon can help with this.
5. Repeat this process until you have a minimum of 1.8ml clear saliva (ie no bubbles)
6. Write on each respective cryovial; (1) your unique ID number, (2) the date and (3) the exact time that you took the sample. E.g F001_070721_06:25. You can record this directly on the cryovial using the pen provided in your kit.
7. The saliva samples that are collected on the first day of bleeding of each menstrual cycle need to be marked up with the coloured pen included in your study kit.
8. Put the clearly labelled cryovial in the storage box provided and place the box in your freezer. It is important that you do this as soon as possible.

Is there anything I shouldn't do?
Prior to collecting your saliva sample you need to:

- Avoid brushing your teeth within 30minutes before collecting the sample
- Avoid eating within 30minutes before collecting your sample
- Where possible avoid eating after 22:00 the night before
- Avoid drinking beverages other than water including; juice, milk, coffee, tea, caffeinated drinks, etc. as this may contaminate the sample.
- Refrain from structured exercise and/or moderate-vigorous physical activity before sample collection.

What if I miss a sample or do it wrong?
If you forget to collect a sample at the correct time, you can still collect it later. Try and do it as soon as you remember, while avoiding any of the things listed in the section above. If you completely miss a sample one day, don't worry just continue collecting the following day. If you think you have collected a sample wrong, just repeat as soon as you can that day and contact the lead researcher if you need more cryovials. Make note of anything you think may have gone wrong on the notes sheet.

What if I have questions?

Thank-you for taking part, if you need to ask any questions regarding sample collection, please contact Natalie Hardaker, email: natalie.hardaker@aut.ac.nz, phone +64 027 898 9023

Appendix IX: Supplementary material for Chapter 6

Supplementary table 6.1: Kendall's tau correlations between biomarker and person-centred data across three consecutive menstrual cycles

	n	Kendall's tau B	p
Slp - Cort	72	0.10	0.27
Slp - Symp	83	0.06	0.48
Slp - miR	56	0.02	0.86
Cort - Symp	72	0.05	0.60
Cort - miR	56	0.10	0.29
Symp - miR	56	-0.01	0.89

Sleep Score (Slp), Cortisol (Cort), Symptom score (Symp), miR ratio (miR)

Appendix X: Conference presentation abstracts

Hume PA, Hardaker N, Kara S, et al. Sprains, strains and SOBI: Can physiotherapists have an increased role in sport originated brain injury assessment and management? In: *Sports Medicine New Zealand 2019 Conference presentation* Dunedin, 2019, p.17. Sports Medicine New Zealand.

Sprains, Strains and SOBI: Can Physiotherapists Have An Increased Role In Sport Originated Brain Injury Assessment And Management?

Patria Hume,[1,2] Natalie Hardaker,[1,3] Stephen Kara,[1,5] Chris Whatman,[1] Duncan Reid,[1] Mark Fulcher,[1,5] Doug King,[1,4] Joshua McGeown,[1] Kennedy Ewan,[6] Dusty Quinn[7]

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The clinical referral pathways for sports related sport originated brain injury (SOBI) (Hume et al., 2019) are varied, complicated and protracted in New Zealand. Given the NZ situation we wish to debate whether physiotherapists could have more of a role in SOBI assessment and management.

Approximately 50% of athletes who sustain a SOBI will experience protracted symptom resolution characterised by one or more post-concussion disorders, based on data from the Axis Sports Medicine Clinic (Kara et al., 2020). A proportion of patients with SOBI require attention from physiotherapists with specialty training in the identification and treatment of cervical and/or vestibulo-ocular pathologies. Physiotherapists therefore can play a crucial role in the management of SOBI. But what about assessment of SOBI rather than a GP referral needing to be made? Could physiotherapists be seen as key providers for assessment of SOBI as they are for musculoskeletal injuries such as sprains and strains?

Physiotherapists are often the first person the athlete sees after a SOBI and have expressed a willingness to be more involved in assessment (Reid et al., 2020). The ”Knowledge, Attitudes and Behaviours” studies (Hume et al., 2019, Reid et al., 2020) have shown that GP’s are not confident or necessarily competent in early management of SOBI. The current system could be failing many people, so we need to consider how to better use capability in NZ.

We currently have a model where a medical doctor must assess the patient to determine if there has been a SOBI. The arguments are that only a medical doctor can assess and provide a return-to-play clearance based on medical diagnosis to rule out other issues other than SOBI. What would happen if a physio missed something more sinister and the patient had adverse consequences? Should physiotherapist be making the SOBI diagnosis and being able to put this onto the Read Code on the ACC45 form to help improves data collection accuracy? Should medical clearance by a physician still be required prior to return to play?

The panel discussion will debate the issues for physiotherapists and sports physicians with the aim of identifying how we can improve clinical pathways for SOBI patients.

McGeown, JP, Kara S, ...Hardaker, NJ. Sex is a key predictor for complicated recovery trajectories post sport originated brain injury In: *The Female Athlete Conference 2019 Poster presentation* Boston, MA, 2019

Sex is a key predictor for complicated recovery trajectories post sport originated brain injury

McGeown, J.P.,[1] Kara, S.,2 Crosswell, H.,[2] Borotkanics, R.,[1] Hume, P.A.,[1,3] Quarrie, K.,[4,1] Theadom, A.[1] Fulcher, M.,[2] Hardaker, N.[5,1]

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Background

Sport originated brain injury (SOBI) as a subset of mild traumatic brain injury accounts for 21% of traumatic brain injury in New Zealand. Adults typically achieve spontaneous clinical recovery within 10-14 days of injury (McCrory et al., 2018 and Ellis & Willer, 2014). However, between 10-15% (Ellis et al., 2014) to 40-50% (Meehan et al., 2014 and Merrit et al., 2015) of individuals will experience symptoms beyond 10-14 days of injury. There is a growing body of research that shows that female athlete's appear to experience a greater incidence, report greater symptom severity and take longer to recover from SOBI. Current diagnosis of SOBI involves the assessment of a range of domains including clinical symptoms, physical signs, cognitive impairment, neurobehavioral features, and sleep/wake disturbance (McCrory et al., 2018). This presents a challenge with diagnosis and effective management as it is still heavily reliant on subjective symptom reports from patients combined with physicians' best clinical judgement. There is a need to identify prognostic models that use information collected during initial clinical assessment of SOBI to predict recovery trajectories for athletes. This study aimed to investigate which aspects of initial clinical assessment for SOBI could be used to predict whether an athlete was likely to experience symptom resolution in less than 14 days, and whether an athletes biological sex played any role in the prediction.

Institutional (ACC and AUT 18/46) ethics committee approvals were obtained for this study. Data were collected prospectively from patients with clinically diagnosed SOBI attending Axis Sports Concussion Clinic between 2017 and 2018. Initial clinical assessment data included demographics, medical history, current medical condition and SCAT-5. The SCAT-5 Post Concussion Symptom Scale (PCSS) produced a positive symptom total (PST) out of 22 symptoms (0 = no symptom, 6 = severe) and a symptom severity score (SSS) out of 132. Global Severity Index (GSI = SSS/22) and Positive Symptom Distress Index (PSDI = SSS/PST) were calculated [516] to produce weighted composite scores for self-reported symptoms. The physician applied criteria for classification of persistent symptoms into predominant symptom clusters (PSC) (Ellis and Willer 2014) to identify whether physiological, vestibulo-ocular, or cervicogenic origin symptoms contributed to participant's symptoms and presentation. Participants were classified as mixed PSC if presentation was not clearly associated with one particular PSC. Patients were categorised into expected (<14 days) and complicated (>14 days) resolution groups based on the number of days to symptom resolution. Initial assessment data were used as predictor variables. A significance level of $p < 0.05$ was set and 95% confidence intervals calculated. Statistical analyses were performed using Python v3.6 and RStudio v1.1.383. Relationships between continuous variables were evaluated using Pearson product movement correlations. Continuous variables were not normally distributed, therefore two sample Kolmogorov-Smirnov tests were used for group comparisons between complicated and expected resolution; comparisons between males and females within each respective group were done using the same method. Chi square tests were used for group comparisons of categorical variables.

Analysis of clinical data for 568 Axis patients diagnosed with SOBI included 133 females (23%). Of the 568 patients; 374 recovered in the expected time frame 55 female (15%) and 319 (85%) male. The remaining 194

patients were classified as complicated resolution cases and of those 78 were female (40%) and 116 (60%) male. These data highlight sex differences for recovery trajectories post-SOBI. Female sex was disproportionately associated with complicated recovery outcomes compared to males. Further 58% (78/155) of all females in the patient cohort experienced a complicated recovery versus just 36% (116/435) of the overall male patient cohort. The complicated resolution group versus the expected resolution group had significantly higher SCAT-5 scores for PST (16.3 ±4.7 versus 7.5 ±5.8), SSS (42.2 ±20.6 versus 13.9 ±14.5), GSI composite (1.9 ±0.9 versus 0.6 ±0.7) and PSDI composite (complicated: 2.5 ±0.8; expected: 1.4 ±0.8). Within the complicated resolution group sex comparisons showed that females had higher initial SCAT-5 scores for SAC (26.3 ±3.6 versus 26.1 ±2.3), PST (16.8 ±4.3 versus 15.9 ±4.8), SSS (46.3 ±20.8 versus 39.5 ±20.0), GSI composite (2.1 ±0.9 versus 1.8 ±0.8) and significantly (p=.008) higher initial PSDI composite scores (female: 2.7 ±0.8; male: 2.4 ±0.8). The complicated resolution group versus the expected resolution group required significantly (p = 0.000) more follow up assessments (complicated: 3.0 ±1.4 versus expected: 1.2 ±0.8;), had more days missed from work/school (3.7 ±5.8; versus 1.3 ±2.1), had more days until asymptomatic (35.8 ±20.0 versus 11.1 ±8.0). This data is consistent with previous findings of more complicated recovery in female athletes with SOBI and is indicative of female sex as a clinical predictor of complicated versus expected recovery from SOBI using a 14-day threshold. SOBI research could benefit from investigations that reflects biological sex. Studies need to be designed that use tighter methodological design to account for fluctuation of hormones across menstrual cycle phases. Symptoms for SOBI overlap with menstrual cycle hormone related symptoms (Wunderle et al., 2014). Therefore, reliance on subjective symptom reporting presents a challenge with diagnosis and effective management of SOBI.

Theadom A, Hardaker N, Henshall K, et al. A brief decision support tool to facilitate assessment and management of suspected mild brain injury/concussion In: 2021.

A brief decision support tool to facilitate assessment and management of suspected mild brain injury/concussion

Alice Theadom,[1,2,3,4] Natalie Hardaker,[1,2,4] Kevin Henshall,[5] Katherine Forch,[6] Kris Fernando,[4] Nusratnaaz Shaikh,[1,3,] Richard Siegert,[3] Doug King,[1,2] Mark Fulcher,[6] Sam Jewell,[7] Renata Gottgtroy,[1,2] Patria Hume,[1,2,3] Charlotte Bray[1,3]

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Sub theme: Clinical research that informs daily practice.
Aim: Currently health care pathways following a mild brain injury vary considerably. This research programme aims to facilitate and standardize health care pathway decision making for patients with suspected mild brain injury.
Method: A brief decision support tool (Brain Injury Screening Tool, BIST) was developed by a multidisciplinary expert panel using a three-step process including: 1) domain mapping; 2) item development; and 3) item testing and review. Evidence-based guidelines and clinical research were used to generate items indicating which patients may need a hospital referral, those who may benefit from early referral to concussion services and those likely to recover when well managed within primary care. Psychometric properties of the tool were investigated using data collected from 114 adults (>16 years). A consultation process with patients and clinicians was conducted to identify any modifications required.
Results: Feedback from patients and clinicians supported clinical utility: “easy for people to understand”, “nice and simple”, “good questions that felt relevant”. The BIST took <6 minutes to complete. Readability statistics indicated a reading age of 6–8 years old. A principal-components factor analysis provided support for use of a total symptom score and symptom cluster scores. These factors demonstrated excellent internal consistency: total symptom scale (α = 0.94), physical/emotional (α = 0.90), cognitive (α = 0.92) and vestibular-ocular (α = 0.80). Spearman’s correlation coefficients determined high concurrent validity between the BIST symptom scale and the Rivermead Postconcussion Symptoms Questionnaire (r = 0.91) and SCAT-5 symptom scales (r = 0.90). BIST produced a satisfactory fit (2(15) =21.67, p>0.05) to the Rasch model, with good reliability (PSI = 0.92).
Conclusion: This study provides provided evidence to support the psychometric properties of the BIST. A revised version including refinements made following consultation and psychometric review is now available.

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