Characterizing reproductive health, bone health markers, and musculoskeletal injuries in Canadian Armed Forces members and across the female lifespan

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Abstract

Reproductive health characteristics (e.g., parity status, menstrual regularity) are demonstrated to have an impact on the occurrence of musculoskeletal injury (MSKi) and bone health across the female lifespan. As well, these relationships extend to female military members; however, the association between reproductive health, bone health and the risk of MSKi have yet to be elucidated in female Canadian Armed Forces (CAF) members. This multi-stage, interdisciplinary approach examining the relationship between reproductive health and injury risk across the female lifespan included: i) a self-report questionnaire where reproductive health and MSKi data were collected and examine among female CAF members; ii) the validation of an ultrasound device to assess bone mineral density (BMD); iii) physical testing where physical fitness (e.g., VO_{2max}, bench press), reproductive health hormones, and their influence on BMD; iv) a cross-sectional study that examined the impact of objectively measured behavioural (e.g., physical activity and sleep) and reproductive factors and their association with BMD in a pregnant population; and v) a five-year longitudinal study examining the impact of the menopause transition on BMD. It was determined that female CAF members who experience endometriosis and menstrual irregularity were more like to have an acute injury and repetitive strain injury, respectively. With the use of a linear regression to transform the output from the UltraScan650™, the ultrasound device was both a valid and reliable way to assess BMD and was used to determined that female CAF members who are parous and used hormonal birth control (HBC) have higher BMD compared to their nulliparous and non-HBC using peers. Further, cardiorespiratory fitness was the greatest predictor of BMD, and the ratio of estradiol/progesterone was a significant predictor of BMD. Among pregnant individuals, longer sleep was linked to lower BMD and age predicted BMD among those in their third trimester and who experienced exposure to HBC. In the menopause cohort, having higher follicle-stimulating hormone (FSH) across their menopause

transition was linked to a greater decline in BMD and serum calcium was negatively associated with BMD. Thus, reproductive health should be considered when developing injury prevention programs for female CAF members. More research in CAF population is required as it relates to pregnant and menopausal populations to develop injury prevention programs suitable for the life-stage.

Co-authorship and formatting note statement

Related to co-authorship, a contribution statement has been included in the title page of each chapter.

Please be aware that the formatting for each chapter reflects the requirements of the journal where the article was submitted, thus multiple citation styles and formatting requirements have been implemented

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List of Abbreviations

1,25(OH)$_2$D – 1,25-dihydroxyvitamin D

ACL – Anterior cruciate ligament

ACSM – American College of Sports Medicine

AIC – Akaike information criterion

ANCOVA – Analysis of covariance

ANOVA – Analysis of variance

aOR – Adjusted odds ratio

BIC – Bayesian information criterion

BMD – Bone mineral density

BMI – Body mass index

CAF – Canadian Armed Forces

CEGEP – Collège d'enseignement général et professionnel

CI – Confidence interval

COI – Conflict of interest

COVID-19 – Coronavirus disease 2019

DND – Department of National Defence

DXA – Dual-energy X-ray absorptiometry

E2 – Estradiol

ELISA – Enzyme-linked immunosorbent assay

FFM – Fat-free mass

FM – Fat mass

FSH – Follicle-stimulating hormone

GnRH – Gonadotropin-releasing hormone

HBC – Hormonal birth control

HPL – Hand positioning locator

ICC – Intraclass correlation coefficient

IDEaS – Innovation for Defence Excellence and Security

IQR – Interquartile range

IUD – Intrauterine device

LBM – Lean body mass

LH – Luteinizing hormone

LM – Lean mass

LOA – Limits of agreement

MONET – Montreal–Ottawa New Emerging Team

MPA – Moderate-intensity physical activity

MSKi – Musculoskeletal injury

NCM – Non-commissioned member

NS – Not significant

NTD – Net time delay

P4 – Progesterone

PA – Physical activity

PAEE – Physical activity energy expenditure

PCOS – Polycystic ovary syndrome

PMOS – Polyendocrine-metabolic ovarian syndrome

PTH – Parathyroid hormone

REB – Research Ethics Board

RED-S – Relative energy deficiency in sport

RM – Repetition maximum

RSI – Repetitive strain injury

SD – Standard deviation

SE – Standard error

SMM – Skeletal muscle mass

SPSS – Statistical Package for the Social Sciences

UPL – Ulna position locator

VO_2max – Maximal oxygen uptake

Chapter 1 : Introduction

The interplay between reproductive health, bone mineral density (BMD), and musculoskeletal injuries (MSKi) presents critical implications for female operational readiness in the military setting. This thesis examines these relationships, recognizing that hormonal fluctuations influence skeletal integrity and injury susceptibility, across key reproductive stages: i) pre-menopause; ii) pregnancy; iii) menopause. While the primary focus of this thesis is on female Canadian Armed Forces (CAF) members, the limited representation of pregnant and menopausal individuals in the CAF sample necessitated the inclusion of civilian cohorts to explore these reproductive stages.

Musculoskeletal injuries among female military members

Presently, females make up approximately half of the Canadian workforce; however, they account for only 15% of the CAF members. [1] The CAF aims to increase female representation among their membership to 25% by 2026, [2] to meet this goal potential disparities in MSKis must be addressed. MSKi represent a significant and persistent challenge within military populations, compromising operational readiness and increasing healthcare costs.

MSKis are musculoskeletal disorders resulting from mechanical energy transfer, which cause pain and/or a reduction in function (e.g., muscle strain, fracture, ligament tear); these injuries can be acute or due to repetitive strain. [3] Both in Canada and in other western countries, [4,5] MSKi are the leading health issue within the military and the most prevalent cause of medical release and medical downgrade [6]. While MSKi are common among all military personnel, female members experience unique risks and consequences due to anatomical, physiological, and occupational differences. These differences can leave female members at a disadvantage when expected to

complete the same training exercises and lift the same weight as men in the military context. [7] Reports suggest that female military members are at greater risk for experiencing an MSKi compared to their male counterparts; [8,9]however, physical fitness is an important consideration, as when physical fitness was controlled for there was no difference in injury rates between male and female recruits. [7] Moreover, females are more likely to require time-off as a result of MSKi compared to their male counter parts. [10] These findings highlight the importance of understanding the sex-specific risk factors and elucidating targeted prevention strategies to reduce the burden of MSKi among female military members.

With the exception of physical fitness, the risk factors associated with risk of injury among female military members are disputed. [8,11] A scoping review conducted by our research group found that rank, BMI, vitamin D, and calcium supplementation were linked to MSKi[11] among female military members (**Figure 1.1**); however, a 2022 systematic review found the relationship between BMI and stress fracture to be contentious, with BMI's outside the normal range (i.e., 18.5-25.0 $kg.m^2$) not consistently being linked to injury. [8] A study investigating anterior cruciate ligament (ACL) injuries found that Commissioned Officers (i.e., members who require a bachelor's degree), had fewer ACL injuries compared to enlisted members;[12] enlisted members are similar to non-commissioned members in the Canadian context. In the same study, multivariable regressions determined that female Officers were more likely to experience an ACL injury compared to male Officers, but the opposite was true for their enlisted counterparts. Further, an American study found that calcium and vitamin D supplementation was linked to a decreased risk of stress fracture among female navy recruits. [13] This information highlights rank, BMI and vitamin D and calcium supplementation as potential mediators of MSKi risk among female military members.

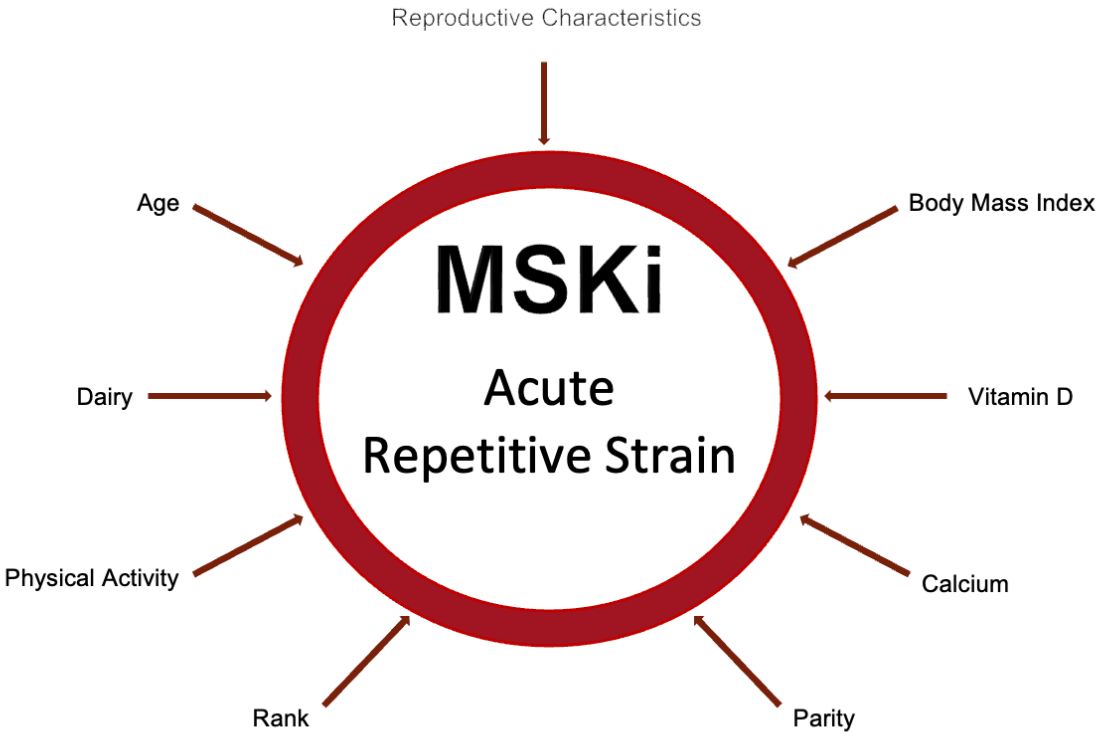

Figure 1.1. Factors that influence risk of musculoskeletal injuries (MSKi). Figure adapted from Hughes et al.

Injury-related data within the Canadian military context remain limited. A 2020 report by the Department of National Defence (DND), examining injuries among CAF personnel identified comparable rates of both acute and repetitive strain injury (RSI) between male and female members. However, the underlying causes of these injuries were not investigated and therefore may differ by sex. A noted limitation of the report was the relatively small representation of female personnel in the sample (n = 325, 9.3%), which may have constrained the generalizability of the findings. The report recommends that future research incorporate open-ended yet targeted questions concerning injury mechanisms to enhance the understanding of sex-specific injury

patterns.[14] Thus, the underlying causes of MSKis must be investigated in a sufficiently robust female sample.

Reproductive health characteristics and musculoskeletal injuries

In both military and civilian populations, many female reproductive health parameters have been linked to MSKi. Pregnancy and the postpartum stage are linked to higher rates of MSKi among female military populations. [15] Other reproductive health characteristics such as: late age of menarche, amenorrhea,[11] endometriosis, menopause, and irregular menstruation[16] are related to MSKi both within and outside of the military setting. Thus, it is possible that a relationship between reproductive health parameters and MSKi exist due to reproductive hormones' (**Table 1.1**), such as estrogen, progesterone, and follicle stimulating hormone (FSH) fluctuations across the menstrual cycle and female life span. Reproductive hormones and their relationship to MSKi have yet to be investigated in a female CAF population.

Table 1.1. Reproductive health biomarker characteristics.[17-31]

Biomarker	Cell/Organ of origin	Mechanism of release	Main Target	Role in MSKi
Relaxin	Corpus luteum located in the ovaries	Menstruation: the pituitary gland releases luteinizing hormone that targets the ovaries to stimulate the release of relaxin Pregnancy: placenta releases human chorionic gonadotrophin that targets the ovaries stimulating the release of relaxin	Uterus	Decreases ligament elasticity, increasing risk of injury
Estrogen	Corpus luteum located in the ovaries, placenta	Luteinizing hormone and FSH are released from the pituitary gland into the blood to stimulate the ovaries that start releasing estrogen.	Uterus	Inhibits bone resorption, stimulates bone formation, decreasing risk of fracture
Progesterone	Corpus luteum located in the ovaries, placenta	Luteinizing hormone is released from the pituitary gland into the blood to stimulate the	Placenta if embryo implantation occurs, degraded if not	Excites bone formation (concentration dependent),

		ovaries and subsequent release of progesterone.		decreasing risk of fracture
Follicle-stimulating hormone (FSH)	Gonadotropic cells located in the anterior pituitary gland	The hypothalamus releases gonadotrophin-releasing hormone to bind to gonadotropic cells in the anterior pituitary gland thereby secreting FSH.	Ovaries	Decreases BMD, increases risk of fracture

FSH: Follicle-stimulating hormone. MSKi: musculoskeletal injury

Estrogen, with respect to skeletal health, is meant to decrease bone remodeling by lowering bone resorption and maintaining formation. [17] With the decline of estrogen, particularly the 85-90% reduction in serum estradiol associated with the menopause transition. [18] a decrease in bone mineral density (BMD) is also observed, particularly during the perimenopausal stage. [19,20] Lower estrogen levels, such as those observed during amenorrhea, have been associated with increased risk of bone stress injuries. [21] Progesterone, increases during the luteal phase of menstruation and more significantly during pregnancy, [22,23] binds to specific receptors on osteoblasts (i.e., cells responsible for bone formation) to stimulate bone matrix synthesis. [24] However, this effect is dose-dependent, as excessive progesterone can impair osteoblastic differentiation. [25] There is growing evidence to suggest that progesterone is most effective in ameliorating bone formation *in vivo* in the presence of sufficient estrogen. [26] FSH rises significantly during the early menopausal transition, independently of estrogen. [27,28] and is positively correlated with markers of bone resorption (e.g., c-terminal peptide and β -I collagen) and negatively correlated with BMD. [29] FSH levels also fluctuate throughout the menstrual cycle, peaking at the luteal-follicular transition, [30] and evidences has demonstrated that FSH inhibition results in increased bone mass. [31] Given the well documented influence of female reproductive hormones on bone turnover (**Figure 1.2**) in the civilian population, it is worth investigating whether this relationship impacts MSKi rates and BMD among female CAF members.

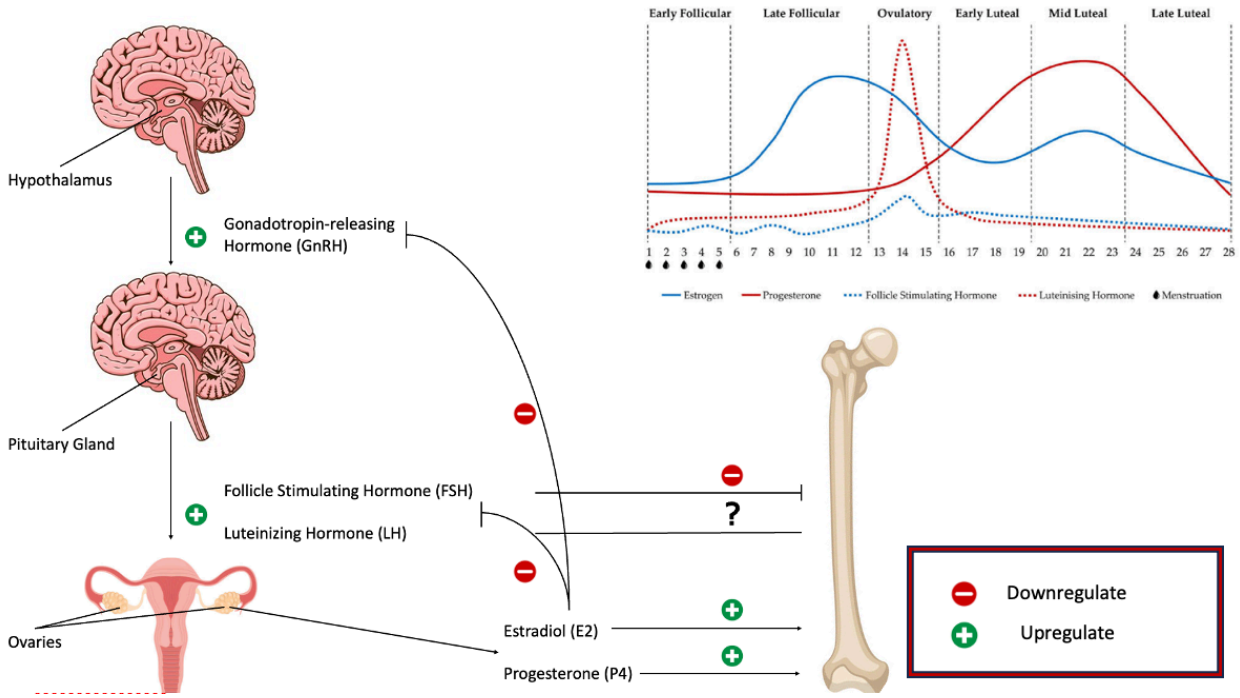

Figure 1.2 Hypothalamic-Pituitary-Gonadal Axis influence on bone health and female hormone fluctuation across the menstrual cycle. Shown in this figure are the flow of female hormones through the hypothalamic-pituitary-gonadal axis and their influence on bone health and bone health markers. Figure adapted from Carmicheal et al. and Mills et al, The Relationship Between Bone and Reproductive Hormones Beyond Estrogens and Androgens, Endocrine Reviews, 2021, under the CC BY 4.0 license.

Pregnancy and Postpartum

While we have initiated research into the risk of MSKi associated with pregnancy and postpartum return to duty in the CAF, several important gaps remain that require further examination. Currently, there is limited information surrounding the reproductive health of female CAF members as it relates to MSKi. A report by Guerin *et al.* highlighted that more attention is required with regarding the reproductive health of female CAF members, given the majority serving are in their reproductive years. [14]

Pregnancy, in the military or otherwise, is a period within the female life span that increases the risk of injury. [32,33] During the pregnancy and postpartum period, hormone levels fluctuate significantly – particularly with increases in estrogen and progesterone. Additionally, relaxin, an

insulin-like hormone produced mainly by the ovaries, [34] may contribute physiologically to the heightened risk of injury. During gestation, relaxin increases in tandem with estrogen to elevate the elasticity of ligaments by lowering the density of collagen fibre bundles. While relaxin levels peak in early pregnancy and decrease across the gestational period, they remain higher than in the non-pregnant state. [34] This mechanism exists to allow the pelvic floor to become relaxed during the delivery of the fetus; however, a secondary outcome is that joints become more flexible, increasing the risk of ligament injury during pregnancy. In the US military, female service members show lower fitness test success rates at 6- and 12-months postpartum, [35] suggesting they may not be fully physically prepared for returning to work, potentially increasing their risk for MSKi. Further, in the UK Army Service, a higher risk of injury has been demonstrated in their first year of service after returning to duties postpartum compared to before giving birth. [36] These findings from the American and British military context suggest that the period after gestation is linked to a higher risk of MSKi among female military members. While pregnancy and the postpartum period are linked to a decrease in bone mineral density (BMD), this effect is not permanent. [37] Evidence suggests that parous females tend to have higher BMD compared to their nulliparous counterparts. [38–40] While the underlying mechanism remains unclear, one possibility is that the higher estrogen levels during pregnancy may enhance intestinal calcium absorption. Based on the evidence, further investigation is warranted into the effects of pregnancy and the postpartum period on both MSKi and BMD - particularly within the Canadian military context.

Menstrual Cycle

Like pregnancy and the postpartum period, other reproductive stages/characteristics have been associated with MSKi among the female population. Late age of menarche and periods of amenorrhea (i.e., irregular menstrual cycles) have been found to be positively correlated to

fractures [16,41]; however, the quality of evidence has been called into question with respect to secondary amenorrhea and late age of menarche. [42] Despite this, the US Army has identified these reproductive health factors as areas that require further investigation. [43] In the civilian population, females that experience irregular menstrual cycles are at a higher risk of vertebral fracture compared to those with consistent menstrual cycles. [44] Moreover, among female civilians research has demonstrated that oral contraceptive users in those 25 to 34, there is a greater risk of ACL injury compared to those who do not use birth control. [45] It is thought that the relationship between oral contraceptive use and ACL injury exists because of slower tendon collagen synthesis in young female individuals, [46] resulting in more flexible ligaments, increasing injury risk. Given most female CAF members are premenopausal, and menstrual cycle factors may influence risk of injury, it is reasonable to explore menstrual cycle regularity, hormonal contraceptive use, and menarche as risk factors for MSKi and fracture among female CAF members.

Reproductive health disease and menopause

Endometriosis is an inflammatory disease characterized by the growth of endometrial glands and stroma outside the uterus in areas such as the ovaries, thus negatively impacting female reproductive health. [47] It is believed that endometriosis impacts approximately 10% of reproductive aged females and reported symptoms include pelvic pain, dysmenorrhea, and infertility [48]. Among civilians, higher levels of FSH are linked to endometriosis and can lead to lower BMD in the postmenopausal population. [29,49] Although symptoms of endometriosis can resolve with menopause, postmenopausal females are at an increased risk of MSKi due to a decrease in reproductive hormones needed to maintain skeletal health (i.e., estrogen and progesterone). [50] Information on menopause and the menopause transition remains limited among female military members. In response, the United States military has recently issued a call to

action for research focused on menopause in active duty members, aiming to enhance their health, wellbeing and retention rates. [51] Meanwhile, in civilian populations, the menopausal transition has been linked with a greater risk of acute injury. [52] These findings highlight the need to for further investigation into reproductive states characterized by reduced ovarian function in both military and civilian populations.

Vitamin D and Calcium

Fluctuations in vitamin D and calcium - critical markers of bone health - throughout the female lifespan can negatively influence bone strength and increase the incidence of MSKi. [53] Clinically relevant reductions in BMD can lead to low bone mass or osteoporosis. Osteoporosis, the more severe condition, is defined as a BMD that is 2.5 standard deviations or more below the average for a young and healthy population of the same sex and ethnic group. [54] Low bone mass refers to a BMD between 1 to 2.4 standard deviations below normal. [55] Both conditions increase susceptibility to fractures, particularly among the female population. [54]

Imperative to calcium absorption of *in vivo*, the active form of vitamin D – 1,25(OH)2D – is predominantly produced in the kidneys. The primary role of Ca^{++} in the body is the maintenance of skeletal integrity, which is regulated *via* intestinal absorption, renal absorption, and bone remodeling. [56] Parathyroid hormone is a hormone secreted by the parathyroid glands that regulate the production of calcium and vitamin D. When vitamin D levels in serum are low, PTH will increase to compensate, stimulating bone turnover in favour of bone resorption, potentially leading to low BMD and osteoporosis. [57] Similarly, when serum calcium is low, PTH is released to amplify bone resorption to release calcium from the bone, thus increasing calcium serum levels. [58]

Reproductive characteristics, such as pregnancy and menopause, have been shown to influence the metabolism of calcium. Vitamin D deficiencies are commonly observed during

pregnancy due to an elevated physiological demand. In response, the expression of vitamin D-binding protein expression increases to accommodate fetal development. [59] Further, calcium absorption in the intestines doubles during gestation. [60] Despite these physiological adaptations, the maternal skeleton is often resorbed to meet the calcium requirements for milk production, resulting in reductions in BMD during the post-partum stage. [60] During the menopause transition, insufficient levels of vitamin D are linked to menopausal symptoms. [61] In postmenopausal populations, there is a positive association between serum vitamin D level and BMD [53] and negative relationship with PTH. [62] During menopause, the notable decline in estrogen, leads to heightened osteoclast activity (i.e., bone cells responsible for bone resorption) and reduced calcium absorption in the intestines. These shifts result in an efflux of skeletal calcium into the extracellular fluid. [18] Thus, maintaining sufficient vitamin D serum concentrations is necessary to mitigate the estrogen-related reduction in calcium absorption and minimize bone loss throughout the menopausal transition.

Assessing vitamin D and calcium will help determine female CAF members that may be susceptible to bone loss, as there is a lack of available information on how these bone markers impact the occurrence of MSKi in the female military population. A study conducted in male and female US military recruits found that supplementation of calcium and vitamin D increases BMD. [63] This knowledge underscores the need to better understand the role of these nutrients in supporting bone health among female CAF members. However, as few active members have entered the menopausal transition, it is also valuable to study changes in BMD during menopause within a civilian cohort. Early characterization of bone health may help prevent bone resorption before it becomes pervasive, ultimately supporting long-term health and improving retention rates among female CAF personnel.

Mechanical forces and bone mineral density

Exercise, in appropriate doses, has an osteogenic effect on bone through a processes theorized as mechanotransduction. [64] Mechanical forces are exerted on the bone through ground reaction forces, [65] and create force on the bone lining cells and osteocytes, leading to osteoblastic differentiation. [66] This same effect can also be elicited through muscle contractions[67]. These effects are site-specific, with sites closest to the contracted muscle group and/or gravitational force experiencing the greatest increases in BMD. [68] In both the premenopausal (i.e., female individual in their reproductive years) [69,70] and postmenopausal[71,72] populations, resistance and aerobic training have been linked to higher BMD. Further, in a premenopausal military population, 3 days of resistance training per week was found to be associated with increases in BMD at the femur neck, lumbar spine, and radius. [73] With physical fitness mitigating the risk of MSKi in the military population, [7] physical activity/exercise is an important variable to consider when investigating BMD and MSKi in both military and civilian female populations.

Dual-energy x-ray absorptiometry and Ultrasound measurements of bone mineral density

Osteoporosis is a burden to the Canadian healthcare system. In the 2007 fiscal year alone, approximately $3.5 billion were associated with osteoporosis related care. [74] It is estimated that 5.6-10.5% of Canadians over the age of 50 have been diagnosed with osteoporosis, with females comprising the majority of cases. [75] In some populations, the prevalence among females is up to 5-times higher than in males. [76]

The key to prevention of osteoporotic fracture is early detection. [77] Despite this knowledge, osteoporosis detection/diagnosis remains costly. Presently, dual-energy x-ray absorptiometry (DXA) scanners are the gold standard to measure BMD; [78] however, purchasing a new DXA can cost around $160,000 CAD. Moreover, DXA scanners are approximately the size of a twin bed,

making them immobile. In Canada, the single-payer government health insurance system covers a BMD scan only when a patient is deemed at risk for low BMD by a physician, [79] possibly hindering early detection and implementation of preventive care. Although DXA scans involve minimal radiation exposure, this still poses a barrier for certain populations, such as pregnant individuals and people receiving cancer treatment. Ultrasound has emerged as an alternative to DXA. [80] Specifically, the UltraScan650™ (Cyberlogic, Inc., New York, NY, USA), is a desktop ultrasound device designed to estimate the bone mass at the 1/3rd distal radius, [81] a clinically relevant anatomical site. [82] Relative to the DXA, this is a low cost ($12 000 USD), user-friendly, radiation-free option to assess BMD. In an older population composed of males and females, Stein *et al.* (2013) conducted a validation study comparing the UltraScan650™ to the DXA, using correlations ($r=0.93$, $p<0.001$) and coefficient of variation of (2.1%). While a reasonable foundation, several methodological shortcomings of Stein's study should be addressed to strengthen its reliability/validity : These include: i) incorporating a more robust statistical analyses; ii) increasing the sample size; iii) ensuring greater homogeneity within the sample population (e.g., healthy premenopausal females); and iv) implementing a cross-validation approach.

Given the economic and clinical limitations of DXA, the development and validation of alternative technologies such as the UltraScan650™ represent a promising step toward improving accessibility to early osteoporosis detection. However, further validation is needed in specific populations, particularly healthy premenopausal females, to establish its clinical utility. Enhancing the methodological rigour and sample specificity of prior studies will be critical for advancing ultrasound as both a viable scalable option for BMD assessment. This device also holds potential for integration into military clinical settings, where it could facilitate early detection of individuals

at increased risk for MSKi/fracture, allowing for pre-emptive approaches to be taken for those deemed at risk of fracture, improving overall operational readiness.

Thesis Objectives

Given the CAF's objective of increasing female representation within their ranks, there is an urgent need better understand the impact of reproductive health characteristics on MSKi and bone health across the female lifespan. Despite the recognition from both Canadian and US military bodies [14,43] that reproductive health is an important factor influencing operational readiness and injury risk, data specific to female CAF members remain limited. This thesis aims to address this gap by examining the relationship between female reproductive health as it relates to MSKi (acute and repetitive strain), as well as the impact of female reproductive hormones on bone health in the CAF. Given the under-representation of pregnant and menopausal individuals in military health research, civilian cohorts were also incorporated to enable exploration of pregnancy-related and menopausal bone health changes. Through this work, we aim to identify physiological and occupational factors that may serve as future intervention targets to reduce MSKi risk and improve bone health across reproductive stages.

The studies in this thesis are organized into five objectives:

i) Investigate the association between reproductive health parameters and the occurrence of repetitive strain injury (RSI) and acute injury in female CAF members.

ii) Assess the validity of a novel ultrasound-based bone measurement device (i.e., the UltraScan650™) against the gold-standard, DXA.

iii) To characterize the relationship between the reproductive health profile and bone health of female CAF members.

iv) Evaluate the relationship between objectively measured physical activity and bone health during pregnancy.

v) Examine the relationship between physiological markers and bone mineral density across the menopause transition in civilian cohort.

Together, these objectives aim to generate foundational knowledge that informs evidence-based strategies for MSKi mitigation, bone health optimization, and retention support for females in the CAF.

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Chapter 2 : Reproductive health and musculoskeletal injury in military personnel

Preamble

An important initial step to examining the relationship between reproductive health, MSKi, and bone health is to explore the types of injuries present in a healthy nulliparous population. The findings from this chapter will inform the subsequent chapters of this thesis that explore reproductive and bone health across the female lifespan.

Appendices 1-3, not included in the published manuscript, highlight the questions used in the survey to define independent variables, covariates, and dependent variables, respectively.

Study title: Association between reproductive health factors and musculoskeletal injuries in female Canadian Armed Forces members

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Authorship Confirmation Statement

All authors confirm their authorship. J.L.P. and D.S. contributed to the writing and analysis of the manuscript with direction from all authors. J.L.P., D.S., K.S., and K.B.A conceived the study and oversaw direction and planning. All authors discussed the results, reviewed the manuscript and approved the final version.

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Abstract

Background: Musculoskeletal injuries (MSKi) play a role in member retention in the military. In general, female military members have higher rates of MSKi than males and female reproductive health characteristics may be contributing to these disparities. This study seeks to characterize reproductive health factors in female Canadian Armed Forces (CAF) members and their relationship with MSKi.

Materials and Methods: An electronic survey (SurveyMonkey®) was made available to present and former CAF members aged 18-65 years. Responses were collected between September 2020 and February 2021. Seven female reproductive characteristics were assessed: age of menarche, menstrual cycle regularity, birth control use, having given birth while serving, endometriosis, early menopause, and secondary oligomenorrhea/amenorrhea. Binary logistic regressions were used to analyze associations between reproductive characteristics with repetitive strain (RSI) and acute injuries.

Results: A total of 2,001 participants consented to the survey with 855 respondents being female. Females reporting menstrual cycles as never regular, irregular for a few months, who never had a period, and whose periods stopped while serving, presented a greater likelihood of reporting RSI compared to their peers who reported regular menstrual cycles (adjusted odds ratio [aOR]: 1.898, confidence interval [CI]: 1.138; 3.166). Participants who have experienced endometriosis presented a higher likelihood of reporting acute injuries than those who did not (aOR: 2.426, CI: 1.030; 5.709).

Conclusion: This examination of females within the CAF suggests that irregular menstrual cycles or absent periods increase the likelihood of experiencing MSKi namely those categorized as repetitive strain injuries and reporting endometriosis was associated with greater rates of acute injuries.

Introduction

The Canadian Armed Forces (CAF) aims to increase the presence of female members from 16% (August, 2020) to 25% representation by 2026. [1] With the goal of meeting this rate of female representation, the CAF is committed to developing solutions to increase the recruitment and retention of its female members. [2] To assist the CAF in meeting their target, one must gain a better understanding of the obstacles and barriers female members face. This discrepancy between male and female recruitment and retention rates is not unique to the CAF, it is common in militaries throughout the world. For example, the US military has identified several barriers to retention of female members, including policy surrounding reproductive health care and injury. [3, 4]

The Canadian Surgeon General recognizes musculoskeletal injuries (MSKi) as a fundamental factor impacting operational readiness and military members' health. [5] In 2017, Canada allocated ~ $200 million towards building strategies aimed at reducing injury prevalence in the CAF, [6] and this effort revealed that females are more likely to be medically released due to MSKi when compared to their male counterparts. [7] Furthermore, other military bodies, like the British Armed Forces also face retention challenges, as overuse injuries are the most common cause of medical released and the rates of MSKi are found to be higher in women. [8] With the adoption of gender-free policies and standards, the disparity between rates of MSKi in male and female members has grown even larger, with the likelihood of overuse injuries rising from 4:1 to 7.5:1, for female to male recruits respectively. [9] This disparity persists, with a report in 2016 demonstrating female trainees may have up to six-times higher overuse injury rates, depending on location, than their male counterparts. [8]

Our research team conducted a scoping review to synthesize factors that may result in higher risk of MSKi and identify gaps in the current literature. [10] The findings highlighted that being female (vs. male) was associated with a greater risk of MSKi, stress fractures, and general

injuries. Furthermore, the scoping review identified that reproductive health factors are implicated and should be taken into account when studying risk of MSKi in female military members. Similarly, a recent report on injuries in the CAF highlighted a need to explore reproductive characteristics as most female CAF members are in their reproductive years. [11]

Reproductive health factors that have been previously linked with MSKi in female military members include a history of amenorrhea, having no or irregular menses and late age at menarche. [10] The reasons for the late age of menarche and menstrual irregularities as predictors of increased MSKi risk are not fully understood. It has been proposed that these irregularities might be due to shorter lifetime exposure to endogenous estrogen. [12] The literature is somewhat inconsistent in this regard, as the findings of a recent qualitative systematic review exploring the relationship between late age of menarche, secondary amenorrhea, and MSKi in military populations did not support late age of menarche as a risk factor for MSKi. Furthermore, there is insufficient scientific evidence for the association between secondary amenorrhea and MSKi. [13] We have accounted for the results of our scoping review, [10] and other findings from the literature [14, 15, 16] linking reproductive health factors with MSKi in civilian (an individual not in the armed services or police force) or military females. Based on this information, we created an electronic questionnaire to explore the association between seven female reproductive health factors and i) repetitive strain (RSI) and ii) acute injuries (described in **Table 2.1**). The questionnaire was developed in consultation with an expert panel composed of various military and non-military authorities (e.g., female reproductive health specialists, doctors, physiotherapists, physiologists, and project stakeholders).

Table 2.1. Selected reproductive health factors and reasons for inclusion in the study questionnaire.

N	Reproductive health factor	Examples of previous findings suggesting a relationship with MSKi
1	Age of menarche	Predictors of MSKi, particularly stress fractures in military populations. [10]
2	Menstrual cycle periodicity	Predictors of MSKi, particularly stress fractures in military populations. [10]
3	Use of hormones for birth control	Female civilians between the ages of 25-34 years who use oral contraceptives were at an increased risk of anterior cruciate ligament (ACL) injury compared to women who do not use oral contraceptives. [15]
4	Given birth while serving in the CAF	Pregnancy and the postpartum period have been associated with a high risk of MSKi in female soldiers.[17, 18] Relaxin is an insulin-like hormone produced by the ovaries that works with estrogen throughout pregnancy to reduce elasticity of the ligaments.[19, 20]This leads to joints becoming relaxed, increasing risk of injury. [21]
5	Endometriosis	Among people with endometriosis, the mechanisms that facilitate normal ovulation are impaired, [22] with follicle-stimulating hormone (FSH) levels being significantly higher in female individuals with stage III/IV endometriosis. [23] FSH has been found to be negatively correlated with bone mineral density, [24] which may increase the risk of MSKi.
6	Early menopause	Menopause was associated with a notable decrease in estrogen production by the ovaries. Given that estrogen promotes bone formation and inhibits bone resorption, [25] there is a decrease in bone mineral density leaving female individuals more susceptible to acute fractures. [16]
7	Secondary amenorrhea	It was positively related with fracture rates among army recruits [14] and was associated with an increased risk of stress fractures in military populations. [10]

MSKi – Musculoskeletal injuries

Knowing that most female CAF members are in their reproductive years, [11] investigating their reproductive health, is essential for reducing the burden of MSKi and could inform future mitigation strategies to improve retention rates. To our knowledge, the reproductive profile of female CAF members and its role in MSKi has yet to be characterized. Therefore, this study explores how the reproductive characteristics and related conditions may contribute to the MSKi rates in female CAF members.

Materials and Methods

Study Design and Sample

This study is a retrospective case-control study. The data presented are a planned sub-analysis of a larger study examining sex-disparities related to MSKi, reproductive health, recruitment, and retention in the CAF. The inclusion criteria for the larger study were being: i) a current regular (including reserves), retired, or medically released, member of the CAF ii) between the ages of 18 and 65 years, iii) able to provide informed consent to participate, and iv) indicated biological sex. For the current study, we only included participants who answered "female" for biological sex. Participants were instructed to report MSKi experienced while they were "a serving member".

The questionnaire, developed explicitly for the CAF, was available electronically via the online cloud-based survey development software SurveyMonkey Inc. (San Mateo, USA). All participants provided their informed consent on the online platform. The survey was released in both English and French between September 2020 and February 2021. This study was approved by the University of Ottawa Research Ethics Board (H-04-19-3442) and all the procedures adopted were in accordance with the Declaration of Helsinki.

Participants were recruited using a variety of methods. The research team utilized social media accounts (e.g., lab website, Facebook, Twitter), and the social networks of the researchers involved, in addition to project stakeholders (e.g., Canadian Forces Morale and Welfare Services, Personnel Support Programs, Defence Fitness), and electronic communications.

The minimum required sample size for the overarching project was estimated accounting for: i) representation of the overall CAF population and ii) previously reported odds ratios from studies comparing MSKi rates from females vs. males in the military context. A *post-hoc* sample-size calculation was conducted for the present sub-analyses aiming to include only female respondents. The estimated number of CAF members (including Regular Forces and Reserves) is 103,873 with

16% of female representation (as of August 2020; n = 16,620) [1]. Considering the estimated prevalence of RSI (~ 35%) and acute injuries (~ 19.8%) reported in female CAF members[11], maximum error of ± 5%, and a design effect of 1.5, the minimal sample size for females was n = 508 and n = 355 for RSI and acute injury, respectively.

Variables

Independent variables, covariates, and outcomes selected to assess the impact of reproductive health factors on MSKi are described in **Table 2.2**. We describe in this table the original questionnaire categories and the subsequent breakdown used for the analysis.

Table 2.2. Independent variables, covariates, and outcomes.

N	Independent Variable	Original Categories for Response	Categories and Reference for Analysis
1	Age of menarche	1) 10 years or less 2) 11-15 years 3) 16 years or more 4) I never had a period 5) I don't know	1) < 10 years 2) 11-15 years (reference) 3) ≥ 16 years
2	Menstrual cycle periodicity	1) Regular 2) Never regular 3) Irregular for few months 4) Periods stopped while serving 5) Never had a period	1) Regular (reference) 2) Never regular, irregular for few months, periods stopped while serving and never had a period
3	Use of hormones for birth control	1) Yes 2) No	1) Yes 2) No (reference)
4	Given birth while serving in the CAF	1) Yes 2) No	1) Yes 2) No (reference)
5	Endometriosis	1) Yes 2) No	1) Yes 2) No (reference)
6	Early menopause[a]	1) Yes 2) No	1) Yes 2) No (reference)
7	Secondary oligomenorrhea/ amenorrhea[b]	1) Yes 2) No	1) Yes 2) No (reference)
N	**Covariate**	**Original Categories for Response**	**Categories and Reference for Analysis**
1	Year of enrollment	Continuous variable (year)	Continuous variable (year)

2	CAF rank	1) Junior NCM - Pte/OS/AB/Avr 2) Junior NCM - Cpl/LS 3) Junior NCM - MCpl/MS 4) Senior NCM - Sgt/PO2 5) Senior NCM - WO/PO1 6) Senior NCM - MWO/CPO2 7) Senior NCM - CWO/CPO1 8) Junior Officer - OCdt/NCdt - 2Lt/A/SLt 9) Junior Officer - Lt/SLt 10) Junior Officer - Capt/Lt(N) 11) Senior Officer – Maj/LCdr 12) Senior Officer - LCol/Cdr 13) Senior Officer - Col/Capt(N) - General Flag	1) NCMs 2) Officers (reference)
3	Current BMI classification	1) Continuous variable (combination of current weight and height (kg/m^2))	1) Underweight and normal weight (reference) 2) Overweight 3) Obese
4	Total physical activity volume	1) Continuous variable (combination of 5 physical activity categories; min/week)	1) Continuous variable (min/week)
5	Parity	1) Discrete variable (number of biological children)	1) 0 (reference) 2) 1 3) 2 4) 3+
6	Dairy consumption	1) ≥ 5x/week 2) 3-4x/week 3) ≤ 2x/week	1) ≥ 5x/week 2) 3-4x/week 3) ≤ 2x/week (reference)
7	Vitamin D	1) Never 2) Once/month 3) Once/week 4) Few times/week 5) Daily	1) Never (reference) 2) Once/month, once/week, few times/week and daily
8	Calcium	1) Never 2) Once/month	1) Never (reference)

		3) Once/week 4) Few times/week 5) Daily	2) Once/month, once/week, few times/week, daily
N	**Outcomes**	**Original Categories for Response**	**Categories and Reference for Analysis**
1	RSI[c]	1) Yes 2) No	1) Yes (reference) 2) No
2	Acute injuries[d]	1) Yes 2) No	1) Yes (reference) 2) No

[a]Defined as menopause before aged 46 years old and not due to hysterectomy.
[b]Defined as cessation of regular periods for 3 months or irregular periods for 6 months.
[c]Defined as injuries to muscles, tendons or nerves caused by overuse or repeating the same movement (e.g., ruck marching) over an extended period. For example, carpal tunnel syndrome, tennis elbow, plantar fasciitis or tendonitis.
[d]Defined as serious physical injuries, likely caused by a significant level of exertion or single incident of trauma, which were serious enough to require at least 24 hours off work after it to recover from. For example, a broken bone, a sprain.
CAF – Canadian Armed Forces; NCM – Non-commissioned members; BMI - Body Mass Index; RSI - Repetitive Strain Injuries.

Statistical Analysis

Data Cleaning

The data were made compatible with the analysis, via the recategorization described in Table 2, and performed using the SPSS statistical software, version 23.0 (SPSS Inc., Chicago, US).

Injury rate data were missing at a level of 19.3% for RSI and 20.4% for acute injuries in the female sample. Considering the missing data rates of approximately 20% for the main independent variable and outcomes, we opted for the listwise deletion method for dealing with missing data. Although other missing data methods (e.g., multiple imputation) can reduce the standard error (SE) estimates, the work by Dong and Peng (2013) found that the improvements when multiple imputations were compared to listwise deletion were minimal (e.g., SE: 0.091 vs. 0.086; 0.046 vs. 0.044) for a missing data rate of approximately 20%. More meaningful changes in the SE can be seen when the missing data rate is approximately 60%. [26] In addition, even accounting for the missing data, our sample is adequately powered for the main analysis.

For categorical variables with < 20 observations per category, we opted to combine categories when possible (e.g., Variable: *Menstrual cycle periodicity* – Recategorization: *1)*

Regular; 2) Never regular, irregular for few months, periods stopped while serving and never had a period). This is a common procedure when categorical variables are involved to improve the interpretation of results.

Main Analyses

Descriptive statistics included number and percentage for categorical variables and mean and standard deviation for continuous variables. The descriptive statistics were used to identify the sample characteristics.

We initially tested the bivariate associations (Table 4) between the independent variables selected and the MSKi outcomes (Table 2). The bivariate associations for 2x2 tables were analyzed using a chi-square test or Fisher's Exact test and the bivariate associations for greater than 2x2 tables (e.g., 2x3) were analyzed using a chi-square test or the Likelihood ratio when the assumption of having < 20% of the cells, with an expected count of less than 5, was not met. For the associations of reproductive health factors and MSKi outcomes in which p-values were ≥ 0.05, the logistic regressions were not performed

When a p-value < 0.05 was found in the bivariate association between independent variables and outcomes, logistic regression analyses were performed. The logistic regressions were adjusted for covariates described in Table 2. After performing the regressions, a summary of the main findings was described in a tabular format (Table 5). Adjusted odds ratios, 95% confidence intervals, and p-values are described for each association tested.

Results

A total of 2,253 questionnaires were accessed on SurveyMonkey, and 2001 participants provided their consent and completed the questionnaire. Forty-nine consenting participants did not report their biological sex. We were able to determine the biological sex of two who did not

respond to the question by analyzing their responses (e.g., responded to questions related to female reproductive health), thus including them in the analysis. In addition, only 1 (0.05%) and 5 (0.26%) participants responded 'intersex' or 'preferred not to answer', respectively, to the biological sex question and were excluded from the analysis. A final exclusion was a civilian participant, leaving us with a total of n = 1,947 whose biological sex was known. Finally, from those, n = 855 were females and included in the present study. **Figure 2.1** illustrates the study participant flow.

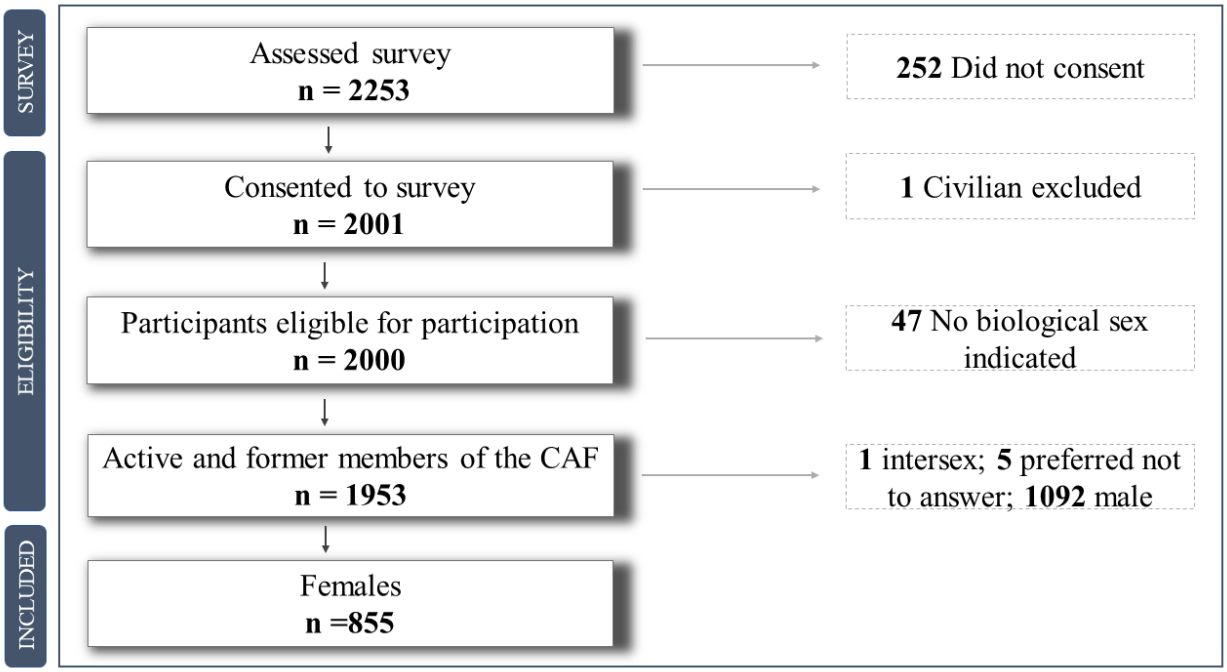

Figure 2.1. Study participant inclusion flowchart.

Demographic characteristics, reproductive health, and injury rates of the available sample are reported in **Table 2.3**.

Table 2.3. Participant demographics, reproductive health characteristics, and injury rates.

Variable	Females	
	N	(%)
Demographics		
Age (years), mean ± SD	845	38.8 ± 9.7
Year of enrollment, mean ± SD	815	2005 ± 9.9

Military branch		
Canadian Army	384	45.8
Royal Canadian Navy	97	11.6
Royal Canadian Air Force	357	42.6
CAF status		
Regular Force	648	75.9
Reserve Force Class A, B, C	117	13.7
Retired	40	4.7
Medically released	49	5.7
Rank		
NCM	482	57.2
Officers	360	42.8
Location		
Eastern provinces	156	18.2
Quebec	202	23.6
Ontario	359	42.0
Western provinces, territories, and outside Canada	138	16.1
Gender		
Man	5	0.6
Woman	846	99.4
Marital status		
Single and other[a]	182	21.4
Married and common law	573	67.3
Widowed, separated and divorced	97	11.4
Education		
Less than high school and high school	183	21.6
Trade certificate and College/CEGEP	217	25.6
University and bachelor's degree	299	35.3
Post-graduate and professional degree	148	17.5
Having children while serving[b]		
Yes	377	44.4
No	472	55.6
Parity		
0	474	56.9
1	112	13.4
2	189	22.7
3+	58	7.0
Current BMI classification		
Underweight and normal	334	41.8
Overweight	270	33.8
Obese	195	24.4
Total Physical Activity (min/week), mean ± SD	625	548.3 ± 376.6
Consumption of dairy products		
≥ 5x/week	246	41.8

3-4x/week	171	29.1
≤ 2x/week	171	29.1
Vitamin D		
Never	278	52.5
1x/month, 1x/week, few times/week and daily	252	47.5
Calcium		
Never	373	73.4
1x/month, 1x/week, few times/week and daily	135	26.6
Reproductive health		
Age of menarche		
< 10 years	49	7.6
11-15 years	554	86.2
≥ 16 years	40	6.2
Menstrual cycle periodicity		
Regular	381	58.1
Never regular, irregular for few months, periods stopped while serving and never had a period	275	41.9
Use of hormones for birth control		
Yes	500	76.1
No	157	23.9
Given birth while serving in the CAF		
Yes	270	41.1
No	387	58.9
Endometriosis		
Yes	57	8.7
No	600	91.3
Early menopause		
Yes	31	4.7
No	626	95.3
Secondary amenorrhea		
Yes	83	12.6
No	574	87.4
Injury rates		
RSI		
Yes	526	76.2
No	164	23.8
Acute injuries		
Yes	418	61.4
No	263	38.6

SD - Standard Deviation; CAF - Canadian Armed Forces; CEGEP - General and professional teaching college; BMI - Body Mass Index; RSI – Repetitive Strain Injuries.

[a]Refers to individuals living alone.

[b]Includes adoption and/or partner giving birth.

The bivariate associations between each reproductive health factor and the MSKi outcomes can be seen in **Table 2.4**.

Table 2.4. Bivariate associations between reproductive health factors and RSI/acute injuries in female CAF members.

Age of Menarche			
Category	**Total N**	**% RSI**	**Chi-square p-value**
≤ 10 years	49	71.4	
11-15 years	554	77.3	0.157
≥ 16 years	40	65.0	
Total N	**603**		
Menstrual Cycle Periodicity			
Category	**Total N**	**% RSI**	**Chi-square p-value**
Yes, regular	381	72.7	0.017
Never regular, irregular for a few months, never had a period and periods stopped while serving	275	80.7	
Total N	**656**		
Use of Hormones for Birth Control			
Category	**Total N**	**% RSI**	**Chi-square p-value**
No	157	70.1	0.048
Yes	500	77.8	
Total N	**657**		
Given Birth while Serving in the CAF			
Category	**Total N**	**% RSI**	**Chi-square p-value**
No	387	71.8	0.002
Yes	270	82.2	
Total N	**657**		
Endometriosis			
Category	**Total N**	**% RSI**	**Chi-square p-value**
No	600	75.7	0.394
Yes	57	80.7	
Total N	**657**		
Early Menopause			
Category	**Total N**	**% RSI**	**Chi-square p-value**
No	626	75.7	0.299
Yes	31	83.9	
Total N	**657**		
Secondary Amenorrhea			
Category	**Total N**	**% RSI**	**Chi-square p-value**
No	574	74.9	0.060

Yes	83	84.3	
Total N	**657**		
Age of Menarche			
Category	**Total N**	**% Acute injuries**	**Chi-square p-value**
11-15 years	554	61.9	
≤ 10 years	49	71.4	0.184
≥ 16 years	40	52.5	
Total N	**603**		
Menstrual Cycle Periodicity			
Category	**Total N**	**% Acute injuries**	**Chi-square p-value**
Yes, regular	381	58.5	0.072
Never regular, irregular for a few months, never had a period and periods stopped while serving	275	65.5	
Total N	**656**		
Use of Hormones for Birth Control			
Category	**Total N**	**% Acute injuries**	**Chi-square p-value**
No	157	61.8	0.931
Yes	500	61.4	
Total N	**657**		
Given Birth while Serving in the CAF			
Category	**Total N**	**% Acute injuries**	**Chi-square p-value**
No	387	60.5	0.517
Yes	270	63.0	
Total N	**657**		
Endometriosis			
Category	**Total N**	**% Acute injuries**	**Chi-square p-value**
No	600	59.8	0.005
Yes	57	78.9	
Total N	**657**		
Early Menopause			
Category	**Total N**	**% Acute injuries**	**Chi-square p-value**
No	626	60.5	0.025
Yes	31	80.6	
Total N	**657**		
Secondary Amenorrhea			
Category	**Total N**	**% Acute injuries**	**Chi-square p-value**
No	574	61.1	0.636
Yes	83	63.9	
Total N	**657**		

RSI - Repetitive Strain Injuries; CAF - Canadian Armed Forces.

The three reproductive health factors associated with RSI and the two associated with acute injuries were moved to the logistic regression analysis adjusting for potential covariates. Regarding RSI, the only reproductive factor that remained as a predictor after the adjustments was menstrual cycle periodicity. The association was adjusted for the year of enrollment, rank, parity, current BMI classification, total physical activity, consumption of dairy products, use of calcium as a nutritional supplement, and use of Vitamin D as a nutritional supplement. Female members with menstrual cycles reported as never regular, irregular for a few months, who never had a period, and whose periods stopped while serving presented a greater likelihood of RSI compared to the ones who had regular menstrual cycles (aOR: 1.898, CI: 1.138; 3.166). The associations between use of hormones for birth control and giving birth while serving in the CAF with RSI were lost after adjustments (**Table 2.5**).

The analysis related to acute injuries demonstrated that those who have endometriosis presented a higher likelihood of reporting acute injuries compared to the ones who did not have endometriosis (aOR: 2.426, CI: 1.030; 5.709). This association was adjusted for the same covariates as outlined above. The association between early menopause with acute injuries was lost after adjustments (**Table 2.5**).

Table 2.5. Binary logistic regression for reproductive health variables vs. RSI and acute injuries after adjusting for covariates in female CAF members.

Outcome: RSI	aOR	95% CI	p-value
(1) Menstrual cycle periodicity (Ref: Yes, regular)			
Never regular, irregular for a few months, never had a period and periods stopped while serving	1.898	1.138; 3.166	0.014[a]
(2) Use of hormones for birth control (Ref: No)			
Yes	1.490	0.843; 2.632	0.170
(3) Given birth while serving in the CAF (Ref: No)			
Yes	0.930	0.180; 4.807	0.931
Outcome: Acute injuries	**aOR**	**95% CI**	**p-value**
(1) Endometriosis (Ref: No)			
Yes	2.426	1.030; 5.709	0.042[a]
(2) Early menopause (Ref: No)			
Yes	1.219	0.302; 4.928	0.781

aOR-adjusted odds ratio; CI-confidence interval; RSI-repetitive strain injury; Ref-reference group; CAF-Canadian Armed Forces
RSI - N = 406 (1); N = 406 (2); N = 406 (3). All analyses were adjusted by: Year of enrollment, Rank, Parity **(except for model 3)**, Current BMI classification, Total physical activity, Consumption of dairy products, Use of Calcium as nutritional supplement, Use of Vitamin D as nutritional supplement.
Acute injuries - N = 406 (1); N = 406 (2). All analyses were adjusted by: Year of enrollment, Rank, Parity, Current BMI classification, Total physical activity, Consumption of dairy products, Use of Calcium as nutritional supplement, Use of Vitamin D as nutritional supplement.
[a]p-value is statistically significant

Discussion

This study aimed to explore how the female reproductive characteristics and related conditions/factors may contribute to MSKi in CAF members. Our main findings were that i) female CAF members with irregular menstrual cycles or absent periods had a higher likelihood of experiencing RSI compared to those who reported regular menstrual cycles and; ii) having endometriosis was associated with greater rates of acute injuries. To achieve an overall goal of 25% female representation in the CAF, understanding why they are at a greater risk to MSKi is important and necessary. Exploring female-specific reproductive health factors associated with MSKi can aid in guide future research and inform changes in practices and policies to improve the experience of female CAF members.

Based on our previously published scoping review [10] illustrating that having no or irregular menses, and late age menarche, were predictors of MSKi in female military members, we explored these reproductive health factors as predictors of RSI and acute injury in the CAF. The lack of association between late age of menarche, secondary oligomenorrhea/amenorrhea and MSKi, support the findings from the systematic review by Sammito *et al.* (2021), [13] who also found no associations between late age of menarche, secondary oligomenorrhea/amenorrhea and MSKi in female military members. We did, however, find a positive relationship between irregular menstruation and MSKi suggesting that irregular menstruation may increase the likelihood of experiencing RSI compared to having a regular menstrual cycle. The association with menstrual cycle periodicity and RSI could be due to the link between relaxin and menstruation. [27] A potential explanation is the shorter lifetime exposure to estrogen, the primary female reproductive hormone, when a menstrual cycle is not regular, [12, 28] which can increase the risk of MSKi, and lower relaxin levels. The physiological changes resulting from short exposure to estrogen can lead to stiffer tendons, [20] causing muscle strain. [29] Estrogen exposure is not, however, the only concern. Low energy availability is also associated with irregular menstruation in female athlete and non-athlete populations. [30] This relationship exists as part of the female athlete triad (i.e., three inter-related conditions: low energy availability, menstrual dysfunction, and low bone mineral density) and can be extended to female military members. [31] It is possible that lower physical performance and higher incidence of MSKi is present in female members due to the female athletic triad. [32] Since energy deficiency is often an underlying cause of menstrual irregularity in physically active women, the association between experiencing RSI and reporting irregular menstruation shown in this study may be related to low energy availability. This relationship needs to be further explored in the female military population.

This study found an association between endometriosis and MSKi in female CAF members. Reporting endometriosis, a disease characterized by the presence of functional endometrial glands and stroma outside the uterine cavity, [33] was associated with greater rates of acute injuries. With endometriosis, normal ovulation is impaired, and follicle-stimulating hormone (FSH) levels are significantly higher in individuals with later stages of endometriosis. [22, 23] Furthermore, FSH has been found to be negatively correlated with bone mineral density (BMD) in females who have experienced menopause. [24] The lower BMD could predispose one to acute injuries. An alternative explanation is that the use of gonadotropin-releasing hormone (GnRH) agonists, a well-known treatment for endometriosis, could have impacted the bone health of female CAF members living with endometriosis. [34] Further exploration of reproductive health markers and bone loss among female military members who experience endometriosis is required.

Together, the findings from the scientific literature and our work reinforce the need to investigate reproductive characteristics in female military personnel. These future investigations can be accomplished by using prospective longitudinal research designs that address the cause and effect of these reproductive health characteristics, and changes over time, to establish appropriate strategies to mitigate the risk of MSKi. Moving in this direction is necessary for the CAF, as most female members are in their reproductive years [35] and can be exposed to different factors (e.g. irregular menstruation, endometriosis) impacting MSKi rates, recruitment, and retention within the military.

This study has important strengths, including incorporating several female reproductive health aspects into the analysis to test their associations with MSKi. Additionally, we had a large sample size providing adequate power to address our primary outcomes. Although the sample

included in the regression for RSI is lower than n = 508 (i.e., the powered sample size), we could test the bivariate association with a sample n > 508 for all independent variables of interest.

In terms of limitations, there were some factors known to influence MSKi which we were unable to take into consideration (e.g., load carriage, PA intensity). Our recruitment did not account for strategies that would allow us to select participants randomly, the survey was subject to participant self-selection. The research team utilized convenience, purposive, and 'snowball' sampling, as well as assistance from project stakeholders (e.g., Canadian Forces Morale and Welfare Services, Personnel Support Programs, Defence Fitness, Defence Women's Advisory Organization social media page, DND CAF Connection website page and Banner, Facebook posts, and Maple Leaf and Defence News) to recruit survey participants. After meeting with our stakeholders, these strategies were deemed the best option for reaching as many members as possible, particularly the female members. Furthermore, we accounted for the possible impact of study design/recruitment within our sample size calculations. These data were self-reported and subject to potential recall bias of the participants. Social desirability could have influenced responses; however, given the anonymous nature of this survey, this effect should be minimal. Lastly, given our study design (i.e., retrospective case-control study), we are unable to establish causation with these data.

Conclusion

Female CAF members with irregular or absent menstrual cycles had a higher likelihood of experiencing RSI than those who reported regular menstrual cycles. Reporting endometriosis was associated with greater rates of acute injuries. Our findings highlight female reproductive characteristics as potential contributors to MSKi in the CAF. Therefore, female members who are affected by these predictors may need to be monitored for MSKi by clinical practitioners and provided with effective preventative strategies. Further research should investigate methods to

mitigate MSKi risks associated with female reproductive health characteristics and create policies to address these barriers in the CAF to improve recruitment and retention of female members.

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Chapter 3 : Validity of an ultrasound device to measure bone mineral density in a premenopausal female population

Preamble

Building on the reproductive characteristics associated with repetitive and acute injury outlined in Chapter 2, the next chapter explores whether these reproductive characteristics were also related to bone mineral density. Given the nature of Chapter 4 (i.e., data collection on military bases) we needed a method to assess bone mineral density that was low-cost and portable. While the Ultrascan650™ was available to us, there had yet to be a validation study conducted in a premenopausal female cohort.

Appendix 4, not included in the published manuscript, highlights the positioning used for the 4 sites measured by the DXA. Appendix 5, not included in the published manuscript, highlight the positioning used for the UltraScan650™ measurements.

Study title: Validity of an ultrasound device to measure bone mineral density

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Authorship Confirmation Statement

All authors confirm their authorship. J.L.P. and V.M.R.W. contributed to the writing and analysis, with direction from all authors. J.L.P., C.M.E., K.S., and K.B.A conceived the study and oversaw its direction and planning. J.L.P, C.M.E, V.M.R.W., and M.A, contributed to data collection and recruitment. All authors discussed the results, reviewed the manuscript, and approved the final version.

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Abstract

Objectives: This study aims to examine the validity and reliability of the UltraScan650™, a portable ultrasound device, used to measure BMD at the 1/3rd radius position.

Methods: Fifty-two female first responders and healthcare providers were assessed using DXA (forearm, femur, lumbar and total body) and the UltraScan650™. Fat and lean mass were also assessed using the DXA. Pearson correlations, Bland-Altman plots, t-tests, and linear regressions were used to assess validity. Intra-class correlation (ICC) coefficients were used to assess reliability.

Results: Inter-rater reliability and repeatability were good (ICC= 0.896 (0.818; 0.942), p<0.001) and excellent (ICC = 0.917 (0.785; 0.989), p < 0.001), respectively. BMD as measured by the UltraScan650™ was weakly correlated to the DXA [r = 0.382 (0.121; 0.593), p = 0.0052]. Bland-Altman plots revealed that the UltraScan650™ underestimated BMD (-0.0569 g/cm^2), this was confirmed with a significant paired t-test (p < 0.001). A linear regression was performed [0.4744*UltraScan650™+0.4170] to provide more information as to the issue of agreement. Bland-Altman plots revealed a negligible bias, supported by a paired t-test (p = 0.9978). Pearson's correlation revealed a significant relationship [r = -0.771 (-0.862; -0.631), p<0.0001] between adjusted UltraScan650™ – DXA and the average of the two scans (i.e., adjusted UltraScan650™ and DXA), suggesting a proportional constant error and proportional constant variability in measurements of BMD from the UltraScan650™.

Conclusion: The UltraScan650™ is not a valid alternative to DXA for diagnostic purposes; however, the UltraScan650™ could be used as a screening tool in the clinical and research setting given the linear transformation is employed.

Keywords: validation, reliability, bone mineral density, ultrasound, diagnostic

Introduction

Osteoporosis affects a large portion of the global population, particularly the female population. In fact, the prevalence of this condition, characterized by decreased bone mineral density (BMD) and bone mass compared to healthy individuals of the same sex and ethnicity, is five-times higher in females compared to males. [1] It is estimated that, at any given time, 5.6-10.5% of Canadians over the age of 50 are living with diagnosed osteoporosis. [2] In the 2007/08 fiscal year, osteoporosis was associated with $1.2 billion in acute care costs and $2.3 billion in outpatient-related costs in Canada. [3]

In the pre-menopausal population, the female athlete triad, more recently referred to as relative energy deficiency in sport (RED-S), is a significant risk to BMD. In 2014, RED-S was defined by the international Olympic committee, acknowledging that the 3 components [4] (i.e., : low energy availability, menstrual disturbances, and changes in BMD) of the female athlete triad did not sufficiently characterize the syndrome, which negatively impacts many aspects of physiology. [5] Bone health is a physiological factor that can be negatively and irreversibly impacted by RED-S among females in their reproductive age. [6] Given females reach their peak bone mass in their early to late 20s, [7] and peak bone mass is a determinant of osteoporotic fracture risk [8] it is critical that low BMD is detected early in this population.

It is well-known that the key to osteoporotic fracture prevention is early detection; [9] despite this awareness, detection and diagnosis is costly and challenging. [10] For instance, the use of dual-X-ray absorptiometry (DXA) scanners represents the gold standard method to measure bone mineral density; [11] however this infrastructure item costs up to $125,000 USD. Additionally,

scanners are approximately the size of a twin bed, rendering them relatively immobile. In Canada, DXA scans are covered by government health care insurance only if a patient is deemed to be at an increased risk of low BMD or osteoporosis by a physician, [12] thereby limiting the early identification of reduced BMD. Moreover, DXA scans require trained personnel to perform the assessment and while minimal, still expose the participants to approximately 0.004 mSv of radiation per scan. [13] Efforts are being made to find more economical and less resource demanding alternatives to measure BMD. For example, ultrasound has been introduced in the clinical and research spaces as an option for the assessment of BMD. [14] One of these ultrasound tools is a desktop device (UltraScan650™, CyberLogic, Inc., New York, NY, USA) developed to estimate the amount of bone present at the 1/3 distal radius position. [15] Of note, the 1/3rd distal radius position has previously been determined to be a useful anatomical site for BMD assessment. [16] Devices like the UltraScan650 are a more affordable (~$12,000 USD), user-friendly (no need for specialized training or qualifications), and radiation-free option which could assist in screening a larger population. In their 2013 study, Stein and colleagues conducted a validation investigation that involved comparing BMD measurements obtained with the UltraScan650™ to the DXA among a mixed-sex populations. The study yielded significant positive correlations ($r = 0.93$, $p < 0.001$) and indicated a 2.1% coefficient of variation. Nevertheless, certain aspects of this prior validation study require enhancement; this includes the application of more rigorous and robust statistical analyses, larger sample size, and ensuring sample homogeneity, specifically for healthy females. Moreover, it would be valuable to explore whether BMD measurements from the UltraScan650™ correlate with those obtained from the DXA in different anatomical regions, such as the lumbar and the femur.

Given the healthcare burden associated with osteoporosis, early detection of the disease is critical for prevention. Thus, the use of a more cost-effective and less cumbersome measurement tool is needed. The purpose of this study is to validate the UltraScan650™ against a gold-standard measurement of BMD (i.e., DXA Prodigy) and test the reliability of the device in a healthy female sample, in their reproductive years. Given the UltraScan650™ employs ultrasound technology, future studies could investigate the use of the UltraScan650™ among groups with contraindications to radiation exposure (e.g., pregnant individuals).

Methods and Materials

Validity was determined by comparing BMD at the 1/3rd distal radius position as measured by the UltraScan650™ to the same anatomical position as measured by the DXA. An *a priori* sample size calculation was conducted for the validation of the UltraScan650™, assuming a large effect size based on Stein *et al.* (2013), 80% power, and an error of 5%, a sample size of 46 was determined.

Stability of the UltraScan650™ was assessed by measuring n = 5 participants, 15 times, in the same session. Of these 5 participants, one was not included in the validation, as they did not complete a DXA scan.

Participants

All participants were recruited through social media (e.g., Twitter, Facebook) and word-of-mouth. Participants were female individuals employed in the following occupations: law enforcement, paramedicine, firefighting, or healthcare provider (as defined by the Province of Ontario) and between the ages of 18 and 55 years old. The study was reviewed and approved by the University of Ottawa Ethics Board (H-02-23-8994).

A secondary sample was used to cross-validate the linear regression developed to transform the raw UltraScan650™ values. Participants were all female individuals between the age of 18 to 30. The study was reviewed and approved by the University of Ottawa Ethics Board (H-01-23-8825).

UltraScan650™ measurement

A daily quality assurance test was performed with a standard test block. For each participant measurement, the length of the right forearm was determined using a retractable vinyl tape measure (from the ulnar styloid process to the elbow). The ulna position locator (UPL) was placed (using its pair of pins) into a pair of holes in the base of the unit that are located along the scale at the same value as the ulna length. A hand positioning locator (HPL) was placed on the UPL, so that the patient could maintain a consistent position and orientation of the hand/forearm throughout the scans. To sanitize the UltraScan650™ and ensure necessary contact, the device sensors and the forearm of the participant were sprayed with 70% isopropyl alcohol. The participant, while in a seated position, rested their elbow on a pad located on the right-side of the device, and their forearm was secured for the scan. To indicate interrater reliability, the scan was repeated 5 times, three-times by the first assessor and two-times the second assessor (n = 44). To examine reproducibility, the scan was repeated 15 times for 5 randomly selected participants (n = 5).

DXA measurement

The DXA scanner model GE Lunar Prodigy (GE Healthcare Lunar, Madison, MI, USA) was used by a trained operator to assess BMD. A quality assurance assessment was performed each day prior to DXA measurements with the quality assurance, phantom lumbar block. Participants were asked to wear fitted clothing and remove all metals from their person.

Participants were instructed to not consume alcohol or engage in strenuous physical activity for 24 hrs prior to the scan. Moreover, participants were fasted for 8 hours before testing, during which time they had nothing to eat and did not consume any liquids for 2 hours before testing. Four scans were performed: i) Full body; participants lay supine on the device with straps to secure the knees and ankles, ii) Right forearm; participants sat in an upright position beside the DXA with their arm on the device and secured to a board by two straps, iii) Lumbar spine; participants lay supine with their legs placed on a positioner box, and iv) Femur; participants lay supine with their feet secured to a positioner by straps. Participants were positioned by a member of the research team.

Statistical Analysis

Statistical analysis was performed using R (R Core Team), unless otherwise stated. Continuous variables were all determined to be normally distributed by way of Shapiro-wilks test.

Validation

Pearson's correlation coefficients with 95% confidence intervals (CI) and simple linear regressions were used to determine the association between the UltraScan650™ and the DXA (i.e., correlations between variables) [17]. Values of r equal to or greater than 0.7 were considered to have a strong relationship and values less than 0.3 were considered to have no correlation, significance was set at $p < 0.01$. Bland-Altman plots were used to determine whether BMD measured by the UltraScan650™ is a valid method compared to the gold standard (i.e., DXA), [18] at the 1/3rd radius position. Bland-Altman plots were created using R (R Core Team) to compare the reference criterion (i.e., DXA) to the UltraScan650™. Differences between the DXA and the UltraScan650™ were plotted against the average of the two devices. Paired *t*-tests were used to support the Bland-Altman plots.

Cross-validation

Unpaired two samples *t*-tests were used determine whether difference exist, with each the UltraScan650™ and DXA at the 1/3rd radius, between the validation and the cross-validation samples. As with the validation, Pearson's correlations coefficients with CI were used to determine the association between the UltraScan650™ and the DXA. Paired *t*-tests were used to determine whether a significant difference exists between the raw and transformed UltraScan650™ BMD and BMD as measured by the DXA.

Reliability

Reliability measurements were conducted in SPSS version 26 (SPSS Inc., Chicago, IL, USA). Intra-class correlation coefficients (ICC) were used to assess reliability of two scans, from two randomly selected raters. Stability was assessed using ICC estimates and their 95% confidence intervals by way of mean-rating (n = 15), absolute agreement, 2-way mixed effects model. Additionally, percent coefficient of variation was used to assess stability. Inter-rater reliability was assessed using ICC estimates and their 95% confidence intervals by way of single-rating, absolute agreement, 2-way random effects model. [19] Significance was set at $p < 0.05$. ICC values less than 0.5, between 0.5 and 0.7, between 0.7 and 0.9, and above 0.9 were considered to have poor, moderate, good, and excellent inter-rater reliability, respectively.

Multiple regression analyses were employed to explore the contribution of lean and fat mass, as measured by the DXA, to BMD as measured by the UltraScan650™. The increase in adjusted R^2 represented the additional variance explained by muscle or fat mass independently of BMD. F-tests were used to compare linear regressions, to determine whether the inclusion of fat and lean mass significantly contributed to BMD as measured by the UltraScan650™. Unless otherwise stated, significance was set at $p < 0.05$.

Results

Figure 3.1 illustrates the inclusion of participants in the assessment of stability, inter-rater reliability, and validation of the UltraScan650™.

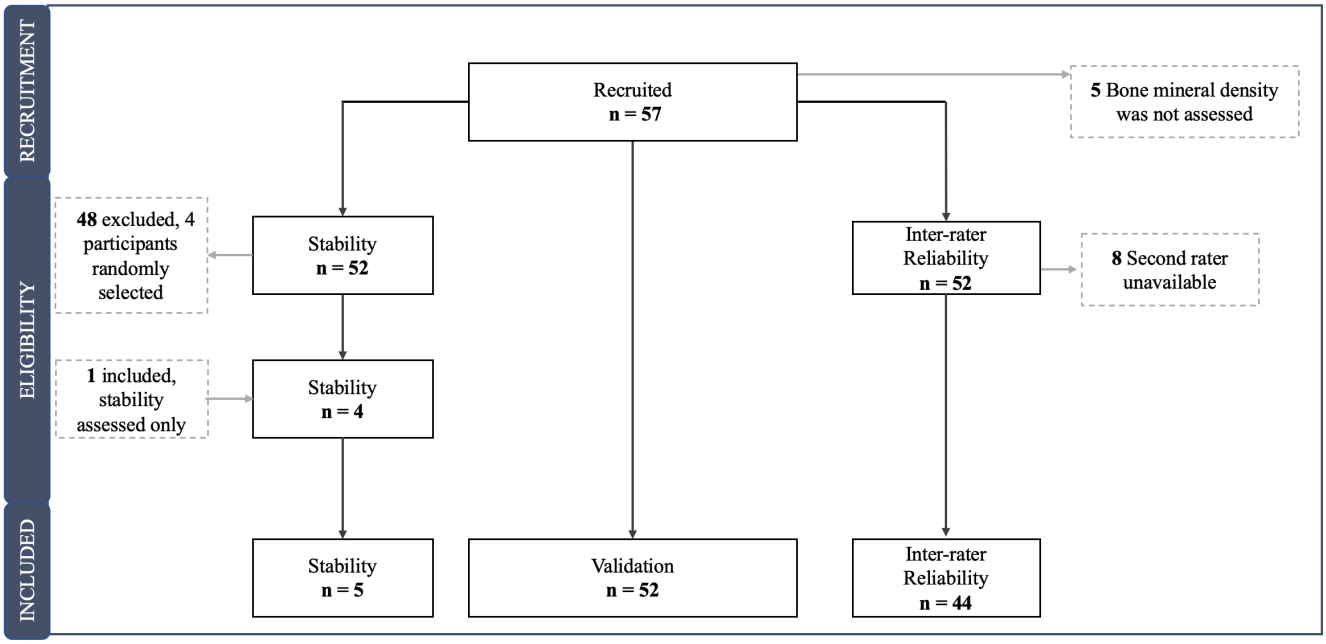

Figure 3.1. Flow diagram of the inclusion of participants in the stability, inter-rater reliability, and validation assessments.

Table 3.1 describes the demographics and BMD per anatomical region of the participants.

Table 3.1. Demographics and BMD of participants.

Variables (n=52)	Mean (SD)	Range (Min – Max)
Age (years)	36.19 (8.21)	21 – 55
Height (cm)	166.4 (6.57)	148.7 – 179.6
Weight (kg)	69.26 (11.11)	46.0 – 99.3
BMD (g/cm^2)		
UltraScan650™	0.652 (0.043)	0.572 – 0.788
$1/3^{rd}$ radius[a]	0.709 (0.051)	0.582 – 0.809
Femur neck[a]	1.032 (0.110)	0.725 – 1.26

L2-L4 lumbar spine[a]	1.262 (0.142)	0.837 – 1.528
Total Body[a]	1.233 (0.102)	0.912 – 1.493

[a]Site was measured using dual energy X-ray absorptiometry (DXA)
BMD – bone mineral density, SD – standard deviation

Reliability

The ICC was used to assess the absolute agreement between the average of 2 measurements from 2 raters in measuring BMD using the UltraScan650™ in 44 individuals. There was a good absolute agreement between the 2 raters, using the two-way random effect models and the "single rater" unit, ICC = 0.896 (0.818; 0.942), p<0.001. These results suggest that the inter-rater reliability of the UltraScan650™ is categorized as 'good' for the measurement of BMD at the 1/3rd distal radius position.

The ICC was employed to examine the absolute agreement between 15 measurements of BMD using the UltraScan650™ in 5 individuals. There was an 'excellent' absolute agreement between the 2 raters, using the two-way fixed effect models and the "single measurement" unit, ICC = 0.917 (0.785; 0.989), p < 0.001. Further, the percent coefficient of variation was 3.3%. These results suggest that the UltraScan650™ provides consistent measures at the 1/3rd distal radius position.

Validity

The 1/3rd radius position as measured by the UltraScan650™ had weak but significant correlations to the DXA [r = 0.382 (0.121; 0.593), p = 0.0052]. Bland-Altman plots revealed a bias of -0.0569 (-0.0715; 0.0422), supported by a paired t-test (p < 0.001), indicating significant mean differences when comparing the UltraScan650™ with the DXA for each individual (see **Figure 3.2**). Further, a Pearson correlation revealed no relationship [r = -0.170 (-0.423; 0.108), p = 0.228] between UltraScan650™ minus DXA and the average of the two scans (i.e., UltraScan650™ and

DXA). These results suggest the UltraScan650™ may not be a valid device for use in healthy female populations. Further, there is likely an issue with precision given that several data points fall outside the limits of agreement.

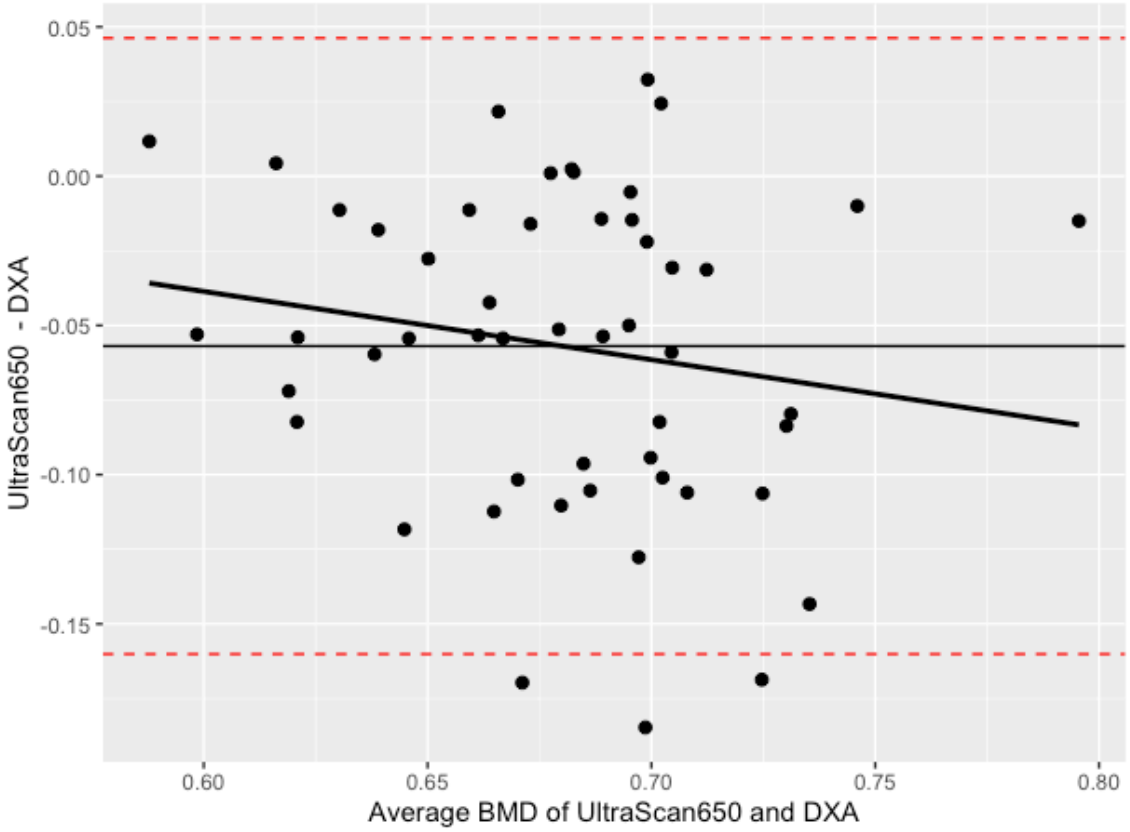

Figure 3.2. Bland-Altman plot with limits of agreement (LOA) relating bone mineral density (BMD) as measured by the UltraScan650™ and dual-energy X-ray absorptiometry (DXA). On the y-axis appears the difference between the UltraScan650™ and the DXA. On the x-axis appears the mean BMD for each participant as measured using the UltraScan650™ and the DXA. A trendline (r = -0.170) is present. The solid line represents how much the UltraScan650™ underestimates the DXA. The dotted lines represent the limits of agreement.

To provide more information as to the issue of agreement between the two measurements, a linear regression (**Table 3.2**) was performed with the predictor variable being BMD as measured by the UltraScan650™ and the outcome being BMD of the 1/3rd radius as measured by the DXA.

Table 3.2. Linear regression.

F statistic: 8.529, adjusted R^2: 0.1286	Coefficient (CI)	p-value

Intercept	0.4170 (0.216; 0.618)	<0.001
UltraScan650™	0.4474 (0.139; 0.755)	0.005

CI - 95% confidence interval

After transforming each UltraScan650™ based on the linear regression presented in Table 2, Bland-Altman plots (see **Figure 3.3**) revealed a negligible bias, supported by a paired t-test (p = 0.9978), indicating no mean differences when comparing the adjusted UltraScan650™ measurement with the DXA for each participant. Further, a Pearson's correlation revealed a significant relationship [r = -0.771 (-0.862; -0.631), p <0.0001] between adjusted UltraScan650™ – DXA and the average of the two scans (i.e., adjusted UltraScan650™ and DXA). Due to the significant change in slope, these results suggest a proportional constant error and proportional constant variability. [20]

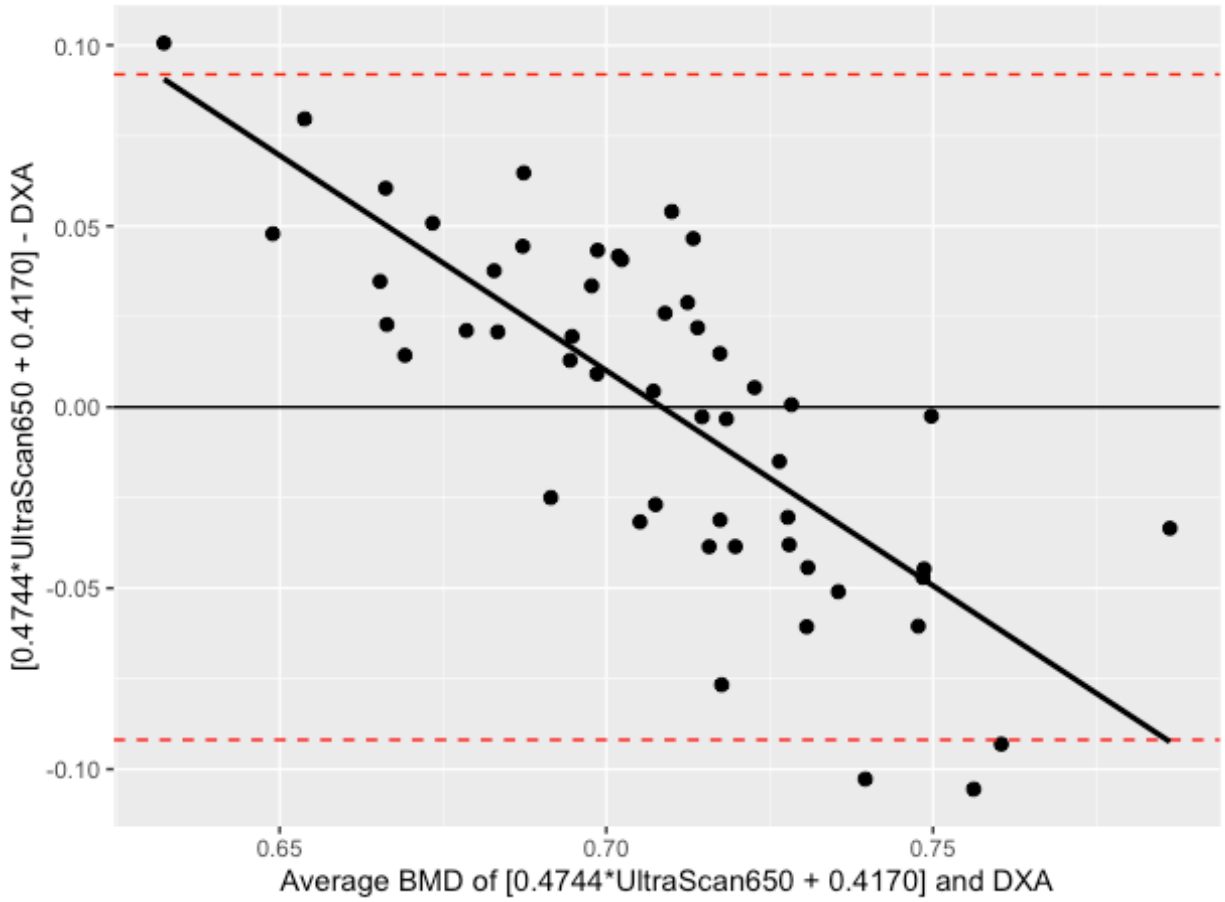

Figure 3.3. Bland-Altman plot with limits of agreement (LOA) relating the difference between bone mineral density (BMD) as measured by the UltraScan650™ and transformed [0.4744*UltraScan650™+0.4170] and dual-energy X-ray absorptiometry (DXA). On the y-axis appears the difference between the transformed UltraScan650™ and the DXA. On the x-axis appears the mean BMD for each participant as measured using the transformed UltraScan650™ and the DXA. A trendline (r = -0.771) is present. The dotted lines represent the limits of agreement.

The demographic information for the cross-validation sample can be found in supplemental file 1. An unpaired two sample *t*-test was employed to determine whether there were significant differences between the validation and cross-validation samples. There were no significant differences in BMD at the 1/3rd radius position as measured by the UltraScan650™ (p=0.4974) and the DXA (p=0.2237). A Pearson's correlation [r=0.336 (0.003; 0.602), p=0.049] revealed a significant but weak correlation between raw UltraScan650™ and DXA 1/3rd radius position measurements. Employing the linear transformation in Table 2, a paired *t*-test was performed. No

significant differences were found between the transformed UltraScan650™ and the DXA measurements of BMD at the 1/3rd radius position in the cross-validation sample (p=0.1855).

Supplemental 1. Demographics and BMD of participants included in the cross-validation.

Variables (n=35)	Mean (SD)	Range (Min – Max)
Age (years)	22.41 (2.72)	18 – 28
Height (cm)	166.2 (8.74)	148.5 – 185.0
Weight (kg)	63.61 (8.88)	50.1 – 85.7
BMD (g/cm^2)		
UltraScan650™	0.645 (0.049)	0.555 – 0.736
1/3rd radius[a]	0.695 (0.047)	0.606 – 0.782
Femur neck[a]	1.093 (0.151)	0.831 – 1.462
L2-L4 lumbar spine[a]	1.231 (0.166)	1.060 – 1.668
Total Body[a]	1.216 (0.111)	1.049 – 1.487

[a]Site was measured using dual energy X-ray absorptiometry (DXA)
BMD – bone mineral density, SD – standard deviation

Pearson's correlations revealed that BMD as measured by the UltraScan650™ did not significantly correlate to the femur neck [r=0.216 (-0.060; 0.462) , p=0.124] or L2-L4 lumbar spine [r=0.153 (-0.125; 0.409) , p=0.278]; however, a weak correlation [r=0.276 (0.004; 0.511), p=0.047] was present between the BMD as measured by the UltraScan650™ and the total body as measured by the DXA. Multiple regression analysis was performed to assess the additional variance (ΔR^2) explained by right-arm lean mass and right-arm fat mass. Right-arm fat mass explained an additional 6.96% (p=0.025) of the variance of BMD at the 1/3rd radius position as measured by the UltraScan650™. Right arm lean mass did not significantly contribute to the variance.

Table 3.3 presents the multiple regression analysis of other anatomical locations (i.e., Total body, femur neck, and L2-L4 lumbar spine) with the UltraScan650™.

Table 3.3. Multiple regression analysis.

	Predictors	Regression	aR^2	p-value
Femur	US	0.5513*USscan + 0.6729	0.0277	0.1237
L2-L4	US	0.5041*UScan + 0.9336	0.0039	0.2779
Total body	US	0.6520*UScan + 0.8086	0.0580	0.0473
$1/3^{rd}$ radius	Predictors	Regression	aR^2	p-value
(1)	US	0.4744*UScan + 0.4170	0.1286	0.0052
(2)[a]	US + FM	$0.5193*UScan + 3.945^{-5}*(FM) + 0.3229$	0.2267	0.0007
(3)	US + LM	$0.4484*UScan + 2.291^{-5}*(LM) + 0.3608$	0.1681	0.0041
(4)[a,b]	US + FM +LM	$0.5241*UScan + 4.147^{-5}*(FM) + 2.518^{-5}*(LM) + 0.2563$	0.2808	0.0003

UScan – BMD as measured by UltraScan650™, FM – Fat mass as measured by DXA, LM – lean mass as measured by DXA, aR^2 – adjusted R^2
[a]Significantly different compared to (1)
[b]Significantly different compared to (2)

Figure 3.4 presented the outputs from the regressions in Table 3 plotted against the DXA measurements of BMD at the $1/3^{rd}$ radius position.

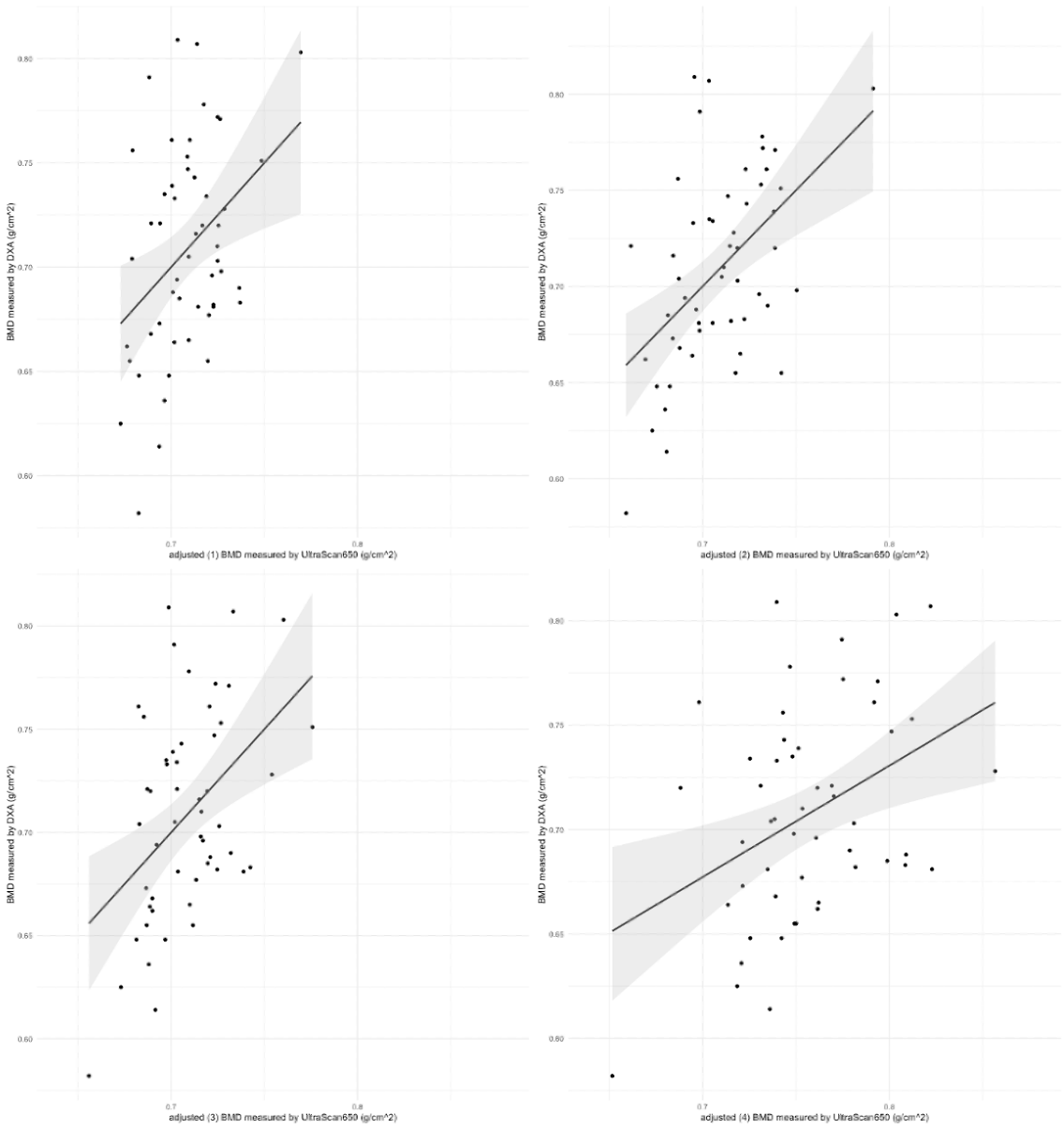

Figure 3.4. Scatter plots with trendlines and 95% confidence intervals. On the y-axis appears bone mineral density as measured by dual-energy X-ray absorptiometry at the 1/3rd radius position. On the x-axis appears BMD as calculated applying the transformations in Table 3. A) r = 0.382 (0.121; 0.593), p=0.005. B) r = 0.507 (0.272; 0.685), p<0.001. C) r = 0.448 (0.199; 0.642), p<0.001. D) r = 0.404 (0.148; 0.610), p=0.003.

Discussion

The female population is at greater risk for low BMD compared to their male peers[1]. While the DXA is the gold standard measurement for assessing BMD, the device is costly, immobile,

and uses X-ray technology, thus precluding certain populations from its use. The aim of this study was to validate the UltraScan650™ for clinical and research use as an alternative option to DXA in the female population, as it is cheaper, portable and uses ultrasound technology. The previous validation yielded a strong positive correlations [r = 0.93, p <0.001] and indicated a 2.1% coefficient of variation. [15] Our data found a weak positive correlation [r = 0.382 (0.121; 0.593), p = 0.0052] and a coefficient of variation of 3.3%. Further, our data suggests that while BMD obtained from the UltraScan650™ are linearly associated with BMD obtained from the DXA at the 1/3rd radius position, there exists a weak coefficient of determination (adjusted R^2 = 0.1286) and an underestimation bias. After employing the simple linear regression to transform each UltraScan650™ BMD measurement (**Table 3.2**), the underestimation bias was eliminated. Further, a cross-validation highlighted no significant differences with the DXA when using the linear regression (**Table 3.2**) to transform BMD as measured by the UltraScan650™ at the 1/3rd radius position; suggesting the model may be sufficiently robust to perform consistently across different samples of pre-menopausal female individuals.

Based on further analysis, we suspect this underestimation without the use of the transformation is a result of several factors, including right-arm fat mass and lean tissue mass. A recent study found that leg muscle and fat mass significantly influenced ultrasonic measurements of bone health outcomes of the leg. [21] While our findings suggest right-arm lean mass did not independently influence BMD as measured by the UltraScan650™, right-arm fat mass in isolation and in combination with lean mass did. Past studies have found that fat mass significantly influences broadband ultrasound attenuation. [22] The additional variance may be related to structural, mechanical and compositional properties of the bone. [21] Steps should be taken to mitigate impact of fat mass on results from the UltraScan650™, including having a measure of fat

mass and adjusting results from the UltraScan650™ based on linear regressions developed for the population of interest. Previous research has stratified validations, of a variety of devices, to measure body fat % across different ages and sexes and found differences between groups. [23] Thus, it is possible that sex and age influence the BMD assessments from the UltraScan650™ and separate validation based on age and sex may be required.

This study also sought to examine whether the assessment of BMD at the 1/3rd distal radius position measured by the UltraScan650™ was predictive of BMD at other anatomical locations (i.e., lumber spine, femur, total body). We determined that BMD at the 1/3rd distal radius position was only predictive of total body BMD as measured by the DXA. It is possible that the 1/3rd distal radius as measured by the UltraScan650™ is not predictive of BMD at the femur and the lumbar spine because they are weight bearing bones. As such, bone at the femur and the lumbar spine may experience different physiological adaptations, compared to the distal radius, a non-weight-bearing bone. Given this information, caution should be exercised when using the UltraScan650™, recognizing its utility for estimating total body BMD while respecting its limited capacity (inability) to appropriately screen for low BMD at the femur or lumbar spine.

While examined in a clinical population (i.e., individuals experiencing kidney failure), hydration has been shown to decrease echo intensity of ultrasound. [24] Thus, it is possible that hydration status played a role with the ultrasound results. Given all participants in the present study were fasted, this could account for an aspect of the underestimation as being fasted was not stated as a requirement for the cohort examined in Stein *et al.* (2013). Moreover, our study population is composed of a much younger cohort (~11 years younger) compared to the previous validation study. [15] Thus, many of the differences exhibited could be due to population demographics.

Strengths and Limitations

The strengths of this study include a homogenous sample; however, having a homogenous sample dictate that this study is not generalizable to the larger population. While the sample size has been met according to the *a priori* sample size calculation, the number of participants remains relatively small compared to other studies of a similar nature. All participants included in the validation had their right forearm measured (dominant n=47, 90.1%). There are reports suggesting that the dominant hand overestimates BMD, recommending that the non-dominant hand be assessed[25]; while we did not consider handedness in our study, we were consistent in assessing the same arm on both devices, regardless of dominance. Therefore, this should mitigate the effect of overestimation. While a linear transformation was performed to decrease the underestimation bias, this adjustment should not be applied to other populations (e.g., male individuals). Thus, we recommend this transformation only be used when considering healthy female individuals. Given we recruited participants via social media and word-of mouth (i.e., snowball recruitment), there is the potential for selection bias. Additionally, this validation was a sub-study within a larger study that recruited first responders and healthcare providers for physical assessments. This could have compounded the effect of selection bias.

Conclusion

Based on the results from the present study, the UltraScan650™ is not a valid alternative to the DXA for diagnostic purposes. It is possible for the UltraScan650™ to be used as a screening tool to determine female individuals who may be at risk for low BMD; however, results should be interpreted with caution. Given it is unlikely the UltraScan650™ will produce a false negative result (i.e., incorrect determination of healthy BMD when BMD is low), those determined to have low BMD by the UltraScan650™ can be referred for further assessment, erring on the side of caution. In the female population, ranging from ages 18-55, we recommend transforming BMD as measured by the UltraScan650™ based on the linear regression presented in Table 2 [BMD 1/3rd

distal radius = 0.4744*UltraScan650™+0.417]. Female individuals with a low BMD measurement of 0.642 g/cm^2 or less (i.e., T-score < -1)[26] at the 1/3rd radius position should be recommended for further assessments.

Acknowledgements

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Conflict of Interest (COI) disclosure:

The authors declare that they have no conflicts of interest to disclose.

Data availability:

Raw data from this study collected were at the University of Ottawa. Derived data supporting the findings of this study are available upon request from the corresponding author KA upon request.

Ethics approval:

The study was reviewed and approved by the University of Ottawa Ethics Board (REB: H-02-23-8994).

Patient consent statement:

All participants provided their informed consent prior to engaging in data collection.

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Chapter 4 : Reproductive health and physical fitness factors as predictors of injury and bone health

Preamble

Given the findings from chapter 2, which demonstrated an association between reproductive health and MSKi risk, this chapter shifts focus to the physical testing of female Canadian Armed Forces members. The aim is to determine how reproductive factors and physical fitness as predictors of MSKi and bone health outcomes. Bone health was assessed using the UltraScan650™, a device whose validity in this context was established in the previous chapter, Together, these analyses provide an integrated understanding of the physiological and reproductive contributors to musculoskeletal resilience in female CAF personnel.

Study title: Investigating the association between reproductive characteristics, injury history, physical fitness, and bone mineral density in female Canadian Armed Forces members

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All authors confirm their authorship. J.L.P. and D.F.d.S. contributed to the writing and analysis, with direction from all authors. J.L.P., C.M.E., K.S., and K.B.A conceived the study and oversaw its direction and planning. All authors discussed the results, reviewed the manuscript and approved the final version.

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Abstract

Introduction: This study examined the relationship between reproductive health, body composition, physical fitness, and bone mineral density (BMD) among female Canadian Armed Forces (CAF) members.

Methods: Ninety female CAF members aged 18 to 55 years completed a demographics and reproductive health survey, objective InBody body composition assessments (e.g., height, weight, fat mass/lean mass), a blood draw (e.g., plasma), and physical testing (e.g., VO_{2max}). The UltraScan650 (CyberLogic Inc., Brooklyn, NY) was used to measure BMD at the one-third radius position. Analysis of variance (ANOVA) examined differences in BMD across reproductive variables. Pearson correlations and forward selection multivariate linear models were used to examine the association of predictor variables with BMD. The estradiol-to-progesterone ratio was forced into the model.

Results: Members who have given birth had higher BMD than nulliparous members ($p = 0.031$). Those using hormonal birth control (HBC) showed a trend toward higher BMD ($p = 0.069$). A two-way ANOVA indicated an interaction between parity and HBC use ($F = 4.718$, $p = 0.033$), with parous HBC use having the highest BMD (adjusted mean = 0.730, 95% CI 0.717-0.743). VO_{2max} ($\beta = 0.003$, $p < 0.001$) and the estradiol-to-progesterone ratio ($\beta = 0.127$, $p = 0.041$) were significant predictors of BMD, explaining 38.3% of the variance.

Discussion: The interaction between parity status and the use of HBC is linked to higher BMD among female CAF members. Further, physical fitness (VO_{2max}) remains the largest influencer of BMD in female CAF members; however, the ratio of estradiol to progesterone is associated with BMD at the one-third radius position.

Introduction

In 2020, the Canadian Armed Forces (CAF) set a goal to increase female membership from 16% to 25% by 2026. Thus, resources have been committed to identifying barriers to recruitment and retention of female CAF members. Two primary barriers highlighted by the United States (US) military were: i) policy surrounding reproductive health and ii) musculoskeletal injuries (MSKi). [1,2] Similar to the US, the CAF has identified MSKi as a significant threat to operational readiness, with female members being more likely to medically release due to MSKi than their male peers. [3]

Bone health is a key factor associated with the risk of stress fracture. [4] Among female military personnel, the risk of MSKi is higher. [4] For example, studies of US Marine Corps recruits have shown that those who experienced a stress fracture had lower BMD compared to recruits remaining uninjured. [5] Since the British Armed Forces implemented gender-free policies (i.e., policies developed without the consideration of gender), injury incidence in females has increased, with the likelihood of stress fracture being 10 times greater. [6,7] These findings highlight the need to uncover contributing factors to the sex-disparity in stress fractures occurring in military settings, apart from the well documented sex-differences in bone strength and structure that emerge at puberty. [8]

A CAF report identified sex-differences in injuries among members and highlighted that most female members are in their reproductive years, identifying reproductive health as a priority research area. [9] Our scoping review [4] established an association between reproductive health characteristics (e.g., irregular menstrual cycle and late menarche), MSKi and stress fracture. While having an irregular menstrual cycle has been linked to MSKi in military populations, [10,11] the quality of scientific evidence is insufficient with respect to secondary amenorrhea, late age of menarche and MSKi. [12] Literature surrounding the use of hormones as birth control (HBC) in

relation to MSKi risk and bone health in female military members is inconclusive, and several recent studies have recommended more research to understand this relationship. [13] While pregnancy and lactation are associated with a temporary loss in bone mineral density (BMD), this effect is not permanent, [14] as demonstrated by parous females having higher BMD compared to their nulliparous peers. [15] The physiological and biomechanical changes that occur during pregnancy are linked to greater injury risk, and parity has further been associated with an increased risk of repetitive strain injury (RSI) among female CAF population. [16] However, it is yet to be explored whether female reproductive factors (e.g., parity status and HBC) and injury history are related to BMD in female CAF members.

Estrogen is the primary sex hormone in females and is beneficial to bone health, as it both promotes bone formation and inhibits bone turnover. [17] Premenopausal female individuals with low serum estradiol levels have a greater chance of experiencing low BMD. [18] Reproductive health characteristics associated with estrogen deficiency, such as amenorrhea, are considered risk factors for fracture among females of reproductive age; [19] however, parity is linked to higher estradiol levels. [20] Progesterone prepares the uterine lining for implantation of a fertilized egg and works in tandem with estrogen to maintain bone health. [21] The effect of progesterone on BMD is dose-dependent, with progesterone being protective of BMD at ideal levels (i.e., during the luteal phase of the menstrual cycle); however, when progesterone levels are outside ideal ranges, BMD may decline [22]. Reproductive stages that increase progesterone levels are pregnancy and the use of HBC during the follicular phase of the menstrual cycle. [23] While the effect of progesterone on bone is variable, FSH has a negative effect on BMD. The main role of follicle stimulating hormone (FSH) is to stimulate the production of estrogen during folliculogenesis, the process by which an ovarian follicle achieves maturation; despite this, FSH is suspected to have direct action on bone.

Menopause is marked by higher concentrations of FSH, shown to be associated with lower BMD, independent of estrogen. [24] Thus, characterizing the vital reproductive health hormone profile may help establish active-duty female CAF members who are susceptible to MSKi and stress fracture.

It is understood that improvements in BMD can exponentially reduce the risk of fracture. [25] Exercise enhances bone health, particularly in the female population, [26] and greater physical fitness (i.e., cardiorespiratory fitness) is related to higher BMD. [26] In premenopausal individuals, physical activity is one of the most important modifiable predictors of BMD. [27] When estrogen is deficient in females, the amelioratory effect of cardiorespiratory fitness and mechanical strain on bone may be nullified. [28] This relationship is particularly important in the female military population as lower BMD is significantly associated with risk of stress fracture. [29] Thus, the combination of physical fitness markers and reproductive health hormones can optimize the prediction of BMD.

Female military members have a unique physical profile, injury experiences and reproductive health patterns, characteristics that cannot be simply extrapolated from the non-military population. Considering reproductive health and physical fitness to BMD in other military and civilian populations, we aim to explore the association of the following independent variables: female reproductive factors; physical fitness; a history of MSKi; and other general characteristics (e.g., years serving, body fat percent) with BMD (dependent variable). We expect i) female CAF members whose reproductive characteristics allow for higher levels of estradiol and progesterone (i.e., parous, regular menstrual cycle, and use HBC) will present higher BMD; ii) individuals with greater aerobic and muscular fitness would experience higher BMD; and iii) a history of MSKi will be associated with lower BMD. To our knowledge, no study has explored estradiol, progesterone, and FSH serum levels and BMD in female CAF members. This study will inform

the effort to reduce the burden of MSKi and help to improve retention rates among female CAF members.

Materials and Methods

Study Design and Sample

This cross-sectional study is a planned sub-analysis of a larger study which assessed the physical and fitness-related differences between female CAF members whose self-reported, MSKi history was believed to have a lower impact on their occupational performance *versus* female members of the CAF whose MSKi history were perceived to have a higher impact on their occupational performance. The inclusion criteria for the larger study were: i) assigned female at birth, ii) 18 to 55 years of age, iii) being an active-duty CAF member, iv) cleared to engage in maximal exercise based on the health appraisal, and v) providing informed consent in English or French. Participants were instructed to report information based on the MSKi sustained while they were "a serving member".

Data were collected from October 2021 to January 2022. A health appraisal questionnaire was completed prior to the physical assessment to gather the following information: i) general demographics (e.g., age, rank, years serving), ii) general health (e.g., blood pressure, diabetes), iii) female specific characteristics (e.g., use of hormones as birth control, parity status), iv) MSKi (e.g., perception of MSKi impact on occupational performance, type of injury). The survey was available on the online cloud-based survey development software SurveyMonkey Inc. (San Mateo, USA) or via a paper format. The physical and physiological assessment was approximately 180 minutes in length. Participants were asked to dress appropriately to perform physical activity and arrive fasted for the purpose of the blood draw and body composition measurements (i.e., nothing to eat or drink for 8 hours prior to testing). Testing was performed in the following sequence as described in

Table 4.1 and has previously been described in a report to Defence Research and Development Canada by our team.[30]

Table 4.1. Description of blood draw, anthropometrics, body composition, and physical testing used in the present study, introduced in sequential order as performed during assessment.

N	TEST NAME	ASSESSMENT DESCRIPTION
1	Blood draws*	The metabolic analysis required the collection of blood samples (10 mL) from the participants, by a phlebotomist, after 8 hours fasting.
2	Anthropometrics	Participants had to remove their shoes and socks. Participants were asked to bring fitted clothing (e.g., t-shirts, tank tops, shorts) to use during the anthropometric assessment. A portable stadiometer (Seca, USA) was used for height, a scale (InBody®, USA) for body weight, and non-extensive measuring tape (former Canadian Obesity Network, Canada) for biceps (relaxed and flexed) and calf (relaxed) circumferences
3	Body composition	Fat mass and lean mass were assessed using bioelectrical impedance assessment (InBody USA) following the validated pre-assessment preparations [30].
4	Bone mineral density	Participants were seated with their forearm relaxed and positioned on a table. The UltraScan650™ (CyberLogic, Inc., USA) quantitative ultrasound was placed on the forearm for this measurement[31]. The right forearm was measured three times and both the bone mineral density (g/cm^2) and net time delay (NTD) standard deviation was determined. If the NTD standard deviation was >0.05, two additional measures were performed. The three closest measures of BMD were averaged and used in the analysis. This measurement lasted ~5 minutes. Raw BMD values as measured by the UltraScan650™ were transformed using a validated linear regression[32].
These tests were completed following a standardized warm-up		
5	Flexibility	The validated sit-and-reach test was performed following that standardized protocol[33]. The best of 3 attempts was used for analyses.
6	Muscular power	For the long jump, the distance was determined by measuring the perpendicular distance from the start line to the back of the closest heal. For the medicine ball explosive power test. The distance measured was from the front end of the subject with their arms straight, to where the medicine ball landed.
7	Strength	Four repetition maximal (RM) were performed in the back squat and bench press exercises. Participants had 7 attempts for each exercise with 3-5min interval between them. Participants had to perform all the four repetitions without assistance.
There was a 20-min interval between strength tests and endurance strength assessment. Video analysis* data collection was performed during the back squat exercise.		
8	Endurance strength	Biering-Sorensen test was performed, and the time was recorded. Single leg wall sit was performed for each leg and the time was recorded. Push-ups were performed and the number of successful push-ups were recorded.
There was a 20-min interval between endurance strength tests and the aerobic fitness test.		
9	Aerobic fitness	A graded exercise test with expired gases collected via indirect calorimetry was performed on a treadmill. The modified Balke protocol was employed.

		VO_{2max} was defined as achieving a combination of standardized criteria including: i) measured maximal heart rate within ±10 bpm of the predicted maximal heart rate; ii) rating of perceived exertion ≥18 in the 6-20 Borg scale; iii) respiratory exchange ratio ≥1.0.

A total of 90 participants from the CAF were assessed; however, blood samples were only collected from 65 participants. The blood draw was a supplementary sub-study and not a required component for the original project. Additionally, we experienced a shortage of blood draw supplies at the assessment location due to the COVID-19 pandemic. Fasting blood samples were collected from active CAF members in serum blood collection tubes. Serum was left to clot at room temperature for 30 minutes and centrifuged at 1,700 relative centrifugal force (Xg) for 15 minutes at 4°C and plasma was processed immediately following the draw. Supernatants were removed and aliquoted into cryovials and stored at -80°C for further analysis. Serum levels of Progesterone, estradiol and plasma FSH were determined using enzyme-linked immunosorbent assays (ELISAs) kits from Enzo Life (catalog ADI-900-011), Invitrogen (catalog KAQ0621) and RayBiotech (catalog ELH-FSH), respectively. All assays were performed according to the manufacturer's protocols.

This study was approved by the University of Ottawa Research Ethics Board (H-11-20-6180) and all the procedures were in accordance with the Declaration of Helsinki. The research team utilized a variety of recruiting methods. Participants were recruited via social media accounts (e.g., lab website, Facebook, Twitter), and the social networks of the researchers involved, in addition to project stakeholders (e.g., Canadian Forces Morale and Welfare Services, Personnel Support Programs, Defence Fitness, Defence Women's Advisory Organization), and CAF internal electronic communications.

Variables

Variables selected to assess the impact of reproductive health factors on MSKi are described in **Table 4.2**. This table outlines the original questionnaire categories, and the reclassifications used for the analysis. Additionally, we described which variables were analyzed as categorical and analyzed as continuous.

Table 4.2 Independent variables, covariates, and outcomes.

#	Independent Variable (Categorical)	Original Categories for Response	Categories and Reference for Analysis
1	Menstrual cycle periodicity	1) Regular 2) Never regular 3) Irregular for few months 4) Periods stopped while serving 5) Never had a period	1) Regular 2) Never regular, irregular for few months, periods stopped while serving and never had a period
2	Use of hormonal birth control	1) Yes 2) No	1) Yes 2) No
3	Parity	1) 0 2) 1 3) 2 4) 3 5) 4+	1) 0 2) 1, 2, 3, 4+
4	History of RSI	1) Yes 2) No	1) Yes 2) No
5	History of acute injury	1) Yes 2) No	1) Yes 2) No
N	**Independent Variable (Continuous)**	**Original Categories for Response**	**Categories and Reference for Analysis**
1	Age	Continuous variable (year)	Continuous variable (year)
2	BMI	Continuous variable (kg/m^2)	Continuous variable (kg/m^2)
3	Years serving	Continuous variable (year)	Continuous variable (year)
4	Body fat	Continuous variable (%)	Continuous variable (%)
5	Skeletal muscle mass	Continuous variable (kg)	Continuous variable (kg)
6	Estradiol	Continuous variable (pg/mL)	Continuous variable (pg/mL)
7	FSH	Continuous variable (pg/mL)	Continuous variable (pg/mL)
8	Progesterone	Continuous variable (pg/mL)	Continuous variable (pg/mL)
9	VO_{2max}	Continuous variable (mL/kg/min)	Continuous variable (mL/kg/min)

10	Flexibility	Continuous variable (cm)	Continuous variable (cm)
11	Long Jump	Continuous variable (cm)	Continuous variable (cm)
12	Medicine ball throw	Continuous variable (cm)	Continuous variable (cm)
13	Relative back squat	Continuous variable (lbs)	Continuous variable
14	Relative bench press	Continuous variable (lbs)	Continuous variable
15	Back endurance	Continuous variable (seconds)	Continuous variable (seconds)
16	Left leg wall sit	Continuous variable (seconds)	Continuous variable (seconds)
17	Right leg wall sit	Continuous variable (seconds)	Continuous variable (seconds)
18	Push ups	Continuous variable (repetitions)	Continuous variable (repetitions)
N	**Outcomes**	**Original Categories for Response**	**Categories and Reference for Analysis**
1	Bone mineral density	Continuous variable (g/cm^2)	Continuous variable (g/cm^2)

RSI – Repetitive Strain Injury; NCM – Non-commissioned members; BMI - Body Mass Index; FSH – Follicle Stimulating Hormone

Statistical Analysis

Data Cleaning

For categorical variables with <20 observations per category, we opted to combine categories when possible (i.e., Variable: *Menstrual cycle periodicity* – Recategorization: *1) Regular; 2) Never regular, irregular for few months, periods stopped while serving and never had a period* and variable: *Parity* – Recategorization: *1) 0; 2) 1, 2, 3, 4+*).

Main Analysis

The data were analyzed using R studio software. Normality was tested using the Shapiro-Wilk test, and non-normal data was reported as median and interquartile range. Equality of variance was assessed using Levene's test. Descriptive statistics were reported using mean and standard deviation or median and interquartile range for continuous variables. For categorical variables, frequency and percentage were reported. Given an analysis of variance (ANOVA) is

sufficiently robust for the analysis of non-normal data with respect to type I errors [31], the outcome variable was compared between reproductive health characteristics using one-way ANOVA. To assess any interactions between significant reproductive factors, a two-way ANOVA was applied. Multiple comparisons were performed using the Tukey method and significance was set at $p<0.05$.

Linear regression analysis was used to determine variables that were associated with BMD at the 1/3rd radius position. Predictor variables (detailed in Table 3) were selected using a stepwise forward selection method. The cut-off for covariate inclusion in the model was set at $p<0.05$. If a variable in a given model had a variance inflation ratio of >5, the variable in question was removed from the model. Further, given reproductive health parameters are a main variable of interest, the ratio of estradiol to progesterone was forced into a model. Significance for the model was tested using the likelihood ratio test with a p-value <0.05.

Results

Participant demographics are presented in **Table 4.3**. A total of n=60 estradiol, n=40 progesterone, and n=53 FSH concentrations were successfully attained due to some samples being outside the detection ranges of the aforementioned ELISAs. Thirty-four participants had all three assays successfully completed.

Table 4.3. Participant characteristics.

Characteristics		
Continuous variables	Mean	SD
Age (years),	33.82	7.30
Years of service (years), median; IQR	9.00	11.00
Height (m)	1.65	0.064
Weight (kg), median; IQR	68.55	15.3
BMI (kg/m^2)	25.71	4.27
Body fat (%)	28.93	8.13
SMM (kg), median; IQR	27.10	4.0

Estradiol (pg/mL), median; IQR	85.25	55.02
Progesterone (pg/mL), median; IQR	270.7	242.43
FSH (pg/mL), median; IQR	32	27.10
VO_{2max} (mL/kg/min), median; IQR	42.5	11.7
Flexibility (cm), median; IQR	35.9	9.75
Long Jump (cm)	163.2	26.7
Medicine ball throw (cm), median; IQR	276.5	49.2
Back squat (lbs)	154.3	50.0
Bench press (lbs), median; IQR	85.0	25.0
Back endurance (seconds), median; IQR	123.0	88.3
Left leg wall sit (seconds), median; IQR	35.8	25.4
Right leg wall sit (seconds), median; IQR	37.3	27.6
Push-ups (repetitions), median; IQR	16.0	12.0
$1/3^{rd}$ radius BMD (g/cm^2)	0.707	0.023
Categorical variables	N	%
Rank structure		
NCM	57	63.3
Officer	33	36.7
Military element		
Canadian Army	25	27.8
Royal Canadian Air Force	2	2.2
Purple Trade*	63	70.0
Use of hormonal birth control		
Yes	38	42.2
No	52	57.8
Parity status		
Parous	39	43.3
Nulliparous	51	56.7
Regular menstrual cycle		
Yes	52	57.8
No	38	42.2
History of RSI		
Yes	77	85.6
No	13	14.4
History of acute injury		
Yes	66	73.3
No	24	26.7

RSI – repetitive strain injury; BMI – body mass index, SMM – skeletal muscle mass, FSH - Follicle-stimulating hormone, BMD – bone mineral density, NCM – non-commissioned member, SD – Standard Deviation, IQR – Interquartile range
*A purple trade is an occupation in the military that is not exclusive to a single environment.

Pearson correlations are presented in **Supplemental 1**. Years serving, body mass index (BMI), body fat percentage, and VO_{2max} were significantly correlated with BMD.

Supplemental 1. Pearson correlation between predictor variable and BMD (n = 89).

Continuous variables	r-value (95% CI)	p-value
Age (years)	0.016 (-0.193; 0.223)	0.883
Years serving (years)	**0.220 (0.013; 0.410)**	**0.038**
BMI (kg/m^2)	**-0.234 (-0.422; -0.027)**	**0.027**
Body fat (%)	**-0.289 (-0.469; -0.085)**	**0.006**
SMM (kg)	-0.092 (-0.295; 0.118)	0.389
Estradiol (pg/mL)	-0.005 (-0.260; 0.252)	0.973
FSH (pg/mL)	-0.055 (-0.323; 0.221)	0.698
Progesterone (pg/mL)	-0.046 (-0.356; 0.274)	0.781
Estradiol: Progesterone	0.148 (-0.176; 0.442)	0.369
VO_{2max} (mL/kg/min)	**0.388 (0.182; 0.561)**	**<0.001**
Flexibility (cm)	0.195 (-0.017; 0.389)	0.071
Long Jump (cm)	**0.294 (0.089; 0.476)**	**0.006**
Medicine ball throw (cm)	0.068 (-0.145; 0.275)	0.532
Relative back squat	**0.350 (0.145; 0.526)**	**0.001**
Relative bench press	**0.376 (0.179; 0.545)**	**<0.001**
Back endurance (seconds)	**0.299 (0.095; 0.480)**	**0.005**
Left leg wall sit (seconds)	0.134 (-0.078; 0.336)	0.215
Right leg wall sit (seconds)	0.202 (-0.009; 0.396)	0.061
Push-ups (repetitions)	0.204 (-0.007; 0.398)	0.058

CI – Confidence interval; BMI – body mass index; SMM – skeletal muscle mass; FSH - Follicle-stimulating hormone

The relationship between predictor variables and BMD were assessed by way of linear regression analysis. Significant findings are displayed in **Table 4.4**.

Table 4.4. Linear regression model to predict Bone Mineral Density

Predictor variables (n = 78)	β	95% CI	p-value

Intercept	0.640	0.613; 0.667	<0.001
VO_{2max} (mL/kg/min)	0.001	<0.001; 0.001	0.019
Relative bench press	0.043	0.007; 0.079	0.020
Years serving	0.001	<0.001; 0.001	0.012

CI – Confidence interval
Adjusted R^2: 0.2308; $p < 0.001$, predicts 23.1% of the model

Given the influence of hormones on BMD in female individuals, the ratio of estradiol to progesterone was forced into the linear regression model displayed in Table 4. At the reduced sample size, VO_{2max} ($p<0.001$) and relative bench press ($p=0.002$) remained significantly correlated to BMD. Insignificant independent were removed from the model, and the resulting regression is displayed in **Table 4.5**. It should be noted that despite a reduced sample size for the Table 5 linear regression, the demographic characteristics remained consistent with those of the overall sample.

Table 4.5. Linear regression model to predict Bone Mineral Density with the ratio of estradiol to progesterone forced into the model.

Predictor variables (n = 33)	β	95% CI	p-value
Intercept	0.590	0.530; 0.649	<0.001
VO_{2max} (mL/kg/min)	0.002	0.001; 0.002	<0.001
Estradiol : Progesterone	0.057	0.002; 0.111	0.041

CI – Confidence interval
Adjusted R^2: 0.3825; $p<0.001$, predicts 38.3% of the model

The relationships between reproductive characteristics (i.e., parity status, use of HBC, and menstrual regularity) and injury history (i.e., RSI and acute injury) were assessed using one-way ANOVA analysis. There were no associations between menstrual regularity ($p=0.809$), RSI history ($p=0.785$), acute injury history ($p=0.427$) and BMD. The relationships between reproductive characteristics were assessed by way of a one-way ANOVA. One-way, reproductive health

ANOVAs with a p-value < 0.1, use of HBC (F=3.400; p=0.069; η^2=0.019) and parity status (F=4.811; p=0.031; η^2=0.052**)**, were assessed using a two-way ANOVA. The findings for the two-way ANOVA are presented in **Table 4.6**. Individuals who had previously used HBC and were parous had significantly higher BMD compared to other groups (i.e., nulliparous/HBC, nulliparous/no HBC, and parous/no HBC).

Table 4.6. Two-way ANOVA, reproductive health factor associations with BMD

		Parity Status		
		Parous (n = 39)	Nulliparous (n = 51)	Total
Use of HBC	Yes (n = 38)	**0.730 (0.717; 0.743)[a]**	0.704 (0.695; 0.713)	**0.712 (0.705, 0.720)[c]**
	No (n = 52)	0.706 (0.697; 0.714)	0.701 (0.692; 0.709)	0.703 (0.697; 0.710)
	Total	**0.713 (0.706; 0.721)[b]**	0.702 (0.696; 0.709)	
Main effects and interaction	Parity Status	**F = 5.276; p = 0.024; η^2 = 0.026**		
	Use of HBC	**F = 5.687; p = 0.019; η^2 = 0.028**		
	Interaction	**F = 4.718; p = 0.033; η^2 = 0.023**		

[a]Significantly different from all other groups in two-way ANOVA
[b]Significantly different from nulliparous (main effect of parity)
[c]Significantly different from no hormonal birth control (main effect of use of HBC)
HBC – Hormones as birth control

Discussion

This study investigated physiological predictors, including those related to reproductive health, of BMD at the 1/3rd radius position among female CAF members. Several relationships between reproductive health and BMD were identified, supporting our hypothesis. Our main findings were: i) parous members have higher BMD compared to their nulliparous peers; ii) those who take HBC have higher BMD compared to those who do not; iii) parous members who use HBC have a higher BMD compared to all other groups (i.e., nulliparous/HBC, nulliparous/no HBC, parous/no HBC); iv) The ratio of estradiol to progesterone and VO_{2max} are predictors of BMD among female CAF members (sub-sample with hormonal profiling). Furthermore, we

determined that cardiorespiratory fitness (i.e., VO_{2max}), relative bench press, and years serving in the CAF are predictors of BMD female CAF members (entire sample). Understanding physiological factors that influence BMD among female CAF members will help guide future research/policies aimed at ameliorating career longevity and achieve the goal of 25% female representation in the CAF.

In the premenopausal population, the relationship between physical fitness and BMD is well-established. The findings from the present study support previous literature indicating the benefits of physical fitness, as we determined that female CAF members with a higher VO_{2max} and relative bench press present with higher BMD. The consensus is that exercise improves BMD, via mechanical stimulation. Physical activity contributes to the maintenance and gain of BMD by mechanical forces exerted on bone through ground reaction forces. At the cellular level, osteocytes (i.e., mature osteoblasts, bone formation cells) rapidly respond to mechanical strain to influence adaptive bone remodeling. [32] Thus, osteocytes would stimulate osteoblasts to upregulate bone formation. Another possible explanation with respect to a higher VO_{2max} being associated with BMD is that female CAF member with higher cardiorespiratory fitness have habitually engaged physical activity and thus maintained this higher level of fitness across their lifespan. Given that exercise in childhood is associated with greater BMD, [33] and those who are physically active in childhood are more likely to be physically active in adulthood thereby reaching a greater peak bone mass, it is possible that these factors would be confounders. Subsequently, it is not just the present exercise habits of participants contributing to current fitness levels being associated with higher BMD. Further, it is possible that other measures of strength/power were not related to BMD because bench press directly applies force to the distal radius whereas the rest of the assessments do not. These findings justify the recommendation for PA in those with low BMD and future

research should prioritize training programs that incorporate both aerobic and resistance training for CAF members deemed to be susceptible to low BMD.

The number of years serving in the CAF, when accounting for VO_{2max} and relative bench press, were found to be a significant determinant of BMD at the 1/3rd radius position among female members. It is possible that a longer service leads to higher BMD partially because of prolonged exposure to military training; however, since the analysis accounted for VO_{2max} (i.e., cardiorespiratory fitness) and relative bench press and multicollinearity checks did not reveal any problematic relationships between predictors, this explanation is less likely as these variables may act as a surrogate for military training. It is posited that those who remain in the CAF longer possess favourable or more resilient physiological profiles, possibly due to adequate training programs, lower injury susceptibility, faster recovery, and/or hereditary factors, while individuals with less favourable profiles may release early for medical reasons. Thus, the observed higher BMD among long-serving members is likely influenced by both enhanced physical conditioning and a selection bias favouring individuals with a better health profile.

Our data corroborate the notion that aspects of reproductive health benefit bone health at the 1/3rd radius position among female CAF members. It is well known that reproductive health hormones, such as estradiol and progesterone, work together to facilitate bone remodeling in the premenopausal female population. [22] Our findings support the relationship between estradiol and progesterone and bone health, with a higher ratio of estradiol to progesterone correlating with higher BMD among female CAF members. Previous research has determined that, *in vitro*, the effect of progesterone is dose dependent, independent of estradiol, with higher doses of progesterone being detrimental for osteoblast differentiation. [34] Further, in the absence of estradiol, progesterone did not have an effect on osteoblast proliferation. [34] With this information in mind,

it is possible that in estrogen-replete female CAF members, with ideal progesterone corresponding to the luteal phase of the menstrual cycle [22] have optimized osteoblastic proliferation and differentiation, leading to higher BMD.

This study also identified links between parity status and HBC with BMD, as well as an interaction between parity status and the use of HBC, with BMD. Being parous was associated with higher BMD when compared to nulliparous female CAF members, aligning with previous literature. [15] A possible explanation is that pregnancy is linked to elevated levels of estrogen caused by production from the placenta. [20] These elevated levels of estrogen may persist into the postpartum period as previous research has demonstrated that pluriparous individuals have higher estradiol levels compared to their nulliparous peers. Estrogen is a key regulatory hormone in females, influencing reproductive function, cardiovascular health, and metabolic homeostasis, while playing a critical role bone turnover. These effects could lead to increases in BMD. In our findings, parous who used HBC had higher BMD compared to all other groups (i.e., nulliparous/HBC, nulliparous/no HBC, parous/no HBC). Oral contraceptives are the most commonly prescribed method of contraception in the United States, with the majority being combined oral contraceptives containing both estrogen and progesterone. [35] Combined oral contraceptives and progestin-only contraceptives function through the same mechanism, whereby production and secretion of FSH and LH are inhibited. Moreover, previous research has found that naturally cycling premenopausal females have lower serum progesterone levels during the follicular phase compared to those who take oral contraceptives. [36] *In vitro* studies on human perimenopausal cell lines have indicated that osteoblast (cells responsible for bone formation) proliferation is not impacted by progesterone in the absence of estrogen. [34] Thus, it is possible that female CAF members who are parous and use HBC have higher BMD because their

reproductive/hormonal profile (i.e., elevated estrogen and progesterone levels on average) provides an environment that supports the proliferation of osteoblasts. Further research examining the effect of combined oral contraceptives *versus* progestin-only contraceptives on BMD are required as the effect of each on BMD may be different.

Strengths & Limitations

This cross-sectional study design did not allow the authors to establish causation. Participants were not randomly selected, rather we employed convenience and "snowball" sampling to recruit our participants. Behaviours related to nutrition, current physical activity status and ongoing military training were not examined. Additionally, the use of health appraisal surveys relied on self-report data, which may be subject to recall bias.

With respect to the linear regression analyses, the assumption of normality was not met for the predictor variables in Table 4. Moreover, the linear regression analysis with the ratio of estradiol to progesterone had a small sample (n=33) and is not sufficiently powered (1-β=0.458). However, in this reduced sample, the assumption of normality for the predictor variables was satisfied. As such, the results of these regressions should be interpreted with caution. We also did not ask participants to specify the type of HBC (e.g., oral contraceptive pill, intrauterine device). Previous reports suggest that BMD may be overestimated when measured in the dominant hand, with recommendations to assess the non-dominant hand. While handedness was not considered in our study, we ensured consistency by assessing the same arm all participants, regardless of dominance. [37] It should be noted that most participants were right-handed.

Important strengths of this study are the sample characterization and size. The ANOVA analysis included a large, well-phenotyped, homogenous sample, providing adequate power to address our primary outcome (i.e., BMD). This study used validated testing protocols, objectively

measured cardiorespiratory fitness (i.e., VO_{2max}), and a validated device to assess BMD. Further, previous research conducted by our team has determined that BMD assessed at the 1/3rd distal radius correlates to BMD of the total body[38], making the assessment important to the understand of overall bone health, but less relevant to load barring bones such as the femur and lumbar spine.

Conclusion

Female CAF members who have given birth, particularly those who use HBC, have higher BMD compared to those who are nulliparous irrespective of their use of HBC. Additionally, those with a higher VO_{2max} and estradiol to progesterone ratio had greater BMD. This study highlights that estrogen-replete, female CAF members with higher cardiorespiratory fitness present with higher BMD. These findings highlight female-specific physiological predictors of BMD. Therefore, female members with a higher risk profile based on these factors may benefit from additional BMD screening and physical training programs aimed at improving bone health. Further research (i.e., longitudinal studies) should seek to understand the relationship between military service, reproductive health hormones, and BMD.

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Chapter 5 : Exploring the impact of physical activity, sleep, and reproductive characteristics on bone mineral density during pregnancy

Preamble

The findings from chapter 4 revealed a significant association between reproductive health factors such as parity status and use of hormonal birth control and BMD. Furthermore, the strongest predictor of BMD among the female CAF population was determined to be physical fitness. Considering these results, it was deemed necessary to investigate these relationships in a pregnant population; however, due to the limited number of pregnant individuals at any one time within the CAF, the subsequent study was conducted in a civilian cohort.

Study title: Bone density at the breaking point: a quantitative look at the role of distal radius density and physical activity in pregnancy

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All authors confirm their authorship. J.L.P. contributed to the writing and analysis of the manuscript with direction from all authors. J.L.P., R.B., and K.B.A conceived the study and were in charge of direction and planning. All authors discussed the results, reviewed the manuscript, and approved the final version.

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Abstract

Introduction: Pregnancy involves rapid changes in calcium metabolism and bone turnover that may affect bone mineral density (BMD) during this period. Evidence linking physical activity (PA), sleep, and reproductive factors to BMD during pregnancy is limited. Previous studies investigating the relationship between PA and BMD have not included objective measures of PA. This study will investigate the relationship between objectively measure PA and sleep, reproductive characteristics, and BMD.

Methods: In this cross-sectional study, 22 healthy pregnant individuals in Madrid, Spain (October 2024–February 2025) completed reproductive health and supplementation questionnaires. BMD at the one-third distal radius was assessed using the UltraScan650™ quantitative ultrasound. PA and sleep were measured objectively with Actical accelerometers over seven days. Associations between BMD and predictor variables were examined using one-way AN(C)OVA.

Results: Longer sleep duration was associated with significantly lower BMD compared with shorter sleep duration ($p=0.008$). An interaction between age and hormonal birth control (HBC) history was observed, with age positively associated with BMD among HBC users but not non-users ($p=0.028$). Age was also positively associated with BMD in the third trimester, but not the second trimester ($p=0.046$). No associations were observed between objectively measured PA and BMD.

Conclusion: This study provides novel evidence that sleep duration, reproductive history, and gestational timing may influence BMD during pregnancy. These findings highlight the importance of monitoring skeletal health with consideration of sleep patterns, HBC history, and trimester, and underscore the need for larger studies to confirm these associations.

Introduction

Osteoporosis affects a large portion of the population in Western world, particularly the female population. It is estimated 5.6-10.5% of Canadians over the age of 50 have been diagnosed with osteoporosis. [1] Similarly, a prevalence study in a Spanish population found that 22.8% and 9.1% aged 50 year or older had osteoporosis at the lumber spine and femur neck, respectively. [2] Reproductive health characteristics, including pregnancy, have been shown to influence bone mineral density (BMD) and markers of bone turnover, the process of bone resorption and formation. In pregnancy, this is due to a shift in the calcium metabolism to accommodate the growing fetal skeleton. Among non-pregnant, pre-menopausal females, physical activity (PA) has been shown to ameliorate bone health. [3] Despite this well-established relationship, the link between PA, particularly objectively measured PA, on BMD has yet to be investigated in the pregnant population.

While studies investigating the effect of pregnancy on BMD are limited, pregnancy is associated with a decrease in BMD. Despite this initial decrease, it has been shown that BMD recovers after lactation. [4] Moreover, pregnancy and lactation have been shown to have a differential effect on anatomical regions of the body. Drinkwater and Chestnut (1991) determined that BMD at the femoral neck, radial shaft and lumbar decreased, while BMD at the tibia increased during pregnancy. During lactation BMD of the lumbar returned to pre-pregnancy levels, while BMD at the femur neck continued to decrease. [5] A separate study found that pregnancy was associated with an increase in BMD of the legs and arms and a decrease at the pelvis and spine region of the gestational parent. [3,6] Pregnancy is a time where bone turnover is high [6] and it has been suggested that the process of bone turnover is not linked, with bone resorption being higher during early and mid-gestation, and bone formation being higher during late pregnancy. [7] These

findings underscore the dynamic nature of bone remodeling across gestation, highlighting critical windows for targeted interventions to preserve maternal skeletal health.

While trimester has been shown to influence BMD during pregnancy, there are additional physiological factors that have been linked to BMD in females of reproductive age. Menstrual regularity, the use of hormonal birth control (HBC), experiencing a reproductive disease, calcium and vitamin D supplementation have all been linked to BMD. Low energy availability, common among elite athletes, is associated with menstrual irregularity and low BMD, though non-athletes are also susceptible. [8] A recent review concluded that combined oral contraceptives and progestin-only HBC have no measurable impact on BMD in the premenopausal population, though further investigations are needed. [9] Reproductive diseases such as endometriosis[10] and previously polycystic ovarian syndrome (PCOS)/now polyendocrine metabolic ovarian syndrome [11] have been linked to lower BMD, due to associated treatments and estrogen deficiency, respectively. Furthermore, among premenopausal women, calcium intake >1500 mg/day (very high) and vitamin D intake of 10 ug/day (high) were linked to better maintenance of BMD across 10 years compared to other intake classifications. [12] Given this information, it is worth exploring these variables (i.e., reproductive and supplementation) among pregnant individuals.

Emerging evidence suggests that lifestyle factors, including sleep, may influence BMD of female individuals across the lifespan. In premenopausal people, sleep quality has been positively associated with BMD, [13] indicating a potential link between restorative sleep and skeletal health. Conversely, in a post-menopausal cohort, excessive sleep duration has been correlated with a higher risk of osteoporosis; a relationship not observed in younger groups. [14] Given the physiological demands and hormonal fluctuations unique to gestation, we believe investigating sleep as a modifiable factor in BMD represents a critical gap in the literature not yet filled.

Physical activity is another key lifestyle factor with implications for BMD during pregnancy. The 2019 Canadian Guidelines for Physical Activity During Pregnancy [15] and the Spanish clinical guidelines on physical activity during pregnancy [16] recommend 150 mins of moderate intensity physical activity (MPA) per week. However, a study found that pregnant women who self-reported exercising 10 or more hours per week experienced significantly less BMD loss across gestation; [17] a level nearly 4x the clinical guideline. Previous research assessing PA during pregnancy relied on self-report questionnaires, primarily to confirm parity with non-pregnant comparison groups. [5] Given that PA questionnaires in pregnancy have been shown to overestimate activity levels, [18] an objective measurement is both necessary and novel.

Furthermore, other existing studies examining BMD during pregnancy have been limited by relatively small sample sizes ($n<20$), [5, 6, 7] potentially undermining the validity of the findings. To address such gaps and shortcomings, this study will use an wearable accelerometer device to objectively measure PA and sleep patterns. With the addition of other related factors, this approach will generate novel insight into the relationship between objectively measured PA, sleep, reproductive factors, supplementation patterns and BMD measures at the 1/3rd distal radius in a healthy pregnant population.

Methods

Study Design and Sample

This study employed a cross-sectional design. Healthy pregnant individuals without contraindication to exercise were recruited; all assessments were carried out by trained hospital staff. The inclusion criteria were: i) Residing in Madrid, Spain; ii) a singleton fetus, iii) Capable of basic communication in Spanish; iv) Over 18 years old; v) No history of preterm birth or miscarriage. Exclusion criteria i) were not planning to give birth in the same hospital as the study setting; ii) Concurrent participation in multiple research studies. Data were collected from October

2024 to February 2025. A reproductive health questionnaire was completed by all participants prior to BMD assessments. Ethical approval was obtained from the University of Ottawa and the Universidad Politécnica de Madrid institutional review boards. All participants provided their written informed consent to participate in the clinical trial.

Demographics Questionnaire

Given the nature of the data collection, height and weight were measured using stadiometers and scales at check-ups with participants' attending physician and self-reported to the research team. A questionnaire developed by the Adamo team was used to assess dietary supplementation of calcium and vitamin D, dominance, and fracture history. Additionally, the questionnaire assessed reproductive health history (i.e., prior use of hormonal contraceptives, parity, menstrual regularity prior to conception, reproductive diseases, and age of menarche).

Bone Mineral Density Assessment

Participants were seated with their forearm relaxed and positioned on a table. The UltraScan650™ (CyberLogic, Inc., USA) quantitative ultrasound was placed on the forearm for this measurement. [19] The right forearm (i.e., 1/3rd distal radius) was measured five times and both the bone mineral density (g/cm^2) and net time delay (NTD) standard deviation was determined. The three closest measures of BMD were averaged and used in the analysis. If the NTD standard deviation of the three measurements was <0.05, this was considered a valid assessment. This measurement lasted ~5 minutes. Raw BMD values as measured by the UltraScan650™ were transformed using a validated linear regression. [20]

Physical Activity Assessment

PA was assessed objectively using an omniaxial Actical® accelerometer (Philips Respironics Inc., Murrysville PA, USA) upon attendance at their first exercise class. Participants wore the Actical accelerometer on the wrist for 7 days straight, including sleep time. Participants

were asked not to wear the Actical in water. At least 3 valid days were necessary for inclusion in the analysis. [21] The main variables assessed were moderate or vigorous physical activity, total physical activity, duration of sleep, and sleep efficiency. Participants were considered active if they engaged in a minimum of 21.4 min/day of moderate PA, equating to 150 min/week or more following the Spanish and Canadian physical activity guidelines in pregnancy. [15,16]

Statistical Analysis

Data Cleaning

We opted to combine categories when possible (i.e., Variable: *Menstrual cycle periodicity* – Recategorization: *1) Regular; 2) Never regular, irregular for few months, never had a period* and variable: *Parity* – Recategorization: *1) 0; 2) 1, 2, 3, 4+*).

Main Analysis

The data were analyzed using R studio software. Normality was tested using the Shapiro-Wilk test, and non-normal data was reported as median and interquartile range. Descriptive statistics were reported using mean and standard deviation or median and interquartile range for continuous variables. For categorical variables, frequency and percentage were reported. Pearsons's correlations were performed to assess relationship between continuous predictor variables and BMD. Given, no relationships were established, predictor variables were divided into high/low categories based on the median. The outcome variable was compared between reproductive health characteristics, supplementation status, and re-categorized sleep/PA groups using one-way analysis of (co)variance (ANCOVA). Significance was set at $p<0.05$.

Results

Participant demographics are presented in **Table 5.1**. A total of 22 participants were included in the table.

Table 5.1. Demographic characteristics.

Characteristics		
Continuous variables	Mean	SD
Age (years)	33.18	5.18
Height (m)	1.66	0.047
Weight (kg)	69.36	9.65
BMI (kg/m^2)	25.09	3.54
Moderate to vigorous PA (min), median; IQR	3.5	13
Daily PA (min), median; IQR	23.5	8.75
Sleep duration (hours)	8.7	0.89
Sleep efficiency (%)	81.78	4.28
$1/3^{rd}$ distal radius BMD (g/cm^2)	0.716	0.025
Categorical variables	N	%
Dominance		
Right	21	95.5
Left	1	0.5
History of right forearm fracture		
Yes	2	0.9
No	20	90.1
Use of hormones as birth control		
Yes	14	63.6
No	8	36.4
Parity status		
Parous	5	22.7
Nulliparous	17	77.2
Regular menstrual cycle		
Yes	17	77.2
No	5	22.7
Reproductive disease		
Yes	5	22.7
No	17	77.2
Calcium supplementation		
Yes	10	47.6
No	11	52.3
Vitamin D supplementation		
Yes	16	72.7
No	6	27.2

PA – physical activity; IQR – interquartile range; BMD – bone mineral density; BMI – body mass index

Pearson correlation analyses revealed no significant relationship between the continuous predictor variables and the BMD; therefore, linear regression models were not developed. Sleep duration was re-categorized into long (mean = 9.29 hrs) and short (mean =7.96 hrs) groups based on whether participants fell above or below the median average sleep of the group. The relationships between transformed reproductive characteristics (i.e., parity status, prior use of HBC, menstrual regularity, trimester, reproductive health disease), supplementation (i.e., calcium and vitamin D), PA, sleep and BMD were assessed using one-way ANOVA analysis and significant relationships were presented in **Table 5.2**.

Table 5.2. AN(C)OVA table, reproductive health characteristics and sleep duration group associations with BMD in pregnancy.

ANOVA – Sleep duration			
	Long Sleep (n=10)	**Short Sleep (n=8)**	
1/3rd distal radius BMD (g/cm^2)	0.704 (0.691; 0.716)	0.730 (0.716; 0.743)	
	F	**p-value**	**η^2**
Sleep group	9.247	0.008	0.003

BMD – bone mineral density, HBC – hormonal birth control\

Figure 5.1 and **Figure 5.2** present an ANCOVA analysis whereby the predicted BMD values across age are stratified by HBC use and trimester, respectively.

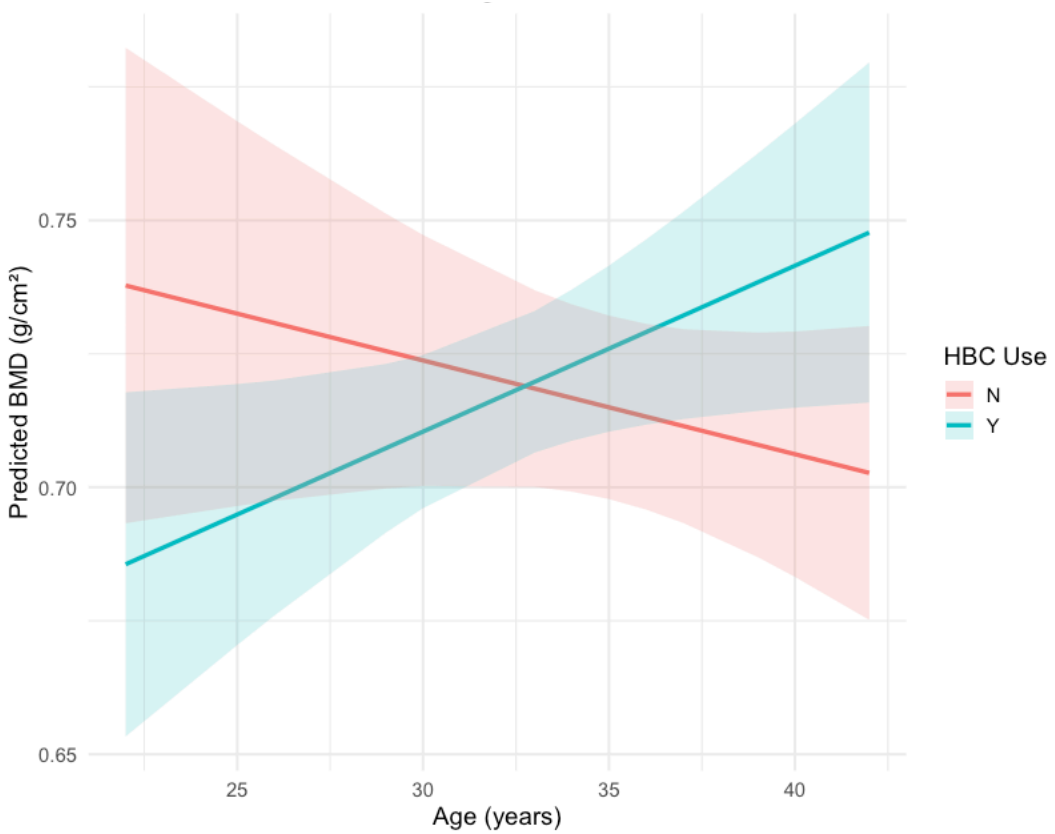

Figure 5.1. Predicted bone mineral density (BMD) as a function of age, stratified by hormonal birth control (HBC) use status. Lines represent estimated marginal means from the ANCOVA model, with shaded bands indicating 95% confidence intervals. A significant interaction between age and HBC use was observed ($p = 0.028$), such that age was positively associated with BMD among HBC users, whereas no such association was observed among non-users.

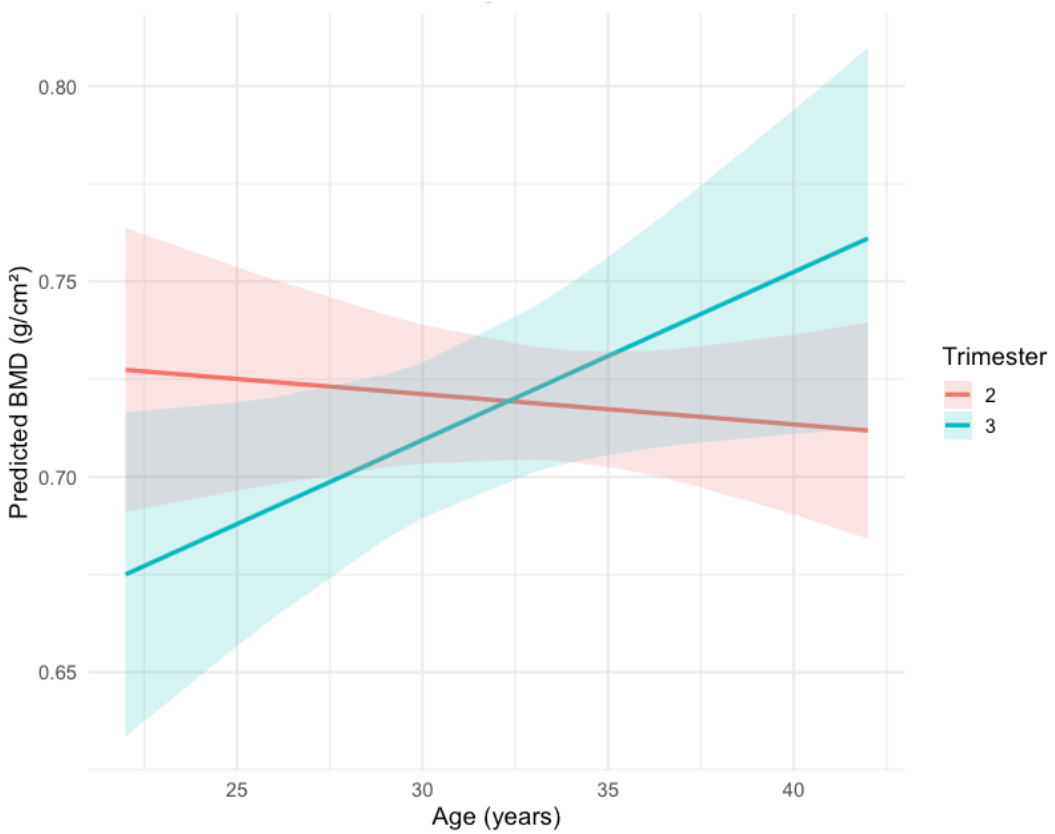

Figure 5.2. Predicted bone mineral density (BMD) as a function of age, stratified by trimester. Lines represent estimated marginal means from the ANCOVA model, with shaded bands indicating 95% confidence intervals. A significant interaction between age and trimester was observed ($p = 0.046$), such that age was positively associated with BMD in the third trimester, whereas no such association was observed in the second trimester.

Discussion

This study investigated physiological predictors, including those related to reproductive health, PA, and sleep, of BMD at the 1/3rd radius position among pregnant individuals. Several relationships between predictive variables and BMD were identified. Our main findings were: i) individuals with longer sleep duration had significantly lower BMD compared to those with shorter sleep duration; ii) age was positively associated with BMD among HBC users, but not non-users; and iii) age was positively linked to BMD in the third trimester but not the second trimester. Understanding the relationship between BMD in pregnancy will help inform strategies to monitor and preserve bone health during this critical life stage. These findings may guide the development

of targeted interventions and recommendations for sleep, reproductive health management, and PA to support optimal skeletal outcome in pregnant populations.

While we did expect to find a relationship between PA and BMD, no such patterns emerged. Previous research found that pregnant individuals who self-reported exercise levels of more than 10 hours per week[17], 4-times higher than current guidelines[15,16] , to have significantly less bone loss across gestation. It should be noted that only n = 3 participants in the present study met/exceeded the current for PA in pregnancy. Thus, further research is required to determine whether the current guidelines are sufficient to have an impact on BMD across gestation.

This study is the first to explore the relationship between BMD and objectively measured sleep parameters in a pregnant cohort. Being in the long sleep duration group was linked to lower BMD compared to the short sleep duration group. Although sleep efficiency, defined as the proportion of time spent asleep relative to time in bed, was assessed, no association with BMD was observed. One possible explanation is sleep quality, which refers to an individual's satisfaction with their overall sleep experience, as a previous study in a premenopausal cohort found that poor sleep quality was linked to lower BMD[13]. It is possible that individuals in the long sleep duration group experienced poorer sleep quality, which may have contributed to their lower BMD. While sleep efficiency did not differ between sleep duration groups and was not linked with BMD, sleep quality may still play a role in BMD. Previous research suggests that the link between sleep quality and BMD may be related to disruptions in endocrine function[22]. Given these findings, sleep duration and quality should be monitored throughout pregnancy to ensure positive outcomes for BMD.

Novel to this investigation was the exploration of the effect of prior HBC use on BMD in the pregnant population. We found a significant interaction between HBC use and age; among

HBC users, age was positively associated with BMD, whereas no such relationship was present in non-HBC users. The most common type of oral contraceptives used in Europe are combined estrogen-progesterone oral contraceptives[23], which work in tandem to maintain bone health[24]. Estrogen is well known for its protective role against bone resorption, while progesterone exerts dose-dependent effects on bone formation. Insufficient progesterone levels may contribute to a decline in BMD[25]. It is plausible that older HBC users benefit from exogenous hormone exposure that contributed to a more favorable endocrine profile, and that even after cessation of use, residual or cumulative effects on estrogen and progesterone balance could promote osteoblastic activity and help preserve bone mass[26]. Future research should investigate the interplay between age, hormonal profile, and BMD in pregnant individuals with a history of HBC use, to determine the potential for lasting effects.

We also investigated the effect of trimester on BMD and determined that in the third trimester, age was positively associated with BMD, but this relationship was not present in the second trimester. This could be due to a shift in the calcium metabolism in the second trimester. Previous research has determined that most of the calcium transfer to the fetus occurs in the third trimester of pregnancy[27] but increased (2-fold) calcium absorption in the intestines starts in the first trimester[28]. As a result, those in the third trimester may benefit from both enhanced intestinal absorption and the heightened mobilization of calcium to support fetal growth. In older pregnant individuals, who may already have more stable skeletal reserves due to a longer life-time exposure to estrogen, this dual mechanism could help preserve maternal bone mass, thereby explaining the positive association between age and BMD observed in the third trimester. In contrast, during the second trimester, calcium demand from the fetus remains relatively modest, and the maternal

skeleton may not experience the same adaptive benefits, leading to the absence of an age–BMD association.

Strengths and Limitations

To the best of our knowledge, this is the first study to investigate objectively measured PA and sleep in a pregnant population. We implemented a robust inclusion/exclusion criterion to reduce any confounding effect of comorbidities on BMD, as well as clearly defined PA guidelines. Furthermore, we used validated tools (i.e., the UltraScan650™) to assess BMD at the 1/3rd distal radius position. The limitations of this study include the sample size (n = 22), reducing the statistical power; as such, results from the present study should be interpreted with caution. The cross-sectional nature of the study design does not allow for causal inference. While height and weight were assessed at periodic check-ups with a physician, the self-reported nature of these variables, supplementation use, and reproductive history variables are subject to recall bias. Lastly, given all participants were healthy, the findings from this study cannot be generalized to clinical populations.

Conclusion

This study offers new insights into factors influencing bone health during pregnancy, identifying associations with sleep duration, prior HBC use, and age across trimesters. Specifically, longer sleep duration was linked to lower BMD, age was positively associated with BMD among HBC users but not among non-users, and age-related increases in BMD were observed in the third trimester but not in the second trimester. These findings highlight the need to account for reproductive history, sleep patterns and gestational timing when monitoring skeletal health in pregnancy.

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Chapter 6 : Investigating bone health throughout the menopause transition

Preamble

Building on the previous chapters that examine reproductive health, physical fitness, and bone health during the early stages of the reproductive life cycle, the final chapter of this thesis focuses on the menopause transition. The menopause transition is a period marked by unmatched hormonal shifts, as well as physiological changes to the musculoskeletal system. This chapter will explore the relationship between biochemical markers (e.g., FSH, calcium) and their effect on bone mineral density across the menopause transition. While it would have been ideal to perform this study in a CAF population, due to insufficient access to female CAF members during their menopause transition, the present study was conducted in a civilian population.

Study title: Calcium, FSH, and the Changing Bones of Menopause: A Longitudinal Look at the Silent Transformation

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All authors confirm their authorship. J.L.P. and V.M.R.W. contributed to the writing and analysis of the manuscript with direction from all authors. J.L.P., E.D., and K.B.A conceived the study and were in charge of direction and planning. All authors discussed the results, reviewed the manuscript and approved the final version.

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Abstract

Introduction: Osteoporosis poses a health risk to post-menopausal females. The menopause transition is associated with hormone fluctuations (e.g., follicle-stimulating hormone (FSH), estrogen), and other biochemical and physiological changes that contribute a decline in bone mineral density (BMD). This study will investigate the relationship between biochemical and physiological markers of BMD across the menopause transition.

Methods: Fifty-six middle-aged females who completed their menopause transition were included in this longitudinal sub-analysis. Participants were recruited from the Ottawa/Gatineau region. Menopause status was classified using menstrual history and FSH plasma levels. BMD and body composition were assessed using dual-energy x-ray absorptiometry (DXA). Additional measures included BMI, cardiorespiratory fitness (VO_{2max}), diet calcium and Vitamin D, and biochemical markers (i.e., FSH, cortisol, calcium). Statistical analysis included repeated measures analysis of variance, linear mixed modeling, and group-based trajectory modeling to identify distinct FSH trajectory groups and their associations with BMD changes over time.

Results: Serum calcium was negatively associated with BMD at the lumbar spine [β: -0.0799, 95% confidence interval (CI): -0.139; -0.020, p = 0.009]. Further, the high FSH trajectory group experienced a greater decrease in BMD across the 5-year period, at the femur neck [β: 0.067, CI: 0.050; 0.084 *vs.* β: 0.035, CI: 0.021; 0.048; p = 0.003] and total body [β: 0.045, CI: 0.035; 0.055 *vs.* β: 0.028, CI: 0.020; 0.036; p = 0.007] than the low FSH trajectory group.

Conclusion: Associations between serum calcium and lumbar spine BMD, and a greater BMD decline in individuals with high FSH levels were revealed. This information suggests that serum calcium and FSH should be monitored throughout the menopause transition to protect BMD.

Keywords: Menopause, bone mineral density, follicle-stimulating hormone, calcium

Introduction

Osteoporosis, characterized by a bone mineral density (BMD) 2.5 standard deviations below that of a healthy sample of the same sex and ethnicity, affects 5.6-10.5% of Canadians over 50 years of age, with female individuals being 5-times more likely to experience low BMD compared to their male-peers. [1] In 2007, osteoporosis-related treatment costs exceeded $3 billion in the Canadian Healthcare system. [2] The menopause transition is a key driver of BMD decline and fracture risk, [3] marked by shifts in follicle-stimulating hormone (FSH) and estrogen [4, 5] – markers that could help identify individuals at greater risk for low BMD and fracture. Estrogen supports skeletal health by lowering bone resorption while preserving bone formation; [6] however, serum estradiol levels drop by approximately 85-90%, accelerating BMD loss in late perimenopause. [7, 8] During the early stages of the menopause transition, serum FSH levels increase independently of estradiol levels, [5, 9] and are positively correlated with markers of bone resorption (e.g., c-terminal peptide of β-I collagen), further contributing to the decline in BMD. [10]

Calcium metabolism is primarily regulated by parathyroid hormone (PTH). Estrogen deficiency has been associated with higher PTH levels, which may disrupt calcium homeostasis. [11] Given the observed inverse relationships between PTH, FSH, and BMD, these hormonal patterns suggest possible alterations in bone metabolism that contribute to low BMD. Although FSH activates pathways responsible for bone remodeling and PTH regulates calcium metabolism, high levels of these hormones likely reflect reduced ovarian estrogen production and may play a role in bone remodeling dysfunction. [12] Thus, reproductive health hormones and their relationship with bone health should be considered during the menopause transition.

Fluctuations in critical bone health markers, such as vitamin D and calcium, influence skeletal outcomes across the female reproductive lifespan. [13] Postmenopausal individuals,

particularly those with a history of osteoporosis and fractures, often experience vitamin D deficiency, [14] which is of concern as its active form, 1,25(OH)2D, is vital for the absorption of calcium *in vivo.* Calcium supports skeletal integrity via intestinal absorption, renal absorption, and bone remodeling, [15] making it an important marker for detecting low BMD. Body composition and cortisol also influence BMD. [16] Typically, during the menopause transition there are gains in fat mass and losses in lean muscle mass. [17] A recent systematic review and meta-analysis in adults, including pre- and postmenopausal women, found that a higher body mass index (BMI) correlates with higher BMD and improved bone microstructure. [18] Similarly, a prior study demonstrated that female individuals in the top tertile of body weight experienced bone loss 35-55% slower than those in the bottom tertile, highlighting BMI's protective role during menopause. [7] Cortisol has also been inversely related to femoral neck BMD post-menopause; [19] however, this relationship has yet to be investigated across the menopause transition. Mechanical loading through physical activity (PA)/exercise promotes bone formation, [20] and across the menopause transition, leisure time PA has been found to mitigate the decline of BMD. [21] Despite the well documented benefits of PA, the menopause transition was been linked to a decrease in energy expenditure [22] with a small minority of females meeting PA guidelines. [23] As such, exploring the relationship between additional biochemical and physiological factors related to bone health is important for the early detection and/or prevention of osteoporosis and related fractures during the menopause transition.

Several longitudinal studies have explored the relationship between plausible predictors and changes in BMD across the menopause transition. For example, a study exploring the relationship between FSH and BMD found that baseline and follow-up FSH levels were significant predictors of BMD at the lumbar spine and total hip [24] and a separate study in the same cohort identified that a higher body weight slowed the rate of BMD loss over a 4-year period. [7] For those

experiencing their menopause transition, calcium absorption fell across the transition without changes to vitamin D metabolites and PTH [25] and those with higher serum calcium at baseline were more likely to develop osteoporosis in a 10-year period. [26] A previous cross-sectional and prospective study found that cortisol levels correlate with BMD at the total femur, femur neck, and lumbar spine but did not influence the rate of change of BMD across the early postmenopausal years. [27] Given this information, the longitudinal impact of these factors should be explored during the menopause transition.

Given the substantial hormonal changes occurring in the transition from pre- to post-menopause - including rising FSH levels - it is essential to understand how these changes affect bone health. While factors like body composition, cortisol, vitamin and calcium are also known to influence bone health, the specific relationships between these factors and BMD during menopause are not fully understood. Therefore, this study aims to examine the relationship between FSH, biochemical and physiological factors (*i.e.,* vitamin D, calcium, cortisol, PA, and body composition) and bone health across the menopause transition. It is suspected that serum FSH concentration will predict changes in BMD during menopause. Further, we expect vitamin D, calcium, body composition and PA to have a positive and cortisol to have a negative impact on BMD during the menopause transition. This study seeks to provide insights into mitigating osteoporosis risk among the post-menopausal population. To the best of our knowledge, no previous study has investigated the effect of cortisol on bone mineral density across the menopause transition.

Methods

Participants

Healthy premenopausal female participants (n=102) between the ages of 47-55 years were recruited to the 5-year Montreal-Ottawa New Emerging Team (MONET) longitudinal study (2004 – 2009). The MONET study aimed to investigate the effects of the menopause transition on body composition and cardio metabolic risk factors. For this sub-analysis we included participants (n = 56) who completed their menopause transition within the 5-year window of the study. Participants were recruited using via recruitment materials in the community and referrals from OB/GYN clinics. This study was approved by the University of Ottawa and Montfort Hospital ethics committees (H 06-03-07) and all procedures adopted were in accordance with the Declaration of Helsinki. All participants provided their informed consent prior to participating in the study.

Participants were included in the main MONET study if they met the following criteria: 1) premenopausal status determined by two menstruations in the last 3 months, no increase in cycle irregularity in the last year prior to testing, and a plasma FSH level of <30 IU/L; 2) aged between 47 and 55 years; 3) non-smoker; 4) no surgically induced menopause; 5) BMI between 20 and 29 kg/m^2; and 6) reported weight stability (± 2 kg) for 6 months of or greater prior to enrollment in the study. Exclusion criteria were: 1) pregnant or planning to become pregnant; 2) medical issues that could potentially impact outcome variables including cardiovascular and/or metabolic diseases; 3) taking oral contraceptives or hormone therapy; 4) high risk for hysterectomy; and 5) history of drug or alcohol abuse.

Design

This sub-analysis employed a 5-year observational design, all variables were measured yearly, except for VO_2max [year 1 (n=54), 3 (n=23), and 5 (n=50)]. The study consisted of several visits, in year 1 two screening visits (3-4 hours each), and 3 half-day visits (3-6 hours) were conducted. For standardization purposes, all measurements were performed in the early follicular

phase (within 8 days) if the participants still had a regular cycle. Participants were contacted periodically by telephone and postcards throughout the year to establish significant changes concerning their menstrual cycle. The study design for the for the main-analysis have been described previously. [28]

Menopausal status

Menopause status was determined by way of self-report questionnaire about menstrual bleeding and regularity. Plasma FSH levels were measured during the early follicular phase of the menstrual cycle at the yearly visit and used to verify menopause status. During the perimenopause/postmenopause phase, FSH levels were measured at the annual follow-up visits and not synchronized with menstrual cycle timing. Individuals were classified as: premenopausal if they reported no change in menstrual frequency; perimenopausal if they experienced changes in menstrual frequency and/or amenorrhea for 3 to 11 months; and postmenopausal based on reporting their final menstrual period, amenorrhea for 12 months, and confirmed with an FSH >30 IU/L. [29]

Anthropometric assessments

Height and weight of the participants were measured using Tanita HR-100 height rod and BWB-800AS digital scale, respectively. BMI (kg/m^2) was calculated based on height and weight. Body composition and BMD were measured using dual-energy x-ray absorptiometry (DXA) GE-Lunar Prodigy module; GE Medical Systems, Madison, WI), as previously described, [30] The coefficient of variation and the correlation for percentage fat mass (FM) measured in of our 12 healthy participants test in the laboratory were 1.8% and r = 0.99, respectively. Additionally, the coefficient of variation for measuring BMD via DXA was <1%.

Cardiorespiratory Fitness (VO_2max)/Physical activity

A treadmill exercise test that consisted of 3-minutes stages, with progressive increases to the grade, was used to assess the VO_2max of participants. Apart from an explanation/safety instruction prior to the test, participants did not receive coaching or advice from the research team on physical activity across the course of the study. Participants were asked to refrain from: 1) any vigorous exercise for 24 hours; 2) alcohol consumption for 6 hours; 3) eating for 2 hours; and 4) drinking coffee for 2 hours prior to the test. After a warm-up, participants took part in the testing protocol. As per ACSM guidelines, [31] the test was terminated when two of four criteria where achieved: 1) predicted maximal heart rate was achieved; 2) respiratory quotient was above 1.1; 3) oxygen consumption remained stable or decreased with increased workload; or 4) rate of Borg-type scale[32] reached 19 or higher. Heart rate and Borg scale were taken at the end of each stage and rest. Breath-by-breath samples of expired air were collected through a mouthpiece during the test and measures (e.g., respiratory exchange ratio) were made automatically using the Vmax 229 series metabolic cart (SensorMedics Corporation, Yorba Linda, CA). The indirect calorimetry unit was calibrated according to manufacturer's instructions.

Blood Sampling

Fasting blood samples were taken after a 12 hour overnight fast. Serum FSH was measured using an automated immunoassay analyzer, the Beckman Coulter Dxl Unicell 800 (Beckman Coutler, Brea, CA). Measures of blood profile presented a coefficient of variation less than 15%. Serum cortisol and calcium were assessed using standardized diagnostic techniques employed by Montfort Hospital, Ottawa.

Dietary

Daily energy consumption/macronutrient intake was assessed via a food diary over 7 consecutive days, which is considered a valid assessment of long-term habitual dietary intake. [33]

Participants were provided with oral and written instructions on recording their food intake. Data were analyzed with FOOD PROCESSOR SQL software version 10.8 including the Canadian nutrient file 2007. Participants were also asked to report if they were taking calcium/vitamin D supplement; however, due to a high degree of variance in supplement type/quantity, this information was not analyzed.

Statistical Analysis

R statistical software for Mac was used to perform statistical analyses. Variables, at all 5 time-points, were assessed for normality, and those that were predominantly non-normal over the 5-year period (i.e., non-normal in 3 or more years) are presented as median and interquartile range in Table 1. Two-way repeated-measures analyses of variance (ANOVA) were used for the determination of the main effect on variables of interest, with year (i.e., years 1 to 5) as a within-subject factor and menopause status (pre-menopause, perimenopause, and post-menopause) as a between-subjects factor. ANOVAs are sufficiently robust for the analysis of non-normal data, with no normalization of the data. [34] Predictor variables that were significantly different between time-points (i.e., FSH, cortisol, blood and diet calcium) were built into linear mixed models using a backward elimination model. Only significant variables remained in the model.

Group-based trajectory modelling was used to identify distinct trajectories of FSH levels over time. Models with two and three latent trajectories were explored with time-point as a predictor. A gamma distribution was specified to account for the non-normal distribution of FSH levels. The model with two trajectories was chosen based on comparing the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC) values. Despite AIC suggesting three latent trajectories was preferable, the difference was <6, indicating a weak preference. Thus, two latent trajectories were selected due to BIC favouring this model. Posterior probabilities from the

selected model were extracted to assign each participant to the trajectory group with the highest probability. To examine differences in the BMD across the identified FSH trajectory groups, linear mixed models were fitted. Each model included group, time-point, and their interaction as fixed effects, repeated measures were accounted for. The significance of main effects and interactions were assessed. $p < 0.05$ was considered significant for all statistical analyses.

Results

Table 6.1 presents the participant characteristics by time-point and a repeated measures analysis of variance was performed to determine areas of significance in biochemical and physiological variables.

Table 6.1. Participant characteristics, time-point, and menopause status were assessed by way of repeated measures analysis of variance.

	Year					Repeated measures ANOVA		
	1	2	3	4	5	Time (y)	Status	Interaction
Premenopause, n	56	12	4	0	0			
Perimenopause, n	0	44	39	21	0			
Postmenopause, n	0	0	13	33	56			
Age, years	50.3±2.0	51.4±2.0	52.5±2.0	53.5±2.0	54.5±2.0			
BMI, kg/m^2	23.3±2.3	23.2±2.4	23.5±2.6	23.6±2.7	23.5±3.0	NS	NS	NS
FM, kg	19.3±5.3	19.0±5.6	19.7±5.9	20.4±6.3	20.8±6.8	NS	NS	NS
LBM, kg	38.0±4.0	38.3±4.0	38.2±3.7	38.1±3.9	38.0±3.5	NS	NS	NS
FFM, kg	40.3±4.3	40.7±4.2	40.6±4.0	40.3±4.2	40.5±3.8	NS	NS	NS
FSH, IU/L	8.0(6.5)	21.5(19.5)	44.0(68)	75.0(39)	85.0(30)	<0.05	<0.001	NS
Cortisol, nmol/L	414±125	396±120	353±119	318±121	297±100	<0.001	NS	NS
Calcium, mmol/L	2.21±0.090	2.19±0.085	2.30±0.091	2.32±0.092	2.37±0.091	<0.01	NS	NS
Diet calcium, mg	888(369)	766(453)	739(305)	700(416)	668(392)	<0.05	NS	NS
Diet vitamin D, µg	3.38(3.47)	3.41(3.62)	3.67(3.02)	3.38(2.91)	3.65(3.22)	NS	NS	NS
VO_{2max}, mL/kg/min	33.4±6.35	-	33.3±6.90	-	32.5±6.34	NS	NS	NS
PAEE, kcal/day	824(324)	785(279)	767(403)	770(292)	791(264)	NS	NS	NS
Outcome								
Femur neck, g/cm^2	0.944±0.107	0.941±0.111	0.934±0.110	0.917±0.109	0.899±0.104	<0.001	NS	NS
L2-L4, g/cm^2	1.20±0.147	1.20±0.148	1.18±0.143	1.15±0.150	1.12±0.148	<0.001	NS	NS
Total body, g/cm^2	1.16(0.110)	1.17(0.110)	1.15(0.095)	1.15(0.110)	1.13(0.110)	<0.001	NS	NS

FM – Fat mass, NS – no significance, PAEE – Physical activity energy expenditure, LBM – Lean body mass, FFM – fat free mass

Linear mixed models, accounting for repeated measures, revealed no significant associations between predictor variables and bone mineral density at the femur neck and total body. However, serum calcium was significantly associated with BMD at the lumbar spine [β: -0.080, 95% confidence interval (CI): -0.139; -0.020, p = 0.009].

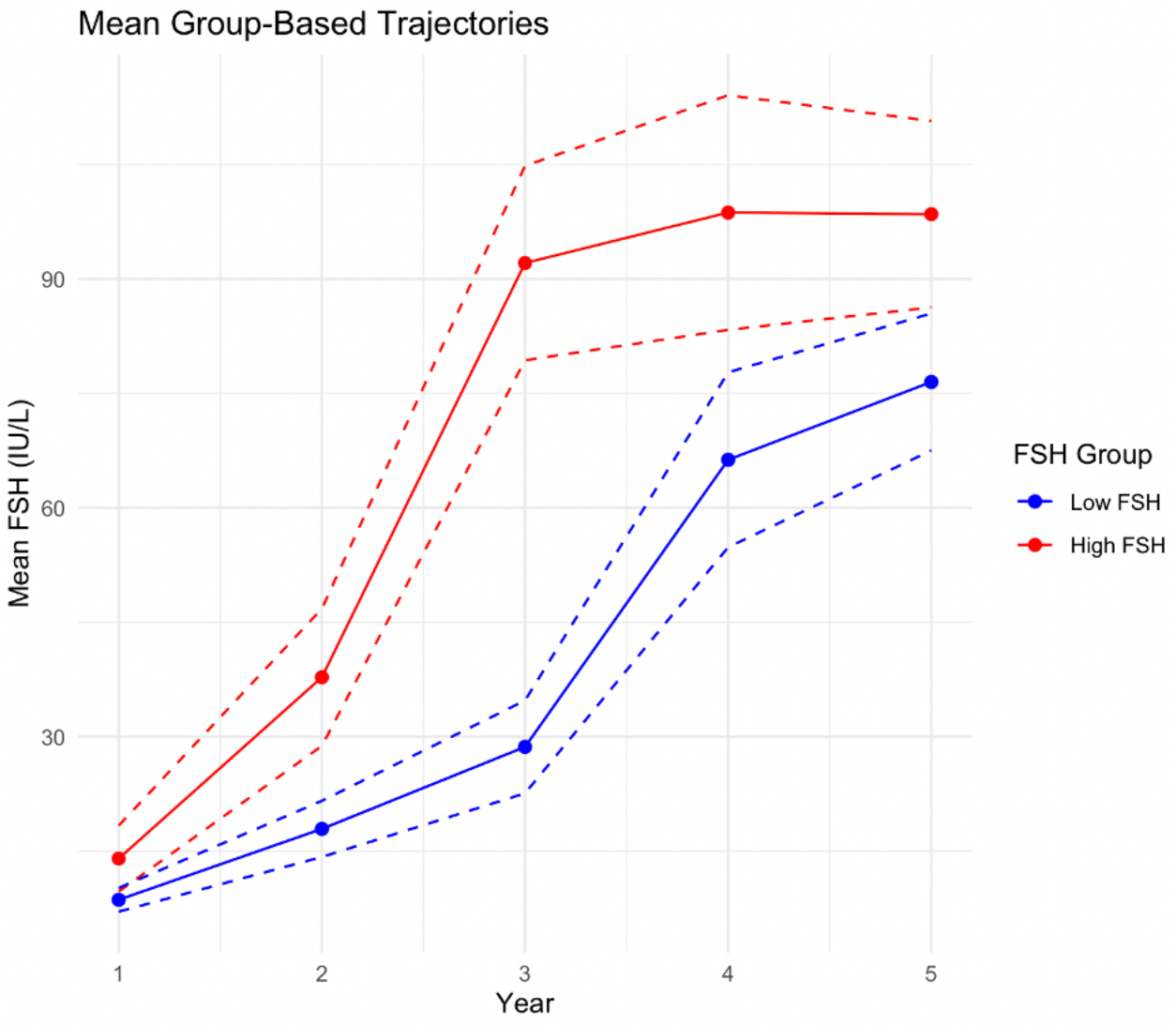

Figure 6.1. Follicle stimulating hormone levels of each trajectory group, with High FSH being n = 21 and Low FSH being n = 35. The dotted lines represent the 95% confidence interval.

At each skeletal-site (i.e., femur neck, lumbar spine, and total body), in both year 1 and year 5, no significant differences in BMD were found between the High FSH and Low FSH

trajectory groups (**Figure 6.1**). Highlighted in **Table 6.2**, BMD at the femur neck and total body decreased significantly more across the 5 year-period in the High FSH group.

Table 6.2. Comparing Estimated Mean Differences (95% CI) in bone mineral density between across the menopause transition from year 1 to year 5 by group.

Trajectory class group	High FSH	Low FSH	p-value
Femur Neck BMD (g/cm^2)			
Year 1 – Year 5	0.067 (0.050; 0.084)	0.035 (0.021; 0.048)	**0.003**
Lumbar Spine BMD (g/cm^2)			
Year 1 – Year 5	0.096 (0.069; 0.123)	0.079 (0.057; 0.100)	0.304
Total body BMD (g/cm^2)			
Year 1 – Year 5	0.045 (0.035; 0.055)	0.028 (0.020; 0.036)	**0.007**

BMD – bone mineral density, FSH – follicle-stimulating hormone

Discussion

To our knowledge this is the first longitudinal study to follow a single cohort of females across the menopausal transition, examining the relationship between follicle-stimulating hormone (FSH), a central reproductive hormone, and a range of biochemical and physiological factors (i.e., vitamin D, calcium, cortisol, physical activity, and body composition), in relation to bone health. Our major findings are two-fold: i) serum calcium levels demonstrated a significant inverse association with BMD at the lumber spine; and ii) although no significant differences in BMD were observed at baseline or after 5 years between participants categorized into high *vs.* low FSH trajectory groups, the rate of decline in femur neck and total body BMD over the 5-year period was significantly greater in the high FSH group.

The observed negative association between serum calcium levels and lumbar spine BMD, despite decreases in dietary calcium intake, aligns with the limited but growing body of research in serum, [35, 36] suggesting a complex interplay between calcium homeostasis and bone metabolism. Similar to serum, urinary calcium levels have been reported to be higher in post-menopause than

in pre-menopause. [25] One study, using both adjusted (e.g., age, sex, and ethnicity) and unadjusted models, identified a negative association between serum calcium levels and lumbar spine BMD, as well as a positive association between vitamin D levels and lumbar BMD. [35] Additionally, a longitudinal study reported that those with higher baseline serum calcium levels were more likely to develop osteoporosis after 10 years, regardless of PTH levels. [36] While calcium is essential for bone health, elevated circulating calcium levels may reflect compensatory bone resorption, possibly driven by PTH dysregulation. Yet, the absence of a relationship with PTH observed in the longitudinal study points to other mechanisms by which calcium may affect bone metabolism, such as alterations in vitamin D metabolism or estrogen-dependent pathways involved in intestinal calcium absorption being diminished due to exposure to an estrogen-depleted state. [25] Estrogen normally enhances calcium absorption through upregulation of intestinal calcium transporters, maintaining calcium homeostasis and promoting skeletal retention. During menopause, these pathways are blunted; however, increased bone resorption may lead to the observed higher calcium serum levels, despite reduced intestinal absorption efficiency. Further, vitamin D is vital for calcium absorption and bone remodelling, and previous studies have reported a high prevalence of vitamin D deficiency among postmenopausal women with a history of osteoporotic fractures. [14] Insufficient levels of vitamin D may exacerbate bone loss at the lumbar spine by impairing calcium absorption, leading to high circulating calcium levels. These findings highlight the need to clarify these mechanisms including the potential impact of vitamin D and calcium supplementation on bone health.

The accelerated decline in femur neck and total body BMD within the high FSH trajectory group is in line with previous research exploring the relationship between FSH and bone health. [10, 24, 37] A longitudinal study by Sowers *et al.* found that FSH at baseline and in subsequent years

was predictive of BMD at the lumbar spine and total hip [24] and other studies have found that decreases in BMD correlated to FSH, independent of estrogen. [5, 9, 38] These consistencies in results underscore the importance of monitoring FSH levels throughout menopause, as elevated FSH levels may signify greater bone loss and thus, higher risk of fractures. Given these implications, counter measures such as hormone therapy and resistance exercise should be considered as potential strategies to mitigate bone loss across the menopause transition.

Unexpectedly and contrary to current literature, [17] there were no changes in PA, cardiorespiratory fitness, or body composition across the menopause transition in the present subsample. Despite a lack of meaningful change in these common predictors of BMD, there was still a reduction in BMD all 3 skeletal-sites (i.e., femur neck, lumbar spine, and total body). The results of the present study indicate that maintenance of PA and body composition alone are insufficient for the preservation of BMD during the menopause transition. Further, it is possible that biochemical markers such as FSH and serum calcium are better predictors of BMD across the menopause transition.

The present study has many strengths including its longitudinal data collection period. To the best of our knowledge, this is the first study to examine BMD at multiple skeletal-sites across the same healthy cohort from pre- to post-menopause. An objective measure (i.e., FSH) was used to determine menopause status, and the study employed the use of linear mixed-models, which account for randomized effects. The cohort examined was entirely Caucasian [39] and inclusion/exclusion criteria aimed to reduce the risk of confounding factors (e.g., smoking, BMI, age). While the sample is a strength, it also presents as a limitation, as the homogeneity of the sample reduces the study's generalizability. Further, the sampling method is subject to selection bias as participants who self-select to participate in the study may be more health-conscious

relative to the general population. The sample size of 56 participants who completed their menopause transition during the study period may have reduced statistical power, increasing the risk of Type II errors. Although dietary intake of vitamin D and calcium were accounted for, other dietary factors were not considered in the analysis.

Conclusion

This study examined the relationship between FSH, biochemical and physiological factors (i.e., vitamin D, calcium, body composition, PA, and cortisol) and bone health across the menopause transition. Significant associations between serum calcium and lumbar spine BMD, as well as a greater femur neck and total body BMD decline in individuals with high FSH levels were revealed. The findings highlight the complex role of calcium regulation in bone health and suggest that elevated FSH may independently contribute to bone loss. Given these associations, monitoring FSH levels and addressing the calcium and vitamin D metabolism could be crucial for osteoporosis prevention. Future research should explore these relationships in larger, more diverse cohorts to refine osteoporosis prevention.

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Chapter 7 : Discussion and Conclusion

This thesis investigated the complex interplay between female reproductive health, MSKi and BMD in both the CAF and civilian populations. Together, these investigations shed light on the physiological and occupational factors that uniquely impact the health and operational readiness of female CAF members, contributing to broader efforts to increase female representation in the CAF. Across the five interrelated studies, additional variables such as fitness status, biochemical markers, and body composition were explored as potential modifiers of the relationship between reproductive health characteristics and MSKi and BMD outcomes. From this body of work, five key findings emerge:

i) **Reproductive health characteristics are associated with elevated risk of MSKi in female CAF members.** Specifically, those experiencing irregular menstrual cycles had greater odds of RSI, while self-reported experience of endometriosis was linked to a higher chance of acute injury within this population.

ii) **The UltraScan650™ is a reliable and valid method for screening BMD among healthy females when used with a transformation equation.** While not suitable for diagnostic purposes, the device demonstrated good inter-rater and excellent test-retest reliability. Although the device tended to underestimate BMD compared to the DXA, the use of the transformation equation enhances its utility in both clinical and research settings.

iii) **Among active-duty female CAF members, reproductive health was associated with BMD.** Parous individuals who have used HBC exhibited significantly higher BMD compared to their nulliparous peers (regardless of HBC history) and parous

individuals without HBC use. Additionally, VO_{2max} and the estradiol-to-progesterone ratio were identified significant predictors of BMD.

iv) **During gestation, reproductive health, alongside age and sleep parameters, are linked to BMD at the 1/3rd radius.** Age was positively associated with BMD in both HBC-users and those in the 3rd trimester**.** Further, individuals reporting a high sleep duration had significantly lower BMD compared to those with shorter sleep duration.

v) **Serum calcium and FSH were key predictors of bone loss across the menopause transition.** In a civilian longitudinal cohort, elevated serum calcium was predictive of lower lumber spine BMD, while elevated FSH levels predicted greater declines in femur neck and total body BMD over the menopause transition.

Reproductive health and MSKi in female CAF members

At the time of publication in September 2022, chapter 2 represented the first and only study focused exclusively on MSKi among female military members in the Canadian context. [1] This work uncovered critical evidence linking reproductive health characteristics with MSKi risk, specifically, irregular menstrual cycles were associated with RSI, while endometriosis was linked to a higher likelihood of acute injury. These discoveries suggest that gender-free policies within the CAF may be insufficiently equipped to detect and address risks that are unique to female service members. To better support this population, the implementation of longitudinal surveillance mechanisms that monitor both reproductive health factors and MSKi incidence is warranted. Incorporating standardized reproductive health assessments into routine health evaluations could facilitate early detection of those with higher chances of injury based on their reproductive profiles.

Further, epidemiological monitoring should be disaggregated not only by sex but also by reproductive status to improve/develop targeted interventions. At the policy and education level, co-development strategies aimed at reducing stigma and normalizing reproductive health discussions within military healthcare settings could enhance reporting and care-seeking behaviours. These institution-level changes would foster a proactive, evidence-informed approach to mitigating MSKi burden among female CAF members and align health surveillance with the realities of reproductive health across the female lifespan.

Reproductive health and BMD

This thesis further elucidated the effect of reproductive health parameters on BMD across three key populations: i) active-duty female CAF members; ii) pregnant individuals; and iii) females undergoing the menopause transition. In the CAF population, parous members, especially those with a history of HBC use, demonstrated significantly higher BMD compared to their nulliparous or non-HBC using peers. Age was also positively associated with BMD, but only among HBC users. Among the pregnant population, the relationship between age and BMD varied by trimester, with age being positively associated with BMD in third trimester participants, but not in the second trimester participants. These findings suggest that advancing maternal age may confer a protective effect on BMD later in gestation. In the menopause transition study, elevated FSH levels were linked to greater declines in BMD at the femur neck and total body, further emphasizing the role of reproductive endocrinology in bone health.

Together, these results highlight the importance of both endogenous hormonal profiles and exogenous hormone use in shaping skeletal health across the female lifespan. Given these findings, the CAF would benefit from implementing proactive educational and policy measures to support bone health in female members. Educational initiatives should aim to raise awareness about the

role of reproductive hormones on skeletal integrity and their fluctuations across life stages. These initiatives should target active-duty members, veterans, and healthcare providers, emphasizing the value of reproductive health surveillance during routine medical appointments. From a policy perspective, integrating reproductive health and bone screening into standard physical assessments, particularly at major reproductive milestones such as pregnancy, postpartum return-to-work, and the menopause transition, would enable early detection of bone loss. Further, deploying validated, low-cost, radiation-free technologies such as the UltraScan650™ could improve accessibility to bone health monitoring without overburdening clinical resources. Collectively, these strategies would help mitigate long-term osteoporosis risk, strengthening health surveillance, and foster career longevity among female CAF personnel.

Physical fitness/activity and BMD

Reproductive health is not the only influencer of BMD, there are interactions with factors such as physical fitness that impact bone health. In chapter 4, it was revealed that a higher estradiol-to-progesterone ratio and superior cardiorespiratory fitness (VO_{2max}) were positively associated with BMD. Additional regression analysis demonstrated that better cardiorespiratory fitness, higher relative bench press, and longer service duration were all positively linked to BMD. This information underscores the dual importance of hormonal regulation and mechanical loading to stimulate skeletal maintenance.

During pregnancy, physical activity did not appear to be associated with BMD. Similarly, across the menopause transition, maintaining physical activity and physical fitness levels did not significantly impact BMD. These observations give rise to several considerations:

i) Physical activity may have a diminished impact on BMD during times of transition in the female reproductive lifespan;

ii) Maintaining physical fitness alone may not be sufficient to preserve BMD across the menopause transition, indicating a potential need for targeted intervention;

iii) Monitoring hormonal profiles during reproductive transitions may be equally, if not more, important than lifestyle factors in maintaining skeletal integrity.

These findings suggest that optimizing bone health in female individuals, particularly during key reproductive transitions, requires a multifaceted approach that integrates both lifestyle and endocrine monitoring strategies.

To address the multifactorial influences on BMD during key reproductive transitions, a coordinated approach involving education, policy, and lifestyle interventions is warranted. particularly those that emphasize the strategic roles of physical activity and physical fitness while recognizing their limitations. While this thesis does not provide sufficient evidence to revise the current physical activity guidelines during pregnancy (i.e., 150 minutes of moderate physical activity per week), [2,3] there is evidence that more is necessary in non-pregnant populations to reap the chronic disease reduction risk, maintaining cardiorespiratory fitness through a combination of aerobic and resistance training is advisable for bone health. [4] During the menopause transition, physical activity alone may not prevent bone loss, particularly when aimed at weight maintenance/weight loss was linked to a greater decline in BMD at the hip. [5] As lean mass loss is common during this period and closely tied to BMD reductions, [6,7,8] interventions should prioritize muscle mass preservation. Accordingly, healthcare providers in both military and civilian settings should routinely prescribe resistance and cardiorespiratory training to stave off bone loss and reduce postmenopausal fracture risk.

Vitamin D, Calcium and Bone health across the menopause transition

Calcium metabolism is a vital aspect of bone maintenance. This thesis identified that calcium serum levels were inversely associated with BMD at the lumbar spine during the menopause transition, suggesting an impairment in calcium regulation. One possible explanation is insufficient vitamin D, which may hinder intestinal calcium absorption and utilization, particularly relevant given the high prevalence of vitamin D deficiency in the postmenopausal population. [9] Educational initiatives aimed at the female population should highlight the importance of vitamin D supplementation for lifelong bone health. Moreover, evidence indicates that, co-administration of vitamin D and calcium during menopause may reduce fracture risk. [10] With this information in mind, healthcare practitioners should consider prescribing vitamin D and calcium supplementation to individuals experiencing their menopause transition as a preventive strategy to promote bone integrity and reduce the risk of fracture long-term.

Limitations

This thesis offers valuable insights to inform education, policy, and lifestyle interventions cross both the civilian and military populations. A key limitation is the inability to evaluate bone health and MSKi during pregnancy and menopause specifically within the female CAF population. Given the relevance of these reproductive stages to musculoskeletal resilience, future CAF-based research should dedicate resources to investigating their relationship to bone health and injury risk. Further, policies and initiatives aimed at supporting these groups within the CAF should be grounded in evidence and tailored to their unique needs.

Integration and Practical Implications

Reflecting on the outcomes of all 5 studies collectively, the findings from this thesis converge on several key conclusions:

i) **Reproductive health, particularly endometriosis, menstrual regularity, parity, and hormone exposure, are critical determinants of both MSKi risk and BMD** in both female CAF members and in civilian populations. The relationships are partly mediated by the hormonal fluctuations in estradiol, progesterone, and FSH. As such, hormonal monitoring should be considered as part of injury prevention strategies in female CAF personnel.

ii) **Physical fitness remains a key modifiable predictor of BMD in the premenopausal population,** yet the protective effect may attenuate during major reproductive milestones like pregnancy and menopause, where associations with BMD were not observed. This information underscores the importance of maintaining physical fitness across the lifespan and suggests a potential need to rebalance training toward muscle preservation during transitional periods.

iii) **HBC appears to positively influence BMD among pregnant individuals into the parous period**, possibly due to the sustained estrogen-rich state of pregnancy. As such, the combined effect of parity and HBC use should be considered protective for bone health.

iv) **Serum calcium and FSH are predictors of bone loss across the menopause transition**, reinforcing the need for biochemical surveillance during key reproductive milestones. Future research should explore these relationships during pregnancy.

v) **Portable ultrasound technology (i.e., the UltraScan650) shows promise as a scalable tool for the assessment of BMD** in both clinical and research settings. However, its limitations must be carefully considered based on the population being

assessed. Additional validation studies are needed, in different populations (e.g., male population)

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Appendices

Appendix 1. Questions used to define independent variables in Chapter 2

Questions	Section	Response	Re-categorization	Observation
What was your sex at birth?	2	1. Male 2. Female 3. Intersex 4. Prefer not to answer	1. Female	Only the original category '2' will be included in the analysis.
Did you have children while in the CAF?	3	1. Yes 2. No	N/A	N/A
During your time in service, did you ever take hormones in the form of birth control (e.g., pills, intrauterine device (Mirena)-IUD) or for hormone replacement therapy (menopause purpose)?	9	1. Yes 2. No	N/A	If used only for hormone replacement therapy, 'no' will be considered for analysis.
How old were you when you had your first period?	9	1. 10 years or less 2. 11-15 years 3. 16 years or more 4. I never had a period 5. I don't know	1. 10 years or less 2. 11-15 years 3. 16 years or more	Only categories 1, 2, and 3 will include in the analysis.
During your time in military service, did you have regular periods (~28 days long)?	9	1. Yes 2. No, they were never regular 3. No, they were irregular for a few months 4. No, my periods stopped while I was serving 5. I never had a period	1. Yes 2. No	Categories 2, 3, 4, and 5 will transform into a 'no' category.
Did you have any of the following conditions while serving (select all that apply)?	9	1. Endometriosis a) Yes b) No 2. Menopause before aged 46 (not due to hysterectomy) a) Yes b) No 3. Secondary amenorrhea (cessation of regular periods for 3 months or irregular periods for 6 months) a) Yes b) No	N/A	N/A

Appendix 2. Questions used to define covariates in Chapter 2

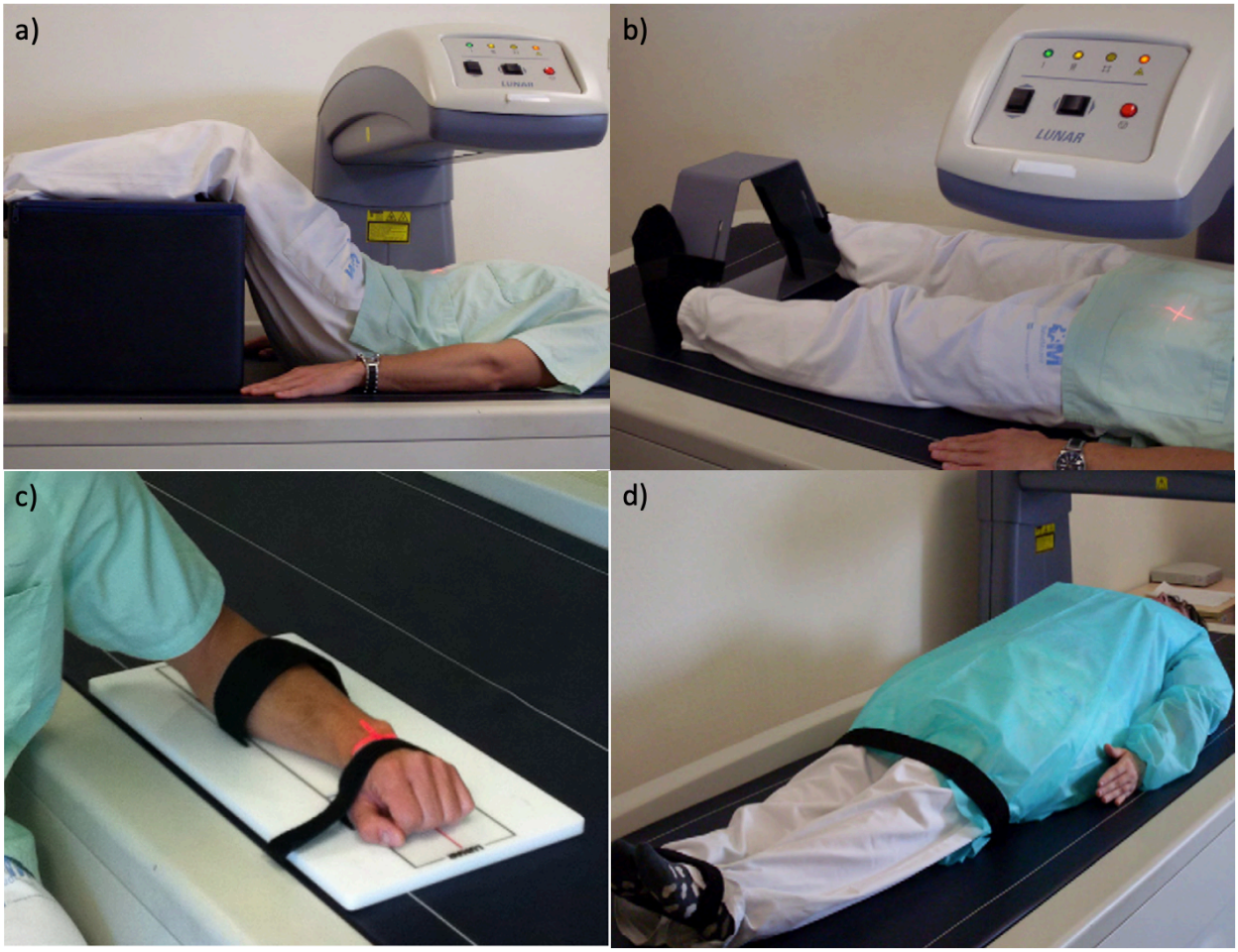
Questions	Section	Response	Re-categorization	Observation
What is your current rank or final rank before leaving the CAF?	1	1. Junior NCM - Pte/OS/AB/Avr 2. Junior NCM - Cpl/LS 3. Junior NCM - MCpl/MS Senior NCM - Sgt/PO2 4. Senior NCM - WO/PO1 5. Senior NCM - MWO/CPO2 6. Senior NCM - CWO/CPO1 7. Junior Officer - OCdt/NCdt - 2Lt/ A/SLt 8. Junior Officer - Lt/SLt 9. Junior Officer - Capt/Lt(N) 10.Senior Officer – Maj/LCdr 11. Senior Officer - LCol/Cdr 12. Senior Officer - Col/Capt(N) - General Flag	1. NCM 2. Officer	Categories 1-6 will merge to create the category 'NCM'. Categories 7-12 were merged to create the category 'Officer'.
What is your current age?	2	1. Fill in the blank	N/A	To be analyzed as a continuous variable.
How many children did you have while in the CAF?	3	1. Biological (fill in the blank) 2. Adopted (fill in the blank)	1. 0 biological children 2.1 biological child 3. 2 biological children 4. 3 or more biological children	N/A
What is your height? Please indicate in inches or centimetres.	4	1. Inches (fill in the blank) 2. Centimeters (fill in the blank)	1. Height (m)	Categories 1 and 2 will transformed into meters and kg, respectively. Then they will used to calculate BMI.
Approximately how much do/did you weigh (not pregnant for female) upon military enrolment and currently or at the end of your military career? Please indicate in pounds or kilograms.	4	1. Currently or at the end of your military career- Pounds: (fill in the blank) 2. Currently or at the end of your military career – Kilograms: (fill in the blank)	1. Weight (kg)	

If yes for any activity below, please indicate how much total time (min) per week you spend:	5	1. Participate in organized or non-organized sports (voluntary), fitness, recreational activities (outside of your occupational demands on the job and non-unit PT time)? (fill in the blank) 2. Use active methods as a mode of transportation (e.g., walking, cycling to work or to the store)? (fill in the blank) 3. Undertake physically demanding household chores (primary or secondary properties) or volunteer work involving physical tasks? (fill in the blank) 4. Does your occupation require you to participate in group physical training and recreational activity sessions? (fill in the blank) 5. Do you participate in CAF lead individual fitness and activity programs (e.g., PSP led PT)? (fill in the blank)	1. Total weekly PA in minutes	Categories 1-5 will re-categorize into a single category. Total weekly PA (mins) will be used as a continuous variable.
Over the past few months, how many times a week did you eat or drink dairy products (e.g., yogurt, milk)?	7	1. 5 or more 2. 3-4 3. 2 or less	N/A	N/A
In general, please estimate your use of nutritional supplements (powders, liquids, bars)? Calcium	7	1. Never 2. Once/Month 3. Once/Week 4. Few times/week 5. Daily	1. Yes 2. No	Categories 2-5 will transform into a single 'yes' category and 1 became 'no'.
In general, please estimate your use of nutritional supplements (powders, liquids, bars)? Vitamin D	7	1. Never 2. Once/Month 3. Once/Week 4. Few times/week 5. Daily	1. Yes 2. No	Categories 2-5 will transform into a single 'yes' category and 1 became 'no'.

Appendix 3. Questions used to define outcome variables in Chapter 2

Questions	Section	Response	Re-categorization	Observation
While serving, did you ever	6	1. Yes 2. No	N/A	N/A

have any injuries that you felt were due to repetitive strain?				
While serving, did you ever have any acute injuries that were serious enough to take at least 24 hours off from work?	6	1. Yes 2. No	N/A	N/A



Appendix 4. Dual energy X-ray absorptiometry. a) Patient's positioning for lumbar spine; b) Patient's positioning for femur; c) Patient's positioning for forearm; d) Patient's positioning for full body. Images taken from Lorente Ramos et al.

Appendix 5. Participant's positioning for UltraScan650™ measurement of the 1/3rd distal radius. Image taken from Stein et al.

Appendix 6. List of complete works and awards during graduate studies.

Awards

Canadian Graduate Scholarship	2023 – 2026
Canadian Institute of Health Research	
PhD Admission Scholarship	2022 – 2025
University of Ottawa	
Médéric Montpetit Travel Award	2024
University of Ottawa	
Michael Smith Foreign Study Supplement	2023
Canadian Institute of Health Research	
CSEP Student Travel Award	2023
Canadian Society for Exercise Physiology	
Special Merit Scholarship	2022 – 2024
University of Ottawa	
Maria and Arthur Roy Scholarship	2020
University of Ottawa	

Abstracts and Conferences

Puranda JL, Edwards CM, Weber VMR, Aboudlal M, Semeniuk K, Adamo KB, Validation of an ultrasound device to measure bone mineral density at the 1/3rd distal radius position in female individuals. American College of Sport Medicine, Boston, United States of America. May 29, 2024.

Puranda JL, Akman A, Edwards CM, da Silva DF, Souza SCS, Miller E, Semeniuk K, Adamo KB, Exploring predictors of bone mineral density at the 1/3rd radius position among female Canadian Armed Forces members. Canadian Society for Exercise Physiology, Calgary, Canada. October 12, 2023.

Puranda JL, da Silva DF, Edwards CM, Nagpal TS, Souza SCS, Semeniuk K, Adamo KB, Sleep characteristics associated with musculoskeletal injuries in female Canadian Armed Forces members. International Congress on Soldiers' Physical Performance, London, UK. September 14, 2023.

Puranda JL, da Silva DF, Darroch F, Edwards CM, Nagpal TS, Souza SCS, Semeniuk K, Mclean L, Adamo KB, Characteristics associated with pelvic floor disorders among female Canadian Armed Forces members. International Congress on Soldiers' Physical Performance, London, UK. September 13, 2023.

Puranda JL, da Silva DF, Souza SCS, Edwards CM, Nagpal TS, Semeniuk K, Adamo KB, Characterizing the associations between reproductive health and musculoskeletal injuries of female members of the Canadian Armed Forces. Interdisciplinary Student Research Conference on Healthcare, virtual conference. May 8, 2021.

Edwards, C.M., da Silva, D., Puranda, J., Souza, S., Nagpal, T., Semeniuk, K., Adamo, K. Between-sex differences of musculoskeletal injury within Canadian Armed Forces Unit Occupation Roles. 7th International NSCA Conference Abstracts, Shanghai, China. 2021.

Edwards, C.M., da Silva, D.F., Puranda, J.L., Souza, S.C.S., Nagpal, T.S., Semeniuk, K., Adamo, K.B. Body Regions Susceptible to Musculoskeletal Injuries in Canadian Armed Forces Pilots: 549. Medicine & Science in Sports & Exercise. 54(9S):p 139, San Diego, CA, United States. 2022. | DOI: 10.1249/01.mss.0000876780.96756.37

Edwards, C. M., da Silva, D.F., Puranda, J.L., Miller, É., Souza, S.C.S., Nagpal, T.S., Semeniuk, K., Adamo, K.B. Does a history of childbirth impact musculoskeletal injury in female military members?. Canadian Institute for Military and Veterans Research, CIMVHR Forum 2022 Halifax, Canada. 2022.

Miller, É., Edwards, C. M., da Silva, D.F., Puranda, J.L., Semeniuk, K., Adamo, K.B. Associations Between Female Reproductive Health and Lower Body Injuries in Female Service Members in

The Canadian Armed Forces. Canadian Institute for Military and Veterans Research, CIMVHR Forum 2022. Halifax, Canada. 2022

Edwards, C. M., da Silva, D.F., Puranda, J.L., Souza, S.C.S., Semeniuk, K., Adamo, K.B. Effects of parity status on physical fitness of Purple Trade members of the Canadian Armed Forces with repetitive strain injury history. Canadian Society for Exercise Physiology, Fredericton, Canada. 2022.

Edwards, C.M., da Silva, D.F., Puranda, J.L., Miller, É., Souza, S.C.S., Nagpal, T.S., Semeniuk, K., Adamo, K.B. Are parity status or injury history related to knee kinematics in a bodyweight overhead squat assessment in military servicewomen? International Congress on Soldiers' Physical Performance, London, UK. 2023.

Miller, É., Puranda, J.L., Edwards, C. M., Semeniuk, K., Adamo, K.B. Associations between body composition and physical performance in female Canadian Armed Forces members. Canadian Institute for Military and Veterans Research, CIMVHR Forum 2023. Gatineau, Canada. 2023.

Publications

Weber VMR, Queiroga MR, Puranda JL, Macdonald ML, Corson AE, da Silva DF, Semeniuk K, Adamo KB (2025, August) What is the optimal level of cardiorespiratory fitness for cognitive outcomes in females: the inverted "U" theory. *International Journal of Sport and Exercise Psychology.* DOI: https://doi.org/10.1080/1612197X.2025.2542550

Edwards CM, Puranda JL, Miller É, , da Silva DF, Semeniuk K, Adamo KB (2025, June) Unmasking the divide: Musculoskeletal injury and physical fitness disparities among military and emergency responders. *Journal of Military, Veteran and Family Health.* DOI: https://doi.org/10.3138/jmvfh-2024-0055

Puranda JL, Edwards CM, Weber VMR, Aboudlal M, Semeniuk K, Adamo KB (2025, January) Validation of an ultrasound device to measure bone mineral density. *Clinical Anatomy*. DOI: 10.1002/ca.24187

Edwards CM, Puranda JL, Miller É, MacDonald ML, Aboudlal M, Adamo KB (2024, October) Low physical fitness indicates future injury, mental health, menstrual cycle disruptions, and burnout in female emergency service personnel and healthcare providers. *Applied Physiology, Nutrition, and Metabolism.* DOI: https://doi.org/10.1139/apnm-2024-0266

Weber VMR, Queiroga MR, Puranda JL, Semeniuk K, MacDonald ML, Dantas DB, da Silva DF, Adamo KB (2024, September) Role of cardiorespiratory fitness, aerobic exercise, and sports practice in female cognition: a scoping review. *Sports Med*. DOI: https://doi.org/10.1186/s40798-024-00776-8

Edwards CM, da Silva DF, Puranda JL, Miller É, Semeniuk K, Adamo KB (2024, June) Are physical fitness, a history of childbirth and injury history related to knee valgus in female Canadian

Armed Forces members during a body weight overhead squat? *BMJ Mil Health.* DOI: 10.1136/military-2023-002653

Edwards CM, Puranda JL, Miller É, Aboudlal M, O'Rourke N, MacDonald ML, Adamo KB (2024, May) Musculoskeletal injuries, exercise behaviors, and reproductive health are related to physical fitness of female first-responders and health care providers. *Women's Health Reports.* DOI: https://doi.org/10.1089/whr.2023.0189

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Edwards CM, Miller É, da Silva DF, Puranda JL, Souza SCS, Semeniuk K, Adamo KB (2023, July) Does a history of childbirth impact injury prevalence and mental health in female military members? *Appl Physiol Nutr Metab.* DOI: https://doi.org/10.1139/apnm-2023-0028

da Silva DF, Edwards CM, Puranda JL, Miller É, Semeniuk K, Adamo KB (2023, June). Sex disparities in self-reported musculoskeletal injuries in the Canadian Armed Forces. *Appl Physiol Nutr Metab.* DOI: https://doi.org/10.1139/apnm-2023-0029

Puranda JL, da Silva DF, Edwards CM, Nagpal TS, Souza SCS, Semeniuk K, Mclean L, Adamo KB (2023, May). Characteristics associated with pelvic floor disorders among female Canadian Armed Forces members. *J. Obstet. Gynaecol.* DOI: https://doi.org/10.1016/j.jogc.2023.05.027

Everest C, da Silva DF, Puranda JL, Souza SCS, Goudreau AD, Nagpal TS, Edwards CM, Gupta R, Adamo KB (2022, October). Physical activity and weight gain throughout pregnancy are associated with umbilical cord markers. *J. Obstet. Gynaecol.* DOI: https://doi.org/10.1016/j.jogc.2022.09.012

Puranda JL, da Silva DF, Edwards CM, Nagpal TS, Souza SCS, Semeniuk K, Adamo KB (2022, September). Association between reproductive health factors and musculoskeletal injuries in female Canadian Armed Forces members. *J Women's Health.* DOI: https://doi.org/10.1089/jwh.2021.0647

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