The influence of sports, energy, vitamin D and calcium intake on bone mineral density in female athletic and sedentary students

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Table of Contents

ABSTRACT

Physical activity, adequate energy, calcium and vitamin D intake all influence bone mineral density (BMD) in young adult females. Unfortunately, it is common to see nutrition deficiencies in this population that may put them at risk for a variety of health issues including lower BMD. ***Purpose:*** To determine the influence of the type of sport on regional and total BMD and its association with dietary energy, vitamin D and calcium intake. ***Methods:*** Seventy-three female students from McGill University were evaluated (average age of 20.8 years old, height 167.4 cm, weight 62.3 kg, and BMI 22.4). Forty-four athletes of 4 different Varsity Teams: basketball (n=13), volleyball (n=11), figure skating (n = 13), and synchronized swimming (n=7); and 29 age-matched sedentary students took part in the investigation. Dietary intake, Vitamin D and Calcium intake were assessed using a 3-day Food Log and a FFQ (short version) then analysed with the Food Processor^{TM} Software. Total Body (TB), lumbar spine (LS) (L1-L4) and femoral neck (FN) BMD were assessed by DXA scanning. A one-way ANOVA explored between-group differences. Linear Regression Analysis, Pearson Correlations and ANCOVA were used to explore the influence of energy, Vitamin D and Calcium intake on BMD. ***Results:*** A significant difference in BMD at all 3 sites was observed across sports (TB = $F(4,72) = 10.2$, $p < .001$; LS = $F(4,72) = 8.6$, $p < .001$; FN = $F(4,72) = 6.3$, $p < .001$). Also, basketball (TB = 2.3 ± 1.2; LS = 1.7 ± 1.53, $p < .001$; FN = 1.7 ± 1.1, $p = .001$) and volleyball (TB = 1.9 ± 1.3; LS = 1.5 ± 1.6, $p = .001$; FN = 1.7 ± 1.7, $p = .002$) players had a significantly higher BMD in all sites compared to their non-athletic counterparts (TB = 0.4 ± 0.9; LS = -0.3 ± 1.2; FN = 0.1 ± 1.0). The figure skating and synchronized swimming athlete's bone densities were not different from the control group ($p = .719$; $p = .246$). No significant association was observed among BMD at all sites and total energy intake, vitamin D and Calcium. There was a significant difference between the delta energy intake (recommended intake minus actual intake) in both BB and SS groups compared to the control group (p = .003 and p = 0.02, respectively). ***Conclusion:*** The type of sport revealed a strong influence on BMD. A significant discrepancy was found between the required versus actual energy intake in some athletes. It is recommended that Sports Dieticians should become part of the multi-disciplinary team that train and work with female varsity athletes.

RÉSUMÉ

L'activité physique, un apport suffisant en énergie, en calcium et en vitamine D ont tous une influence importante sur la densité minérale osseuse (DMO) des jeunes femmes adultes. Malheureusement, les carences nutritionnelles sont fréquentes dans cette population, ce qui les expose à divers problèmes de santé, notamment une réduction de la DMO. ***Objectif:*** Déterminer l'influence du type de sport sur la DMO totale et régionale et son association avec l'apport alimentaire en énergie, en vitamine D et en calcium. ***Méthodes:*** Soixante-treize étudiantes de l'Université McGill ont été évaluées (âge moyen de 20,8 ans, taille: 167,4 cm, poids 62,3 kg et indice de masse corporelle de 22,4). Quarante-quatre athlètes de quatre équipes différentes: basketball (n = 13), volleyball (n = 11), patinage artistique (n = 13) et nage synchronisée (n = 7); et 29 étudiantes sédentaires ont été évaluées selon l'âge. Les apports alimentaires, les apports en vitamine D et calcium ont été évalués à l'aide d'un registre des aliments de trois jours et d'un FFQ (version abrégée), puis analysés à l'aide du logiciel Food Processor™. La DMO totale du corps (TB), de la région lombaire (LS) (L1-L4) et du col fémoral (FN) ont été évalués. Une analyse ANOVA à un facteur a exploré les différences entre les groupes. L'analyse de régression linéaire, les corrélations de Pearson et une ANCOVA ont été utilisés pour explorer l'influence de l'énergie, de l'apport en vitamine D et du calcium sur la DMO. ***Résultats:*** Une différence significative de la densité minérale osseuse aux trois sites a été observée dans tous les sports (TB = F (4,72) = 10,2, p < 0,001; LS = F (4,72) = 8,6, p < 0,001; FN = F (4,72) = 6,3, p < 0,001). Aussi, le basketball (TB = 2,3 ± 1,2; LS = 1,7 ± 1,53, p <0,001; FN = 1,7 ± 1,1, p = 0,001) et le volleyball (TB = 1,9 ± 1,3; LS = 1,5 ± 1,6, p =. 001; FN = 1,7 ± 1,7, p = 0,002) la DMO était significativement plus élevée dans tous les sites par rapport à leurs homologues non sportifs (TB = 0,4 ± 0,9; LS = -0,3 ± 1,2; FN = 0,1 ± 1,0). Les densités osseuses des athlètes de patinage artistique et de la nage synchronisée ne différaient pas de celles du groupe témoin (*p*

= 0,719; p = 0,246). Aucune association significative n'a été observée entre la DMO de tous les sites et l'apport énergétique total, la vitamine D et le calcium dans tous les groupes. Il y avait une différence significative entre l'apport énergétique delta (apport recommandé moins l'apport réel) dans les groupes BB et SS par rapport au groupe témoin (p = 0,003 et p = 0,02, respectivement). ***Conclusion:*** le type de sport a révélé une forte influence sur la DMO. Un écart important a été trouvé entre l'apport énergétique requis et l'apport énergétique réel chez certains athlètes.

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AUTHOR'S CONTRIBUTIONS

A. De la Parra-Sólomon is the principal author of this thesis and collaborated in all aspects of the investigation. She took part in all the recruitment creation, logistics, and planning that involved poster design, social media posts and paper hand outs, emails to different McGill faculties administrations, and presentations to all athletic coaches and their teams. She also followed up by email all female students' interested in participating in the study and developed the calendar for all evaluation dates. She actively screened and evaluated all participants, which involved anthropometric measurements (i.e., height and body weight), dual-energy x-ray absorptiometry (DXA) scanning, and the implementation and explanation of all questionnaires. She collected all data, analyzed and interpreted the research, handed over all results to participants including further nutrition recommendations, and the thesis preparation.

D. Unrau and J. Shaw assisted in some of the participants' evaluations, as well as data collection. S. Beauregard assisted in the statistical analysis, interpretation of data, and on the translation of the abstract to French. And L. Belzile assisted in all the thesis editing.

Dr. Ross E. Andersen, Professor, Department of Kinesiology and Physical Education, McGill University, the candidate's supervisor, was actively involved in the research project design and direction, statistical analysis and interpretation of data, editing of manuscript and guiding the completion of this thesis.

Hugues Plourde Ph.D., RD, Professor, School of Human Nutrition, McGill University, the candidate's co-supervisor, was also actively involved in the research project design and direction, statistical analysis and interpretation of data, editing of the thesis literature review and also guiding the completion of the thesis.

LIST OF TABLES AND FIGURES

1.0 LITERATURE REVIEW

1. Introduction/Rationale

The skeletal system is remarkably active living tissue that serves two primary functions: it provides biomechanical support and protection to soft tissue, and it plays a key role in mineral homeostasis (1). Since bones are metabolically active, they are constantly renewed through a natural process called remodeling, in which new bone cells replace old bone cells (2). This renewal process is critical for bone strength (3). Bone structure and architecture can adapt in response to usage and mechanical loading from external forces as a result of bone remodeling. This adaptation was labeled the "Mechanostat" by Harold Frost in 1996 (4). Thus, physical activity may confer favourable effects on bone mass due to the mechanical loading associated with bone tissue's response to the stimuli generated by physical strength in the bones during exercise (5). Unfortunately, this process becomes increasingly less efficient with age, causing bone tissue to progressively deteriorate (6). Among other systemic factors, estrogen plays a key role in the maintenance of bone tissue homeostasis. Estrogen is responsible for the activation of bone remodeling components; and the suppression of bone resorption via osteoblast and osteocyte apoptosis, which prevents bone resorption and exceeds bone formation by stimulating the latter (1, 7, 8). Though, when slightly less bone cells are deposited than resorbed, it can result in osteopenia and eventually osteoporosis (9). Nevertheless, skeletal health can be affected by a number of factors, including genetics, lifestyle, demographic characteristics, and disease (1).

Osteoporosis is a potentially debilitating disease, and is characterized by an impairment of bone mass and bone health (10). This loss in bone mineral density (BMD) and its associated fragility fractures are a serious public health issue due to their increasing prevalence worldwide, contributing significantly to morbidity, mortality and healthcare spending (3). The economic impact of the disease in the United States, is thought to be approximately $17.9 billion per year, and €37 billion is spent in Europe (11). Within the Canadian health system, the overall yearly cost of treating osteoporosis and fractures caused by this disease was over $2.3 billion in 2010 (12).

Puberty and adolescence are critical periods when skeletal development lays the foundation for bone health in adulthood. As BMD doubles between puberty and young adulthood, bone mass acquisition plays a significant role (13-15). Essentially, the acquisition of a maximal peak bone mass (PBM) is influenced by environmental and genetic factors, in which only physical exercise and nutrition are modifiable (10). As a result, the role of physical activity and nutrition are critical during adolescence and young adulthood.

Weight-bearing activities in conjunction with adequate calcium and vitamin D intake may provide a practically relevant method to improve BMD at all ages, especially during puberty (16, 17). Participation in sports and activity within the age range of growth and skeletal maturity has been found to result in a higher peak bone density later in life (15). Sports that involve high-impact loading (e.g., gymnastics, single figure skating, hurdling, judo, karate, volleyball, and other jumping sports) or odd-impact loading (e.g., soccer, basketball, racquet games, step-aerobics, pair figure skating speed skating) are associated with higher bone mineral composition, BMD, and enhanced bone geometry in anatomic regions specific to the loading patterns of each sport. However, non-impact sports such as swimming, water polo, and cycling may not be associated with improvements in bone mineral composition or BMD (15, 18-22).

Nonetheless, physically active individuals may undertake high training volumes and simultaneously restrict their dietary energy intake. Either in isolation or when combined, these are practices that may put individuals at risk for energy deficiency (23). Consequently, this leads to low energy availability, which is associated with menstrual dysfunction and impaired bone health in female athletes (24).

Nutrition has been found to play an essential role in many aspects of bone health in young female athletes, where calcium and vitamin D are among the key elements that contribute to the maintenance of healthy bones and muscles (25). Adequate calcium intake (1,000 mg/day) has been associated with higher bone mineral composition (BMC) and BMD values (10). Vitamin D plays a key role in optimizing bone development in children, and bone density in young adults (25). Vitamin D status is also indicative of calcium absorption and bone mineralization (26).

Ward et al. (27), documented that calcium intake among adolescent and young adult females is often below the recommended levels (27). Also, a high prevalence of vitamin D deficiency and insufficiency among adults and children is evident worldwide (28-34). Despite these findings, very few relevant studies have focused on athletes due to the lack of current precision on the vitamin D status thresholds for this population, and the accuracy of the quantification methods employed for assessing 25(OH)D concentrations (35-42). Research completed by McClung et al. (43), and Ogan & Pritchett (44), suggests that the prevalence of vitamin D insufficiency among female athletes may range between 33% and 42%, depending on the type of athlete, season, indoor or outdoor training sessions, and geographical latitude (43, 44).

To address the knowledge gap on the impact of the type of exercise and overall nutrition on university athletes' bone health in previous research, this study will focus on the influence of physical activity level, total energy, vitamin D and calcium intake, which are known critical factors of BMD in athletic and non-athletic young adults.

1.1 Purposes

- Primary Aim: Explore the influence of non-impact, odd-impact and high-impact sports among female collegiate students and their sedentary counterparts, on regional (lumbar vertebra and femoral neck) and total body BMD.
- Secondary Aim: Explore the influence among energy availability (delta energy intake), dietary vitamin D and calcium intake with regional (lumbar vertebra and femoral neck) and total body BMD.

1.2 Hypotheses

- **Hypothesis #1:** There will be significant differences in bone health across the 3 different impact sports and the sedentary group on regional and total body BMD.
- **Hypothesis #2:** There will be a significant association among energy availability, dietary vitamin D and calcium intake primarily on regional (lumbar spine and femoral neck) BMD of varsity athletes and sedentary students.

2. Bone Physiology

Our skeleton is a remarkably active living tissue comprised of a myriad of cells, blood vessels, proteins, and minerals (3). It serves two primary functions: it provides biomechanical support and protection of soft tissue, and plays a key role in mineral homeostasis and storage of calcium, magnesium and phosphorus, in particular (1).

During growth and development, skeletal growth undergoes the process of bone modeling when deposition and resorption occurs to allow bones to expand (periosteal apposition of cortical bone) and lengthen (endochondral ossification) into their adult form (45). On the other hand, a complex process defined as remodeling, involves a cycle of three phases where four type of cells (osteoblasts, osteocytes, osteoclasts and bone lining cells) assist on the achievement of this process (Figure 1). 1) The activation of bone resorption by the osteoclasts, 2) the reversal considered a transition phase from resorption to new bone formation, and 3) the formation of bone by the osteoblasts (1, 7). Bone remodeling begins during fetal growth and continues until epiphyseal fusion, usually toward the end of adolescence (45).

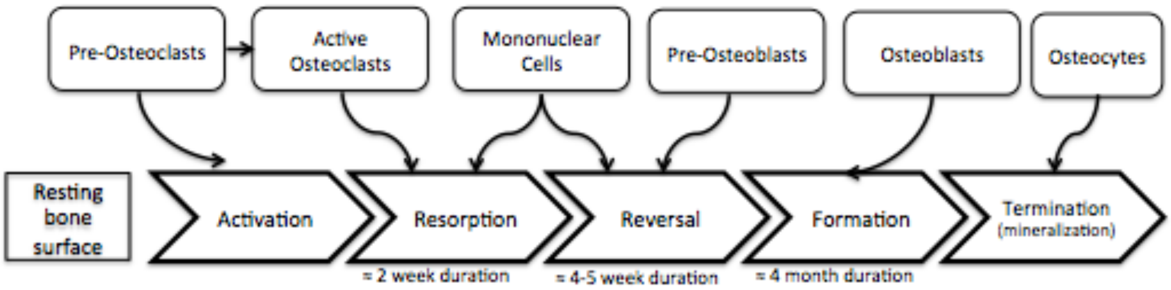

Figure 1. Bone Remodeling Cycle *(Adapted from: Kenkre & Bassett, 2018)*

Bone metabolic functions controlled in part by systemic factors involving calcium-regulating hormones such as parathyroid hormone (PTH), calcitonin (1,25-dihydroxyvitamin D_2) and calcitriol (1,25-dihydroxyvitamin D_3), and estrogen, among others. Basically, they play an essential role in the maintenance of bone tissue homeostasis. PTH helps to control calcium concentrations in its active form. Vitamin D hormones assist on the preservation of calcium and phosphate intestinal absorption, which are then distributed for bone mineralization. And estrogen stimulates the suppression of osteoblast and osteocyte apoptosis (programmed cell death), and impedes that bone resorption exceeds bone formation (1, 7). Considering that bone modeling is promoted and is sensitive to mechanical loading, physical activity throughout growth is imperative (45). For example, a female basketball player may develop greater bone mass in her legs compared to a non-athletic female, due to an increase in bone strength caused by the percussive nature of the sport (46).

Between the ages of 18 and 25 years, the formation of new cells occurs, and bone continually renews itself through bone remodeling (1, 2, 47). Remodeling takes place at different rates in different parts of the body. Even when bones reach their adult shape, the old bone is repeatedly broken down and new bone forms in its place; this clearly demonstrates that bone is a living metabolising organ (2, 47).

The renewal of bone is responsible for bone strength (3). However, this process becomes progressively less efficient with age, causing bone tissue to gradually be lost (6). When slightly less bone is deposited than resorbed, it results in osteopenia and eventually osteoporosis (9). Bone health can be affected by diverse factors, such as genetics, lifestyle, demographic characteristics, and disease (1). The following section will review some of these factors in greater detail.

3. Pathology, Prevalence, and Economic Impact of Osteoporosis

Osteoporosis is a potentially debilitating disease within our society. It is characterized by a bone loss that occurs more rapidly than normal, causing bones to become very thin and weak over time, resulting in impaired bone mass and strength (6, 10). In the United States, about 10 million individuals are estimated to have osteoporosis; 18 million are at risk of developing the disease; and another 34 million are at risk of having low bone mass, which place the population at greater risk for fractures (48). In Canada, almost 2 million individuals are affected and currently one in three women suffer from this disease beginning most commonly after the age of 50 years (49). The condition primarily affects the elderly and women, and is associated with 80% of fractures in people over 60 years of age (50). Fractures are associated with reduced quality of life, long hospital stays, institutionalization, and higher rates of mortality (50). In women, osteoporosis has been defined by the World Health Organization (WHO) since 1994 as a bone mineral density (with the reference site now being the femoral neck and lumbar spine) that is 2.5 standard deviations (SD) or more below the young adult female mean, based on a DXA measurement (3, 51). Although osteoporosis affects primarily elderly populations, young adults must be aware of the long-term impact their lifestyle can have on bone health.

The loss in bone mineral density (BMD) and its associated fragility fractures are a serious public health issue due to their increasing prevalence worldwide, contributing significantly to morbidity, mortality, and healthcare spending (3). In 2000, the prevalence of any fragility fracture, characterized by the number of individuals disabled, was 56 million worldwide (52). Most recently, in 2010, the global incidence of hip fracture was estimated to have increased to 2.7 million cases per year (53). Osteoporosis and related fragility fractures' cost European countries approximately €37 billion per year and the United States spend $17.9 billion on this health problem. In 2010, the Canadian healthcare was spending over $2.3 billions (12).

4. Bone Health Throughout Life

The quality of an individual's skeleton reflects everything that has happened to it from uterine existence into young adult life, when peak bone mass is achieved, and later in life, when progressive loss of bone often predominates (54). Puberty is a particularly critical period for bone mass acquisition, as BMD doubles between this stage and young adulthood (13-15) (approximately from 0.7 to 1.3 g/cm^2 at the lumbar spine site; and from 0.9 to 1.2 g/cm^2 at the femoral neck site)(14). The accrual of bone mass during this phase of life is a considerable ultimate determinant of peak bone mass (PBM) (13, 14). However, profound skeletal changes in bone size, structure, and material properties occur, in addition to increases in bone mass, all of which contribute to overall skeletal strength (13). According to research done by Lu and colleagues (48), this process is generally finalized in the hips between ages 18-20 years in women, and in the lumbar spine between ages of 20-25 years.

Women in particular tend to achieve peak bone mineral density between the ages of 16-20, while men achieve peak bone mineral density between the ages of 20-25. Once PBM has been achieved, the structural integrity of bone is maintained by the remodeling process, which continues throughout life so that most of the adult skeleton is replaced about every 10 years (3). When optimal peak bone mineral density is not achieved, the risk of osteoporosis and related fractures may increase later in life (54). Environmental and genetic factors influence the maximal gains on PBM, where only physical exercise and nutrition are modifiable (10). Consequently, the role of physical activity and nutrition in this stage of life is fundamental.

5. Impact of Physical Activity on Bone Health

Physical activity and adequate nutrition habits have many benefits on bone health and overall physical function. BMD may significantly improve with a combination of weight-bearing activities, and a high calcium and vitamin D intake. The enhancement of BMD may happen at all ages, but particularly during the stage of maturity (16, 17). BMD is an important indicator of skeletal health, integrity and strength. Having a higher BMD is among the many health benefits of vigorous and moderate-intensity physical activity, which applies to both aerobic and resistance-type activities (55). Since bone and muscle mass are indicators that represent the strength in both components of body composition, the "functional muscle-bone unit" theory was developed to measure the association between bone and muscle strength (4). Hence, an increase in mechanical loading (i.e. physical activity involving medium to high-impact loading) may impose positive effects on muscle strength and enhance structural and bone mass adaptations leading to enhanced bone strength (4, 56).

Osteoblasts, lining cells and osteocytes form a cytoplasmic mass to fuse together with the essential purpose of detecting and transferring the skeletal response to mechanical efforts.

Osteocytes generate signals to begin modeling and remodeling responses (1). Physical loading plays a significant role in the process of bone modeling and remodeling (57, 58) and is critical for increasing or maintaining bone mass in all periods of life (15).

Likewise, it has been revealed that among young adults, participation in activities in accordance with the Physical Activity Guidelines can attenuate age-related decreases in BMD and in some cases increase it by 1-2% per year (59). Hence, physical activity is well known to be an important modifiable risk factor for skeletal health (55).

In 1992, Recker and colleagues (60) conducted a longitudinal investigation over 5 years (visits made at 6-month intervals for a total of 9 visits) in 156 healthy female university students between 20-25 years old. The relationship between physical activity, nutrient intake and bone mass was assessed in these participants. Activity was measured with an accelerometer (CALTRAC activity monitor) over a 4-day period of monitoring prior to each visit. Nutrient intake was recorded in a 7-day diary at each 6-month visit and analyzed with Nutripractor software. A positive correlation was found with both calcium intake and physical activity [BMD ($r = .31$; $P = .004$)] and lumbar spine BMD (60).

More recently, Tonnesen and colleagues (61) demonstrated that BMD, assessed with a DXA scan at total hip, femoral neck (bilaterally), and at the lumbar spine (L2-L4) in young healthy adults (29 male between 19-24 years old; and 68 female between 18-23 years old) was positively associated with physical exercise, independent of sex and s-25[OH]D status. BMD at all DXA-scan sites was 0.142 g/cm^2 ($p = .001$), 0.140 g/cm^2 ($p = .0002$), and 0.134 g/cm^2 higher ($p = .01$), for the 0-½ h, ½-4 h, and 4-7 h per week exercise levels, respectively (61).

Sports that involve high-impact loading (e.g., gymnastics, hurdling, judo, karate, volleyball, and other jumping sports such as single figure skating) or odd-impact loading (e.g., soccer, basketball, racquet games, step-aerobics, speed skating, and ice dancing) are also associated with higher bone mineral composition, BMD, and enhanced bone geometry in regions specific to the loading patterns of each sport when compared to non-impact sports. However, non-impact sports such as swimming, water polo, synchronized swimming and cycling may not be associated with improvements in bone mineral composition or BMD (15, 18-22). Taaffe et al. (62), demonstrated significant changes in BMD (assessed with DXA) in university-level female athletes (gymnasts, runners, and swimmers) and non-athletes between 18-29 years old. They observed athlete's activity patterns over an 8-month period (Cohort I) and a 12-month period (Cohort II). Significant increases were found in lumbar spine (LP) and femoral neck (FN) BMD after the 8-month period. Specifically, in Cohort I LP in gymnasts (2.8 ± 2.4%) was greater than runners (-0.2 ± 2.0%) or controls (0.7 ± 1.3%), $p < .001$. FN in gymnasts (1.6 ± 3.6%) was also significantly greater than runners (-1.2 ± 3.0%), $p < .05$ compared with controls (-0.9 ± 2.2%), $p = .06$. Furthermore, after Cohort II, gymnasts gained 2.3 ± 1.6% at the LP, which differed significantly ($p < 0.01$) from changes in swimmers (-0.3 ± 1.5%) and controls (-0.4 ± 1.7%). Similarly, the change at the FN was greater ($p < 0.001$) in gymnasts (5.0 ± 3.4%) compared to swimmers (-0.6 ± 2.8%) or controls (2.0 ± 2.3%). More recently, Stanforth et al. (20), compared BMD on impact (basketball BB, soccer SOC, track sprinters and jumpers TR, and volleyball VB) and non-impact (swimming) sports. They evaluated 212 female university-level athletes (18-23 years old) and the control group consisted of another 85 physically active female college students of the same age. BMD was evaluated in both groups at preseason and postseason, over a period of 3 years. The results revealed a significant change in total BMD for BB athletes (Y-1 pre: 1.315 ± 0.01 to Y-3 post: 1.334 ± 0.02; 1.4%), TR athletes (Y-1 pre: 1.276 ± 0.01 to Y-3 post: 1.305 ± 0.01; 2.3%), and VB athletes (Y-1 pre: 1.276 ± 0.01 to Y-3 post: 1.310 ± 0.02; 2.7%). Despite of what it might be expected from a non-impact sport, SW athletes also showed significant changes in total BMD (Y-1 pre: 1.130 ± 0.01 to Y-3 post: 1.194 ± 0.02; 5.7%). According to the authors this was probably due to the resistance training these athletes were performing all year (20).

Finally, in 2018, Tenforde and colleagues (22) evaluated 239 female athletes (19.9 ± 1.2 years old) participating in 16 collegiate sports from the NCAA DI (swimming/diving, sailing, synchronized swimming, crew/rowing, cross-country, water polo, tennis, fencing, lacrosse, field hockey, track and field, soccer, gymnastics, softball, volleyball, and basketball). DXA scans were performed to measure their BMD at the lumbar spine (LS) and total body (TB) sites. It was found that the lowest average of BMD z-scores were seen in the synchronized swimming (LS, - 0.34; TB, 0.21), swimming/diving (LS, 0.34; TB, -0.06), crew/rowing (LS, 0.27; TB, 0.62), and cross-country running (LS, 0.29; TB, 0.91) athletes. The highest average values were observed among the gymnastics (LS, 1.96; TB, 1.37), volleyball (LS, 1.90; TB, 1.74), basketball (LS, 1.73; TB, 1.99), and softball (LS, 1.68; TB, 1.78) athletes. In summary, the participants from high-impact and multidirectional loading sports had significantly higher BMD values compared with athletes from the other sports (all $P < 0.05$) (22).

6. Low Energy Availability in Sports

Physically active individuals may undertake high training volumes and simultaneously restrict their dietary energy intake. These habits may lead to a higher risk for energy deficiency when practiced either in isolation or in combination (23). Energy availability (EA) has been used to quantify energy deficiency in physically active individuals. It is recognized that dietary energy is utilized through basic physiological processes such as cellular maintenance, thermogenesis, immunity, growth, reproduction, and locomotion. However, when energy is used for one of these processes, it becomes unavailable for the others (63). Thus, these processes can be more or less prioritized in terms of their importance to individual survival, where some of them, such as circulation, cannot be compromised and will be maintained at all costs (64). Consequently, the ones not considered critical including growth, thermoregulation, and reproduction, would likely be the first compromised during times of low EA (LEA) (64).

Energy Availability describes the calories available to the body to optimize physiological and metabolic functions. Hence, it can be defined with the following equation: Energy Availability (EA) = Energy Intake (EI) – Energy Expended in Exercise (EEE) normalized to fat-free mass (FFM), expressed in units of kilocalories per kilogram of FFM (24, 63). This takes into account the energy expended during exercise by this simple equation: energy balance = dietary energy intake minus total energy expenditure (EB = EI - TEE) (63-65). Measuring EA is not an easy task and a gold standard assessment has not yet been acknowledged. Nonetheless, self-reported food and exercise records are extensively implemented to estimate EA, even though they lack objectivity. To reduce inaccuracy reporting dietary intake, some researchers invest time educating the athlete on how to better describe their food intake by teaching them portion sizes and detailed ingredient contents; and even some researchers advise the athletes to send pictures of their meals to better understand portions and ingredients consumed (66, 67). More accurate ways to measure EEE include wearing an accelerometer (68-70) or a heart rate monitor (67, 69), combined with physical activity questionnaires (66) such as the IPAQ. Yet, a more accurate but more intensive assessment is by measuring energy expenditure on a controlled exercise protocol environment in the lab with a calorimeter (63, 68).

Low levels of EA can often result from disordered eating, failing to meet exercise energy requirements due to time constraints, food accessibility issues, and lack of appropriate nutrition knowledge, mostly related to body weight habits (24, 64). Low EA has been associated with menstrual dysfunction and impaired bone health in female athletes and has been previously described as the Female Athlete Triad (Triad) (23, 24, 71). According to

Nattiv and colleagues (24) in the ACSM (American College of Sports Medicine) Position Stand on The Female Athlete Triad, the Triad is defined as the “relationship among energy availability, menstrual function, and BMD that may have clinical manifestations including eating disorders, functional hypothalamic amenorrhea, and osteoporosis” (24).

6.1 The Role of Menstrual Function on Bone Health

In young women, adequate levels of endogenous estrogens, including estradiol, are likely to contribute to the achievement and maintenance of peak bone density (72). Adequate levels of estradiol are a necessary component to the complex processes leading to ovulation and menstruation, due to it’s mechanism for facilitating calcium uptake into bone, and inhibiting bone resorption (1, 72). Nevertheless, female athletes seeking success in their sport, combined with the pressure to achieve a prescribed body weight and poor eating habits may develop the Female Athlete Triad (64).

Menstrual disorders are common among female athletes, especially adolescents. Menstrual disregualtion can be caused by insufficient caloric intake to support basic physiological functions. When compounded with high amounts of exercise female athletes with menstrual disorders may have lower BMD (described in Figure 2), poor cold tolerance and may experience reduced performance in their chosen sport.

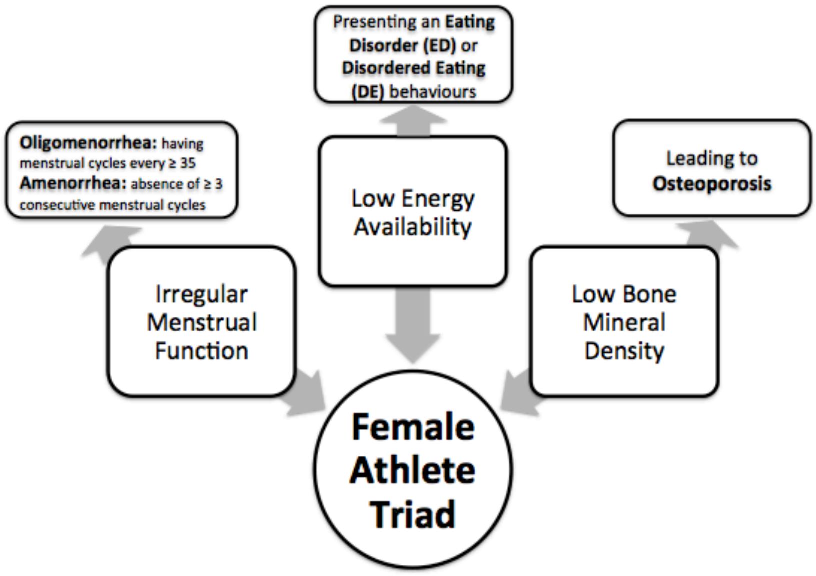

Figure 2. The three aspects that lead an athlete to develop the Female Athlete Triad and the consequences of each aspect. *(Adapted from: Nattiv et al., 2007)*

When athletes present with LEA, dysfunction of luteinizing hormone (LH, secreted by the pituitary gland and key to the reproduction and maintenance of the menstrual cycle) first develops, followed by anovulation and then leading to amenorrhea (73, 74). Amenorrhea is the most common menstrual disorder which has shown to be associated with lower BMD, and is better defined as the functional hypothalamic amenorrhoea (FHA, no cycles for > 90 days) (64). The low BMDs observed in amenorrheic athletes have been attributed to their habitual estrogen deficiency that results in an excessive resorption, leading to a higher risk of fractures (1, 68). In 2011, Ackerman and colleagues (75) evaluated 50 adolescent females [16 amenorrheic athletes, 18 eumenorrheic athletes, and 16 non-athletes (15-21 years old)]. They assessed their BMD (lumbar spine, femoral neck and hip) applying a DXA scanning. They found that the BMD Z-scores for the lumbar spine site lower in amenorrheic athletes compared with eumenorrheic athletes and the non-athletic group ($p = 0.007$). At the femoral neck and hip sites the Z-score was significantly lower in amenorrheic athletes compared with eumenorrheic athletes ($p = 0.02$ and $p = 0.0004$, respectively) (75).

Loucks and colleagues (74) studied the association between EA and LH. The results revealed that periodic secretion of LH can be suppressed when EA decreases to < 30 kcal/kg fat-free mass (FFM)/day over more than 5 days, causing FHA (74). This contributes to having low levels of estrogen, which potentially increases the risk for stress fractures and osteoporosis (8, 24). Comparatively, a rapid or significant fat mass reduction, even over a short period of time (one month), may also compromise menstrual function (76).

In 2004, Ihle & Loucks (68) determined that the prevention of amenorrhea and low BMD in the athletes evaluated (29 healthy, young, regularly menstruating women) involves maintaining energy availability above 30 kcal/kg FFM/day. Plasma osteocalcin (OC) was assessed to observe bone mineralization (p = < .05). Two restricted diets and one balanced diet (10, 20 or 30 kcal/kg FFM/day, respectively) were randomly assigned to participants, and compared with treadmill sessions done at 70% of VO_{2max}. These sessions assessed exercise energy expenditure (EEE) and were performed over a period of 5 days, while controlling for daily dietary energy intake. They found that plasma OC levels were reduced by 28% with the 10 kcal/kg FFM/day, 32% with the 20 kcal/kg FFM/day and 11% with the 30 kcal/kg FFM/day. The significant repression of OC with the 3 different diets, exhibits how bone formation is highly affected (68). Similarly, Pollock et al. (77) performed a longitudinal analysis in seven female elite endurance runners aged between 18-30. A significant association was found between the BMD reduction at the lumbar spine (L2-4 specifically) and training volume (p = .026) after 1-month follow-up. Although the investigation did not include athletes' energy intake or expenditure to confirm an association with LEA, the increased reduction of BMD with an increment in the training volume may be linked with LEA in the population studied (77).

6.2 RED-S

Evidence suggests that the aforementioned clinical conditions are not only a Triad; instead, it can be characterized as a syndrome resulting from relative energy deficiency that influences more than 3 aspects (76). Multiple aspects of physiologic functioning are affected, including; metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular functioning, and psychological health. In addition, it has been shown that men can also be affected (76). Therefore, the International Olympic Committee (IOC) has more recently labelled the syndrome as the Relative Energy Deficiency in Sports (RED-S) (76).

RED-S has many adverse health consequences for both menstrual function and BMD. When peak bone mass has been reached at around 19 yrs in women, two hormonal processes take place: oestrogen increases the uptake of calcium into blood and deposition into bone, while progesterone facilitates the actions of oestrogen through multiple complex mechanisms. Even an imperceptible oestrogen/progesterone imbalance, seen in subclinical ovulatory disturbances with LEA, may produce negative changes in bone health (76).

Stress fracture risk may be increased with changes in bone structure. High-risk stress fractures (e.g., femoral neck) often occur in adolescent athletes with RED-S, and can have severe long-term consequences. Unfortunately, bone loss in some athletes may be irreversible (76).

7. Impact of Nutrition on Bone Health

Nutrition plays an essential part in many aspects of bone health in young female athletes. Calcium and Vitamin D are among the primary elements in the maintenance of healthy bones and muscles (25).

7.1 Calcium

7.1.1 Food sources of Calcium

Typically, calcium is associated with dairy products such as milk, yogurt, and cheese. In the United States and Canada most diets include products that provide the major share of this micronutrient (78). In the United States, it has been estimated that 72% of calcium consumed comes from milk, cheese, yogurt, and other foods that contain dairy products (e.g., pizza, lasagna, dairy desserts). The remaining calcium is derived from vegetables (7%); grains (5%); legumes (4%); fruit (3%); meat, poultry, and fish (3%); eggs (2%); and various other foods (3%) (78, 79).

In the United States and Canada is has become more common to fortify foods that do not naturally contain calcium, such as orange juice, other beverages, and ready-to-eat cereals (78, 80). These practices influences the accuracy of national food composition databases, and may result in some underestimation of actual calcium intake from food sources (78), due to the different amount of calcium added depending on the brand or product.

7.1.2 Absorption and Metabolism

Calcium is the most abundant mineral in the human body and it is absorbed in two ways: 1) by active transport, which depends on the action of calcitriol and intestinal vitamin D receptor (VDR); 2) by passive diffusion across the intestinal mucosa, which involves the movement of calcium between mucosal cells during higher intakes (78, 81).

Hunt & Johnson (82) reported that approximately 25% of total calcium intake is absorbed in men and non-pregnant women across a wide age range (also called fractional calcium absorption) (82). From total calcium intake, mean calcium loss derives from urine (22%) and feces (75%), with minor losses from sweat, skin, and hair (78). However, when calcium intake is very low, fractional calcium absorption may become inverted, and can vary, even during critical periods of life (78). For example, a study done in 1993 by Dawson-Hughes et al. (83), revealed that after an 8-week period, when calcium intake was lowered from 2,000 to 300 mg in healthy women, their fractional whole body retention of ingested calcium was increased from 27% to about 37% (between the baseline measure and at the 2-week period), then the retention was practically reversed after restriction (83).

Calcium balance within the body is closely associated to the hormonal actions of calcitriol (one of vitamin D metabolites). The basis of the calcium homeostatic mechanism in mammals is formed by the vitamin D metabolic system. Total calcium concentration in serum is naturally regulated to maintain between 8.5 and 10.5 mg/dL (2.12 and 2.62 mmol/L). If this level differs slightly, the calcium sensing receptor of the parathyroid gland signals the secretion of PTH (also a vitamin D metabolite). PTH then stimulates the kidney to produce calcitriol, the hormonal form of vitamin D. Additionally, PTH activates bone resorption, which increases extracellular calcium levels (78).

7.1.3 Prevalence of deficiency among young female adults

Adequate calcium intake is associated with higher bone mineral composition (BMC) and BMD values (10). According to the latest updates on dietary intake requirements for calcium and vitamin D from the Institute of Medicine (IoM), the dietary reference intake (DRI) based on the dietary requirements using bone health as an indicator (for male and female between 19-30 years old) is 1,000 mg/d (84). Unfortunately, in both sedentary and physically active individuals, particularly adolescent girls, low calcium is still one of the most frequent nutrients deficiencies (85).

Calcium deficiencies in the young population can account for a 5 to 10% difference in PBM consequently increasing the risk for hip fracture later in life (8). An analysis of NHANES data indicated that dietary calcium intake among females aged 19-30 years was inadequate (median intakes of 686 ± 25 mg) (86). Moreover, the required calcium intake might be greater particularly among female athletes than the general population (27, 87). It has been suggested that exercise may increment calcium losses through sweat and urine (88). Baker and colleagues (89) collected sweat through whole-body wash-down (WBW) in 8 athletes (4 men, and 4 women) who cycled for 90 minutes in a plastic open-air chamber in the heat. They evaluate their sweat calcium and other mineral losses and found they lost in average approximately 45 mg/L, which is a high amount (89). Consequently, athletes who train for a long time and have excessive sweat rates may lose a higher amount of calcium and therefore their requirements may increase (88). Still, further research is needed. However, the average intake among female athletes appears to be insufficient and similar to that of same-age peers in the general population (27).

Ward and colleagues (27) compared two different assessment tools to measure calcium intake in 76 female collegiate athletes (aged 17-21 years old) from basketball, cross-country, field hockey, soccer and volleyball teams. The results demonstrated that average calcium intake within the sample was significantly below the recommended daily amount (823 mg/d) (27). In another study, five collegiate athletic teams (basketball, golf, tennis, track/cross-country, and volleyball) from the women's NCAA Division 1-A (age range of 18-23 years old) were assessed. Similar results were observed, as calcium intakes were below recommended levels with an average of 898 mg/d (90).

The benefits of protein and its association to calcium intake have also been validated. The influence of protein on bone health may differ, according to calcium consumption (91). In the Framingham Offspring Cohort, a significant interaction between the energy percentage from protein intake and total calcium intake in women at all bone sites was found. Protein intake was positively associated with BMD, only among women with low calcium intakes (<800 mg/day) (91, 92).

Recently, there has been an increasing trend for avoiding dairy products to control or lose weight. Unfortunately, it is all too common for young women in particular, to fear gaining weight. As a result of avoiding dairy products, these women may become more vulnerable to calcium deficiencies (14, 54).

7.2 Vitamin D

7.2.1 Food Sources of vitamin D

Vitamin D (calciferol) is composed by a group of fat-soluble seco-sterols, where vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) represent the major forms. Vitamin D_2 is primarily produced by the human body, and also added to foods. Vitamin D_3 is synthesized in human skin, and also obtained by consuming certain animal-based foods (78). Dietary sources of vitamin D are slightly limited, and include fatty fish such as salmon, mackerel, and herring, fish liver oil, egg yolk (78), and shiitake mushrooms (93); however, some foods are now fortified. In Canada, under the Food and Drug Regulation, it is mandatory to fortify milk in both, powder and fluid forms (35-45 IU vitamin D per 100 mL), as well as margarine (530 IU per 100 g). Moreover, plant-based beverages must also be fortified with a similar amount of Vitamin D (78, 80).

Currently, dietary supplements containing vitamin D in either form (D_2 or D_3), are more frequently consumed. In general, they contain 400 IU per daily dose, although levels in supplements have recently been increasing. Daily doses of 1,000 IU in a single supplement or in a multi-vitamin/multi-mineral formulation are now more commonly sold to consumers. In Canada, a prescription is required for dosage levels above 1,000 IU (78).

7.2.2 Absorption and Metabolism

Dietary Vitamin D, in either D_2 or D_3 form, is absorbed with other dietary fats in the small intestine, due to its fat-soluble nature (94, 95). In order for Vitamin D to be absorbed efficiently, the presence of fat in the lumen is necessary, which activates the release of bile acids and pancreatic lipase (95). In spite of this, the optimal amount of fat required for maximal absorption of vitamin D is yet to be determined. Within the intestinal wall, vitamin D binds with other lipids (cholesterol, triglycerides, lipoproteins, etc.) into chylomicrons. These reach the systemic circulation via the lymphatic system and are metabolized in peripheral tissues, especially in adipose tissue and skeletal muscle. These tissues can take up a fraction of vitamin D released during hydrolysis of the chylomicron triglycerides. After lipolysis, another fraction of vitamin D is present in the triglyceride-depleted particle (78).

Vitamin D is considered biologically inactive until it undergoes two enzymatic reactions. When vitamin D accesses the blood stream, it is converted into 25OHD (precursor of calcitriol and major circulating form of vitamin D). Thereafter, the liver or other storage tissues take a few hours to release it. Subsequently, 25OHD binds to a specific plasma carrier protein, the vitamin D-Binding Protein (DBP), where it can flow into the circulatory system. When there is a lack of calcium or phosphate, 25OHD is converted enzymatically (1α-hydroxylated) in the kidney to its active form (calcitriol) (78).

Two counteracting hormones regulate the renal synthesis of calcitriol: 1) up-regulation via parathyroid hormone (PTH), 2) down-regulation via fibroblast-like growth factor-23 (FGF23). Calcitriol binds to DBP after its synthesis in the kidney, and further carried to target organs (78).

The development and maintenance of bone health is the result of the actions of vitamin D. Due to the functions of the active metabolite (calcitriol), the regulation of serum calcium and phosphate homeostasis take place (78).

Metabolic vitamin D products are excreted through the bile into the feces, where small amounts are eliminated through urine. The renal uptake of vitamin D metabolites bound to DBP contributes to this process (78).

7.2.3 Prevalence of deficiency in young adult females

Vitamin D plays a key role in optimizing bone health. It is an important factor in children's bone development, and correlates positively with bone density in young adults (25). Vitamin D status is indicative of calcium absorption and bone mineralization. A considerable expanse of knowledge describes the association between this micronutrient deficiency and bone health (26).

Several reviews have reported a high prevalence of vitamin D insufficiency and deficiency among children and adults worldwide (28-34). Approximately one billion individuals across all ethnicities and age groups are thought to have low vitamin D levels (30). A recent report on Brazilian adolescents and young adults (78 male and 82 females aged 16-20 years old), found that there was no significant difference (p = .444) in calcium and vitamin D intake between males and females. In addition, only 3.8% of participants met the daily adequate intake recommendation for calcium, and none for vitamin D (682.2 ± 132.2 mg/day and 124 ± 28 IU/day, respectively) (96). According to Cycle 2 data from the Canadian Health Measures Survey (CHMS) collected between August 2009 and November 2011, approximately one third (32%) of Canadians had concentrations of Vitamin D below the cut-off point (< 50 nmol/L according to the suggested cut-off points by the IoM). This rate also includes those who were vitamin D deficient (< 30 nmol/L according to IoM), meaning that a total of 10% of Canadians were deficient (97, 98).

Although numerous population studies have documented a high prevalence of vitamin D insufficiency, few studies have focused on athletes (35-41). The prevalence of vitamin D insufficiency among female athletes may range between 33% and 42%, depending on the type of athlete, season, and latitude of residence (43, 44). Overall, these studies have found that in athletes, 25[OH]D concentration varies by both population and season. The lowest status found in athletes might be due to low intakes (< 600 IU/d in individuals between 19-30 yrs old corresponding to serum 25[OH]D levels of <20 ng/ml/<50 nmol/L according to the IoM recommendations), and a lack of endogenous synthesis due to scarce UVB exposure, which may be influenced by skin pigmentation, clothing requirements, early or late day training, indoor training, geographic location, and sunscreen use (43, 99, 100).

One of the most significant sources of vitamin D for most humans is casual exposure of the skin to sunlight (16). Seasonal and latitude differences can have a dramatic influence in vitamin D status, due to the amount of solar UVB exposure and the cutaneous vitamin D_3 production (101). Sunscreen with a sun protection factor (SPF) of 30 can also reduce the capacity of the skin to produce vitamin D_3 (102). Additionally, the effectiveness of cutaneous synthesis of vitamin D_3 is determined by the skin pigmentation (101, 102).

Nutritional parameters are often difficult to evaluate due to possible response bias in dietary records, which can impact the significance of results. Additionally, small short-term changes on dietary behaviours may not have any impact on bone health. Future research may benefit from longitudinal intervention studies in which explore the interactions among physical activity, Vitamin D and Calcium consumption and whether supplementation may positively influence BMD.

8. Assessment of Bone Health

Bone mass is commonly measured by bone mineral content (BMC) and areal or volumetric BMD. Dual-energy X-Ray absorptiometry (DXA) provides a two-dimensional assessment of areal BMD (aBMD), and is commonly used to assess clinically sites, such as the proximal femur and lumbar spine (13). DXA is the most widely used imaging devices in bone health research and is considered a safe and relatively fast assessment for individuals of all ages (13). Thought, certain limitations have to be recognized. Since DXA measures aBMD in a two dimensional assessment, it relies on bone size, which can diverge greatly due to body width. Thus, it tends to superimpose soft tissue and calcified structures. Likewise, DXA does not have the ability to measure tissue thickness, hence variations between thick low-density bone and thin high-density bone are difficult to identify. Also, standardization on the position regarding the regions of interest can be inadequate (International Atomic Energy

Agency 2011, Gluer 2017), and it's precision can fluctuate depending on the site of measurement and on the type of equipment implemented (103). Hind et al. (103), have demonstrated that the in vivo precision of GE Lunar iDXA is more precise compared to previous DXA models. It has been reported to be approximately 0.4% for the lumbar spine (L1-L4), around 0.6% for total-body BMD, and between 1-2% for the femoral neck (103).

8.1 Statistical Analysis of DXA scanning

The International Society for Clinical Densitometry (ISCD) has recommended that Z-scores should be used, rather than T-scores adjusted for ethnicity or race, especially to report BMD in pre-menopausal women. Z-scores of -2.0 or lower are classified as low BMD for chronological age (or less than the expected age range), and those above -2.0 fall within the expected age range (104, 105).

An athlete's BMD reflects their cumulative history of energy availability and menstrual status, as well as their genetic endowment and exposure to nutritional, behavioural, and environmental factors (24). Consequently, their current and subsequent BMD assessments must be considered (24). The DXA should be used to measure BMD in athletes with LEA, DE, ED, or amenorrhoea for over 6 months (90, 91). In adolescents, DXA scans should include the whole body (head excluded), in addition to the lumbar spine (6, 92). Considering that athletes in weight-bearing sports should have 5-15% higher BMD than non-athletes (93, 94), a BMD Z-score < -1.0 SD merits further attention. In athletic populations, low BMD is defined as a Z-score between -1.0 and -2.0 SD, which also represents a risk for osteopenia. Additional compounding factors for low BMD include a history of nutritional deficiencies, hypoestrogenism, stress fracture, or other secondary clinical issues. Moreover, a value below – 2.0 SD is considered a classification for osteoporosis along with secondary clinical risk factors (6). In this case, a reassessment of BMD via DXA scan is recommended for athletes at risk, or those being treated for low BMD (12 months later in adults and a minimum of 6 months later in adolescents) (76).

9. Assessment of Dietary Intake

The Food Processor™ Software is one of the most commonly used instruments to assess overall energy intake, as well as macro and micronutrient consumption in clinical nutrition investigations (106). To measure energy intake, this software utilizes the IoM Estimated Energy Requirement (EER). The EER is defined as the average dietary energy intake that is required to maintain energy balance in healthy individuals of a specific age, gender, weight, height, and level of physical activity. In this particular assessment, relative body weight (i.e. loss, stable, gain) is the preferred indicator of energy adequacy (107). For example, for a healthy normal weight woman, the following equation is used: Estimated Energy Requirement (kcal/day) = Total Energy Expenditure (EER for women = 354 – (6.91 x age [y]) + PA x [(9.36 x weight [kg]) + (726 x height [m])] (107). This equation was based on an extensive doubly labeled water database (considered the gold standard for assessing Total Energy Expenditure (TEE) measurement) (108). The TEE may be calculated as the sum of the basal metabolic rate, thermic effect of food (TEF), physical activity and the energy expended in depositing new tissues and in producing milk (the latter in case of growth, development, and pregnancy) (107).

When using the EER equation, it is important to distinguish between the "Physical Activity Coefficients (PA)" and the "Physical Activity Levels (PAL)". The physical activity coefficients are used in the EER equation to estimate energy requirements, and are based on participants' physical activity levels. The Physical Activity Level (PAL) is the ratio of total energy expenditure to basal energy expenditure (TEE:BEE) (107). The Physical Activity

Level categories were operationalized as sedentary (PAL 1.0-1.39), low active (PAL 1.4-1.59), active (PAL 1.6-1.89), and very active (PAL 1.9-2.5) (107).

10. Lifestyle effects on Bone Health

College students undergo a period of adaptation in which they adjust to the new university environment (109). For many of them, it is their first time living away from their home and newfound independence can be stressful, increasing vulnerability to negative health behaviours, including poor eating habits (110). Students may also be lacking in knowledge regarding dietary sources, as well as the importance of energy availability, calcium, and vitamin D to optimize bone health (111, 112). They may also lack experience in healthy food preparation, and due to highly demanding academic workloads, may not prioritize exercise and good nutrition. Additionally, many students have reported unhealthy habits such as smoking, excessive alcohol (binge drinking), and physical inactivity (21, 110, 113-115). These are practices can lead to a nutrient imbalance that might not appear acutely; however, they may cause bone and long term health problems (113-116).

10.1 Sedentarism

Sedentary behaviour is a recently recognized public health problem, and is typically characterized by long periods of sitting. This can include watching TV, playing passive video games, or using a computer. Sedentary behaviours have been associated with poor health outcomes and decreased fitness, which can directly influence bone health (117). The Canadian Society for Exercise Physiology, with the support of the Public Health Agency of Canada (PHAC), released Canada's first evidence-based sedentary behaviour guidelines in 2011. They recommend limiting screen time to no more than two hours per day for children and youth (117). Recently, it has been also been recommended that adults should strive to get breaks in sitting time and seek limit sedentary behaviours both at work and in the leisure hours (118).

Physical inactivity has been identified as an important public health concern for Canadians of all ages (119). More than three quarters (77.8%, or 20.1 million) of Canadian adults 18 and over are not meeting the Canadian Physical Activity Guidelines according to the 2012-2013 Canadian Health Measures Survey (CHMS) (117). These guidelines recommend that adults accumulate 150 minutes or more of moderate-to-vigorous physical activity each week, in bouts of 10 minutes or more (119). However, college students are specially at risk due to decreased physical activity and prolonged periods of sitting during their first years attending university (109).

Physical inactivity plays a key role in the risk of osteoporosis, affecting both the achievement of peak bone mass in youth, and the subsequent rate of bone deterioration later in life (1). The reduction of habitual physical activity and increased sedentary behaviours in modern Western populations have been suggested as a primary explanation for the increasing incidence of osteoporotic fractures (1).

10.2 Smoking and alcohol consumption

According to the Canadian Tobacco, Alcohol and Drugs Survey (CTADS) (120), the prevalence of smoking among young adults aged 20 to 24 was 18% (452,000) in 2015, with females representing a 14% (171,000) rate. Young female adults (aged 20 to 24) who reported smoking daily, consumed an average of 11 cigarettes per day; alarmingly in the province of Quebec where the average was 14.1 cigarettes per day (120). Smoking has been linked with lower BMD values, though body weight, the amount of cigarettes, and the duration of smoking may influence the effects (116). Ward & Klesges (121) conducted a meta-analysis including 86 cross-sectional and prospective studies evaluating 40,753 subjects

(74% were women). In summary, this data revealed an increased rate of bone loss in smokers compared to non-smokers mostly at the hip site, and it was estimated that fracture risks later in life were increased by 13% in women (121).

Alcohol consumption has also been found to be related to fracture risk (115, 122). Ganry and colleagues (123) defined light alcohol consumption between 1-10 g of ethanol per day, moderate consumption between 11-30 g of ethanol per day, and heavy chronic alcohol consumption >30 g of ethanol per day (123); although, variations are common among authors. Consumption level categorization may also depend on the age, sex and hormonal status, as well as the type of beverage consumed (115, 122).

Excessive alcohol consumption among college students remains a significant health concern on many college campuses (110). A study done by Steptoe & Wardle in 2001 (124), revealed that among college students almost 63% in Western Europe, and 70% in Eastern Europe considered themselves occasional and/or regular drinkers (124).

More recently, The Canadian Tobacco, Alcohol and Drugs Survey (CTADS) reported that in 2015, the rate of alcohol consumption among young Canadian adults aged 20 to 24 was 83%. According to Canada's Low-Risk Alcohol Drinking Guidelines, 28% of this age group exceeded the chronic risk guideline, based on alcohol consumption in the previous 7 days (120). The Guideline's recommended drinking limit, to reduce long-term risks (chronic risk), is 0-2 standard drinks per day for women (no more than 10 standard drinks per week). In order to reduce short-term risk (acute risk) no more than 3 drinks in one day should be consumed (125). Although there have been advances in understanding the complex action of alcohol on bone, much remains to be determined (122).

Physical activity and adequate nutrition are essential for bone health in young female students. It is clear that not all types of physical activity can impact bone strength at a significant level and that mechanical loading plays an important role to show the highest benefits. On the other hand, a lack of healthy nutrition practices, which are common among varsity students, may cause an imbalance in their energy availability, vitamin D and calcium deficiencies. This can directly impact their menstrual function, leading to negative effects on their bone health (higher risk of stress and spontaneous fractures; and a higher risk of developing osteopenia and osteoporosis later in life). This study may provide valuable insight on the current physical activity and nutrition status of Female Canadian University Students.

2.0 MANUSCRIPT

The influence of sports, energy, vitamin D and calcium intake on bone mineral density in female athletic and sedentary students

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2.1 Abstract

Purpose: Determine the influence of the type of sport on regional and total BMD and its association with dietary energy, vitamin D and calcium intake. ***Methods:*** Seventy-three female students from McGill University were evaluated (age 20.8 ± 1.9). Forty-four athletes [basketball (n=13), volleyball (n=11), figure-skating (n = 13), and synchronized-swimming (n=7)], and 29 age-matched sedentary students. Dietary, vitamin D and calcium intake were assessed using a 3-day Food Log and a FFQ, then analysed with the Food Processor^{TM} Software. Total Body (TB), lumbar spine (LS) and femoral neck (FN) BMD were assessed by DXA scanning. ***Results:*** A significant difference in BMD at all 3 sites was observed across all sports ($p < .001$). Basketball (TB = 2.3 ± 1.2; LS = 1.7 ± 1.53, $p < .001$; FN = 1.7 ± 1.1, $p = .001$) and volleyball (TB = 1.9 ± 1.3; LS = 1.5 ± 1.6, $p = .001$; FN = 1.7 ± 1.7, $p = .002$) players had a significantly higher BMD in all sites compared to their non-athletic counterparts (TB = 0.4 ± 0.9; LS = -0.3 ± 1.2; FN = 0.1 ± 1.0). No significant association was observed among BMD at all sites and total energy intake, vitamin D and calcium across all groups. There was a significant difference between the Delta Energy Intake in both basketball ($p = .003$) and synchronized-swimming ($p = 0.02$) groups compared to the control group. ***Conclusion:*** The type of sport revealed a strong influence on BMD. A significant discrepancy was found on the Delta Energy Intake in some athletes. Female varsity athletes should work closely with sports dieticians to promote healthy eating and optimize bone health. ***Key Words***: BONE MINERAL DENSITY, SPORTS, FEMALE ATHLETES, DELTA ENERGY INTAKE, ENERGY, VITAMIN D, CALCIUM.

2.2 Introduction

Osteoporosis is a potentially debilitating disease, and is characterized by an impairment in both bone mass and strength (10). This loss in bone mineral density (BMD) and its associated fragility fractures represent a serious public health issue due to their increasing prevalence worldwide, contributing significantly to morbidity, mortality and healthcare spending (3). The economic impact of the disease in the United States can be measured in approximately $17.9 billion per year, and €37 billion in Europe (11). Within the Canadian health system, the overall yearly cost of treating osteoporosis and fractures caused by this disease was over $2.3 billion in 2010 alone (12).

Our skeleton is made up of active tissue that serves two primary functions: it provides biomechanical support and protects soft tissue, it also plays a key role in mineral homeostasis (1). Since bones are metabolically active, they are constantly renewed through a natural process called remodeling, in which new bone cells replace old bone cells (2). This renewal process is responsible for bone strength (3). Unfortunately, this process becomes gradually less efficient with age, causing bone tissue to progressively deteriorate (6). When less bone cells are deposited than resorbed, it results in osteopenia and eventually osteoporosis (9). Nevertheless, skeletal health can be affected by a number of factors, including genetics, lifestyle, demographic characteristics, and disease (1).

Puberty and adolescence are critical periods when skeletal development lay the foundation for bone health in adulthood. As BMD doubles between puberty and young adulthood, bone mass acquisition plays a significant role (13-15). Essentially, the acquisition of a maximal peak bone mass (PBM) is influenced by a complex interaction of environmental and genetic factors, in which only physical exercise and nutrition are modifiable (10). As a result, the role of appropriate physical activity and nutrition are critical to optimize bone health at this stage of life.

Weight-bearing, percussive activities alongside adequate calcium and vitamin D intake may provide a practical method to improve and optimize bone health at all ages, especially during puberty (16, 17). Participation in sports within the age range of growth and skeletal maturity may result in a higher peak bone density later in life (15). Primarily, as Tenforde & Fredericson described (15), high-impact loading sports such as gymnastics, hurdling, judo, karate, figure skating (singles), volleyball and other jumping sports; or odd-impact loading including basketball, soccer, racquet games, step-aerobics, pair skating and speed skating, are correlated with a higher BMC and BMD (15). However, non-impact sports such as swimming, water polo, and cycling are not associated with improvements in BMC or BMD (15, 18-22).

Physically active individuals may undertake high training volumes and simultaneously restrict their dietary energy intake. Either in isolation or when combined, these are practices that may put individuals at risk for energy deficiency (23). Consequently, this leads to low energy availability, which is associated with menstrual dysfunction and impaired bone health in female athletes (76), also recognized as the Female Athlete Triad (23, 24, 71). Physiologic factors are affected not only in female but also in males as well, such as metabolic rate, immunity, protein synthesis, cardiovascular function, and psychological health; the International Olympic Committee classified the syndrome as the Relative Energy Deficiency in Sports (RED-S) (76).

Nutrition has been found to play an essential role in many aspects of bone health in young female athletes, where calcium and vitamin D are among the key elements that contribute to the maintenance of healthy bones and muscles (25). Adequate calcium intake (1,000 mg/day) has been associated with higher BMD values (10). Vitamin D plays a key role in optimizing bone development in children, and bone density in young adults (25). Vitamin D intake is also indicative of calcium absorption and bone mineralization (26).

However, Ward et al. (27), documented that calcium intake among adolescent and young adult females is often below the recommended levels (27). Also, a high prevalence of vitamin D deficiency and insufficiency among adults and children is evident worldwide (28-34). Despite these findings, very few studies have focused on athletes (35-41). Research completed by McClung et al. (43), and Ogan & Pritchett (44) suggests that the prevalence of vitamin D insufficiency among female athletes may range between 33% and 42%, depending on the type of athlete, season, and geographical latitude (43, 44).

To address the knowledge gap in previous research the purpose of this study was to explore the influence of mode of sports activity, overall energy intake, vitamin D and calcium consumption on measures of bone health in athletic and non-athletic young female adults.

2.3 Methodology

2.3.1 Research Design

A cross-sectional study was performed at the Health & Fitness Promotion Lab in the Department of Kinesiology & Physical Education at Currie Gymnasium of McGill University. All procedures were in accordance with the ethical standards and were approved by the McGill University Institutional Review Board from the Faculty of Medicine and all participants provided written informed consent. Participants' names were protected for the purpose of confidentiality. Furthermore, all data is kept in locked filing cabinet in the Health & Promotion Lab. After a period of 7 years the data will be disposed off in accordance to University research procedures.

2.3.2 Recruitment Procedures and Eligibility

Seventy-six healthy female students (age 18-25 years old) were recruited for this investigation. Forty-six athletes from four different McGill Varsity Teams (basketball n = 14; volleyball n = 11; figure skating n = 14; and synchronized swimming n = 7) were evaluated prior to the beginning of their competition season. The evaluations took place in August and September of 2018. The athletes reported training on average 2-3 hours per session 4-6 times per week. These training volumes were representative of their in-season preparation. A second group of 29 age-matched, sedentary female students from McGill University community were also assessed.

The sedentary group was recruited via word of mouth, online posts on social media and the internal McGill Undergraduate Society webpage, classroom announcements, and posters on lower campus, with permission by the McGill IRB. An International Physical Activity Questionnaire (IPAQ) was implemented as part of the inclusion criteria to document low levels of physical activity. Specifically, their physical activity level had to be below 150 minutes of moderate to vigorous intensity per week, without participating in regular structured exercise over the past year. Participants had to be healthy females between 18-25 years old, and current students at McGill University. Within the athletic groups, inclusion criteria required participation in the 2018-2019 season, without current or previous injuries restricting their involvement in their sport or the study.

Data collected from three participants (1 sedentary and 2 athletes) was not included in the analysis due to failure to meet the inclusion criteria. One sedentary participant reported having an eating disorder during the evaluation. One athlete reported having a recent injury and inability to train and play during the season, and the other athlete was under the predetermined age range. As a result, a total of 73 participants' data were included in the analysis for this investigation.

Data Analysis

2.3.3 Assessment of Dietary Intake

Total dietary energy, calcium, and vitamin D intake were analyzed with the Food Processor^{TM} Software using a three-day Food Log. All participants recorded food and beverage intake for two weekdays and one weekend day, to provide insight into their eating habits. They were instructed to give detailed information on the amounts they were consuming, specifically the type of foods and beverages consumed (e.g. brown or white bread, rice or pasta; fat free or whole yogurt, milk or cream; etc.), and the brand of each product they recorded. Additionally, their Vitamin D and calcium supplement intake was reported in the Food Logs. Participants were asked to send pictures of the type of supplement and brand to have the specific amounts of these micronutrient intakes. All supplement data was also counted and analyzed with the Food Processor™ Software.

In addition, a previously validated short version of the Food Frequency Questionnaire (FFQ) (126) was also administered to all participants to complement the information on calcium and vitamin D intake. The nutrient analyses were conducted using the 2018 Canadian Nutrient File database (127). Supplement intake was also included in this analysis.

The FFQ short version was applied to compare vitamin D and calcium estimated intakes of our participants, with the analysis obtained from the Food Logs with the Food Processor™ Software. Though, this instrument cannot replace the complete analysis of the Food Logs administered, which provides a more extensive nutritional assessment (126).

2.3.4 Assessment of Menstrual Function and Contraceptive Use

Menstrual history and contraceptive use were assessed in both groups using one part of the Low Energy Availability in Female Questionnaire (LEAF-Q) (128). Participants responded to questions regarding menstrual function (age of first period, intensity of flow, length of monthly period, menstrual irregularities such as oligomenorrhea/amenorrhea) and contraceptive use (reasoning for use, type of contraceptive, length of use). In this study, oligomenorrhea was defined as having menstrual cycles every 35 days or more, and amenorrhea of having an absence of 3 or more consecutive menstrual cycles (24).

2.3.5 Assessment of Physical Activity

To assess daily physical activity levels, the International Physical Activity Questionnaire (IPAQ) was administered (129). For the purpose of this study, the long self-reported version was applied to provide more detailed information regarding participants' activities during the day. The IPAQ consists of 5 different sections: 1) Job-related physical activity, 2) Transportation physical activity, 3) Housework, house maintenance, and caring for family, 4) Recreation, sport, and leisure-time physical activity, and 5) Time spent sitting.

2.3.6 Assessment of Bone Mineral Density

Bone mineral density (total body, lumbar vertebrae from L1 to L4, and femoral neck) was evaluated in both assessment groups using dual energy x-ray absorptiometry (GE Lunar iDXA scan). Strictly standardized procedures were followed, which included measuring all participants with the same technician, and taking into account quality control measures such as daily system calibration (130). In addition to being a non-invasive procedure (3), DXA is considered the diagnostic gold standard for identifying individuals with osteoporosis (3, 6, 13).

The World Health Organization (WHO) established four categories relating to BMD in postmenopausal women, particularly for epidemiological classification, yet they become essential for the clinical diagnosis for osteoporosis: 1) Normal: value within 1 SD of young healthy female mean, later referred to as a T-score < -1; 2) Low bone mass (osteopenia): value more than 1 SD below the young healthy female mean but less than 2.5 SD below this value, later referred to as a T-score in the range of -1 to -2.5; 3) Osteoporosis: value of 2.5 SD or more below the young healthy female mean, later referred to as a T-score < -2.5; and 4) Severe osteoporosis: value more than 2.5 SD below the young healthy female mean in the presence of one or more fragility fractures (3, 51). Though, for the purpose of this study, we just implemented for our population the ISCD recommendations using Z-scores for pre-menopausal women. Were a Z-score of -2.0 or lower is classified as low BMD for chronological age (or less than the expected age range), and above -2.0 falling within the expected age range (97: ISCD Official Positions-Adults International Society for Clinical Densitometry, 2013; Lupsa & Insogna, 2015).

Before each DXA scan, participants responded to questions concerning their bone fracture history (site and age of fracture, treatment received, if it was a spontaneous or stress fracture previously explained to the participant, family bone disease background and if there was a personal diagnosis of any bone disease). This was done in order to determine the influence of prior injuries on their current bone status.

2.3.7 Assessment of Lifestyle Variables

Food Frequency Questionnaire included a section for lifestyle habits that may negatively influence bone mass, such as alcohol consumption (frequency of consumption and type of alcohol consumed) and cigarette smoking (frequency and amount of cigarettes consumed). Additionally, sunlight exposure time during summer and frequency of sunscreen use was also part of the questionnaire.

2.3.8 Statistical Considerations and Power Analysis

All analyses were conducted using the IBM Statistical Package for Social Science (SPSS) version 24.0 software (IBM SPSS Statistics for Macintosh, Version 25.0 Armonk, NY: IMB Corp). Descriptive statistics on all measures and tests of normality were done on all data. In the event of non-parametric distributions, logarithmic or root square transformations were applied to normalize data. One-way ANOVA was used to identify between-group differences across groups. Tukey Kramer post hoc analyses were performed to interpret between-group differences, in the event of a significant F ratio. Finally, multiple linear regression analysis was performed to explore predictors of each BMD measurement and overall energy, vitamin D and calcium intake across all groups.

Sample size calculations were conducted using the G*Power^{TM} sample size calculation software (131). A total of n = 73 was chosen based on a series of power analyses using a pilot study of three samples. Normal parameter estimates needed for a one sample t-test power analysis were estimated by splitting the 95% mean confidence interval from the pilot observations into five equidistant points as well as expanding the pilot standard deviation by magnitudes of 20%, 50%, and 100%. Sample size estimates ranged for 3 to 16 per group to achieve a power of 0.80.

2.4 Results

A total of 73 female participants with a mean age of 20.8 years completed all questionnaires and DXA scan evaluations. The participants were identified as Caucasian (65.8%), Asian (12.3%), Black (11%), and other (6.8% mixed raced American-Asiatic, 2.7% Hindu, and 1.4% mixed race American-Indian). The characteristics of all participants are presented in Table 1.

After evaluating menstrual function characteristics, more than half of participants (67%) reported taking oral contraceptives. Additionally, 84.9% of all participants reported using them to regulate their menstrual cycles. Many participants (27.4%) stated that their periods had stopped for 3 consecutive months or longer (13.7% of sedentary participants, 6.8% of figure skaters, 1.4% of basketball players, 2.7% of volleyball players, and 2.7% of synchronized swimmers). In 4.1% of them, those menstrual irregularities were currently happening (in 1.4% of sedentary participants, 1.4% of the figure skaters, and 1.4% of the volleyball players).

A one-way ANOVA on the anthropometric characteristics revealed a significant difference in height ($F = 15.83$, $P < 0.01$), weight ($F = 5.93$, $P < 0.01$), fat mass ($F = 9.55$, $P < 0.01$), and lean mass ($F = 20.41$, $P < 0.01$) between groups. Particularly, basketball and volleyball athletes were heavier but leaner, meaning that they had higher muscle mass and less fat mass, which may also be beneficial for bone health. However, no significant differences were found in age and BMI characteristics.

The details of nutrient consumption are presented in Table 2. There was a significant difference between the recommended intake minus the actual intake (Delta Energy Intake) in the basketball and the synchronized swimming athletes compared to their sedentary counterparts ($p = .003$ and $p = 0.02$, respectively). A total of 11% of participants reported consuming multivitamin supplements that contained vitamin D and calcium (15% of participants reported taking Vitamin D supplements, and 4% of participants reported taking calcium supplements). The highest levels of supplement consumption were reported by the figure skating athletes (23% multi-vit, 27% vitamin D, and 8% calcium) and the sedentary students (38% multi-vit, 36% vitamin D, and 67% calcium).

Effects on the type of sport

A one-way ANOVA (**Figure 1**) revealed significant differences in BMD of the total body ($F = 10.2$, $P < .001$), femoral neck ($F = 8.6$, $P < .001$), and lumbar spine ($F = 6.3$, $P < .001$) sites, across all sports (**Table 3**). Basketball players (total body and lumbar spine $P < .001$; femoral neck $P = .001$) and volleyball players (total body and lumbar spine $P = .001$; femoral neck $P = .002$) had significantly higher BMD at all sites, compared to their sedentary counterparts. Particularly, BMD Z-scores at the total body site in basketball players was in average ±1.39 higher than in synchro swimmers, and ±1.88 higher than the sedentary group. At the femoral neck site ±0.58 higher than in synchronized swimmers, and ±1.62 higher than the sedentary group. And at the lumbar spine site ±1.64 higher than in synchronized swimmers, and ±1.97 higher than the sedentary group. However, no significant differences were observed in BMD in the figure skating and synchronized swimming athlete groups, compared to the sedentary group (P = .719; P = .246).

Regression Analysis

Linear regression was used to explore the association among BMD measurements and energy, vitamin D, and calcium intake, adjusting for known confounders (ethnicity, physical activity, menstrual irregularities, BMI, lean mass, sun exposure, sunscreen use, and unhealthy habits such as smoking and drinking). The only significant predictor was physical activity at the total body ($R^2 = 0.157$, $t = 3.63$, $P < 0.01$), femoral neck ($R^2 = 0.124$, $t = 3.18$, $P < 0.01$), and lumbar spine ($R^2 = 0.122$, $t = 3.15$, $P < 0.01$) sites.

2.5 Discussion

This cross-sectional pilot study first investigated the influence of the type of sport on regional and total BMD and second, the association of BMD with dietary energy, vitamin D and calcium intake in young Canadian female athletes, compared to sedentary students. The results provided considerable insight into the bone health and eating habits of young adult Canadian female athletic populations. Additionally, these findings contribute to the knowledge that high and moderate-impact loading sports benefit the development of greater bone strength as previously described by other authors, which confirms our first hypothesis. On the nutrition aspect, albeit we did not find a significant association among energy availability, dietary vitamin D and calcium intake on BMD at all sites, we did found that low energy intake and high training loading were reported in some of our participants, which matches with previous literature describing how low energy availability may affect bone health and other aspects of physiological health in young women, as well as vitamin D and calcium deficiencies.

A significantly higher BMD was found in basketball and volleyball athletes, compared to their sedentary participants. The high differences resulting from the BMD measurements in the high-impact group compared to the low-impact and the sedentary group impose a physiological advantage in their bone strength. Demonstrating once more that high-impact sports may be beneficial for decreasing the risk of osteoporosis later in life. Among other factors that will be explained shortly, ethnicity has shown to play a highly significant role on BMD (132). According to cross-sectional comparisons between white and black female populations, black women revealed a higher BMD at the hip and femoral neck sites than white women (132). Almost 50% of the basketball athletes participating in our research were black and had a higher BMD than their white teammates. In contrast, participants involved in synchronized swimming (non-impact sport) demonstrated having a lower BMD at all 3 sites, but specially at the lumbar spine site compared to the other athletes and the sedentary group. This confirms work done by Tenforde and colleagues, in which non-impact and low impact sports such as cross-country running, swimming/diving, and synchronized swimming were significantly associated with lower BMD, compared to 16 other high-impact and odd-impact sports (22). Additionally, through longitudinal and cross-sectional data, Standforth et al. (20), determined that swimming athletes (non-impact sport) showed lower values of BMD compared to athletes in other sports (basketball, volleyball, soccer, track sprinters and jumpers and swimming) and sedentary participants (20).

Additionally, our figure skating athletes, participating in singles and ice dancing disciplines, did not revealed any difference in their BMD values compared to the sedentary group. Figure skating is considered an aesthetic sport where athletes may restrict their diets to maintain a lean body. Correspondingly, our participants' food records showed that energy intake was lower than the recommended amounts for the level of physical activity regularly performed in relation to the IPAQ records. The skaters also reported the highest frequency of menstrual irregularities, which involved the use of oral contraceptives to regulate their menstrual cycles and a more frequent reporting of an absence of their period for more than 3 consecutive months. A study conducted by Prelack and colleagues (133), evaluated BMD in 36 female adolescent skaters in single, pairs, and ice dancing disciplines. They took into account dietary energy, calcium and vitamin D intake as controlling factors. They reported very low BMD values at the lumbar spine site (negative Z-scores), confirming our findings. According to the dietary records, the average intake was below the reference population recommendations (500 kcal below standard intakes). The vitamin D intake was also below the recommended DRI, and calcium intake levels in 33% of the athletes was also below the recommendation (133).

We found that almost all the athletes were consuming a lower amount of total energy compared to their recommendation based on their activity level. There was a significant difference between the delta energy intake (the recommended intake minus the actual intake) in the basketball and synchronized swimming athletes compared to the sedentary group. Similar to our results, Schaal et al. (67), evaluated the energy availability (EA) of 9 synchronized swimming female athletes. Since the baseline measurements they found that EA was already significantly lower than 30 kcal.kg/LBM/day. This suggests that their dietary energy intake was inadequate for their intense training loads (67). Additionally, Melin and colleagues (69) assessed 40 endurance female athletes (average age of 26 years old) exercising in average 11 h/week. Fifteen with optimal EA (≥ 45 kcal/kg FFM/day), 17 reduced EA (between 30-44.9 kcal/kg FFM/day), and 8 with low EA (< 30 kcal/kg FFM/day). They reported that athletes with low EA had a significantly lower energy intake compared to the athletes in the reduced EA and optimal EA groups (22% and 32%, respectively) (69). Even though we did not report EA in our participants, these results are similar to the significantly lower energy intake we found in our basketball and synchro swimming athletes compared to the sedentary group (20% and 19%, respectively) Furthermore, 60% had menstrual dysfunctions [Optimal EA: 3 oligomenorrehic (Oligo), 1 with primary functional hypothalamic amenorrhea (FHA) and 4 with secondary FHA; reduced EA: 3 oligo, 1 with primary FHA and 7 with secondary FHA; low EA: 2 with primary FHA and 3 with secondary FHA], and 45% had reduced bone health [15 had low BMD (6 in optimal EA group, 8 in the reduced EA group and 1 in the low EA group) and 3 had already osteoporosis (1 in the reduced EA group and 2 in the low EA group)] (69). These results are similar to our findings, since our 7 synchronized swimmers, found to have significantly lower energy intake compared to the sedentary group, had low BMD at the lumbar spine site, and our figure skating group also reported the greatest menstrual irregularities.

Participants in our study had low vitamin D intake, which confirms previous finding in female athletic populations. Halliday and colleagues (37), evaluated the dietary vitamin D intake of 41 young adult athletes (18 men and 23 female) from the NCAA Division I (outdoor athletes: football, soccer, cross-country or track and field and cheerleading/dance; indoor athletes: wrestling, swimming, and basketball) from the University of Wyoming. They found that a very small percentage of athletes (5% in the fall, 6% in the winter, and 4% in the spring) were meeting the prevailing RDA (600 IU) from food alone. When supplements were included, this percentage increased to 27% in the fall, 18% in the winter, and 20% in the spring with most not even reaching half the daily recommendation (37). Internal and external factors may also contribute to low level of vitamin D include the lack of sun exposure during winter in northern countries and ethnicity. Black athletes may also be at a higher risk of vitamin D deficiencies than white athletes, due to racial differences that may be related to genetic variations in Vitamin D-binding protein (primary carrier of vitamin D) (134, 135).

According to the self-reported calcium intake in our participants, we found the lowest amounts reported among basketball players. Leachman et al. (90), assessed 59 American female collegiate athletes from the NCAA Division 1-A, and revealed that their calcium intake was below the RDA (90). Similarly, Anderson (136) found that 50% of 15 female NCAA Division II volleyball players also reported calcium intakes below the RDA. They recorded and analyzed their intake as we did, with a 3 day food record and the Food Processor Software (136).

When we analyzed the IPAQ records of our sedentary participants, according to the standards for cataloguing an individual sedentary in the Canadian physical activity guidelines (< 1,000 MET/min/week or < 150 min of moderate to intense physical activity) (119); we found that some were not indeed classified as sedentary. Their physical activity in MET/min/week was very close or a bit higher than 1,000. Though, their BMD Z-scores especially at the lumbar spine site were close to being classified as osteopenia. Consequently, future studies should aim to incorporate objectively measured physical activity to complement self-reported measures.

On the other hand, the LEAF-Q applied to record menstrual function in our participants, showed us that more than half of our participants had their first period between the ages of 12-14 years old. Menarcheal age has been linked with osteoporosis later in life considering that a late menarche age may be associated with a lower BMD at the lumbar spine and femoral neck, increasing the risk of fracture particularly at the hip and vertebral sites (137). Since puberty is a period were estrogen, one of the hormones involved in growth and development, may be responsible for accelerating skeletal maturation (138). Hence, a delay in menarche may reduce estrogen levels leading to negative effect on bone mineral accretion (138, 139). This information is worth mentioning since McKay and colleagues (138), conducted a 6-year longitudinal study of bone mineral accretion in 53 girls (from 8 to 16 years of age), and revealed the association between a greater peak bone mineral content velocity and earlier peak height velocity, when comparing with girls with a delayed maturity (according to their late menarche age) (138). Furthermore, Galuska & Sowers (72) showed after evaluating the association of 6 characteristics of menstrual history with BMD at the lumbar spine and the femoral neck in 182 white female students (19-26 years old) with a cross-sectional design; that late age at menarche was significantly associated with a lower BMD at the lumbar spine (-0.023 g/cm^2 for each year that menarche was delayed) (72). More recently, Gibbs and colleagues (139), also demonstrated from their retrospective cross-sectional data on 437 exercising women, that a delay in menarche (having the first period after the age of 15) is associated with a lower BMD (139). Only 15% of our participants had their first period after this specific age.

In addition, half of our participants reported using oral contraceptives (OC), mostly for regulating their menstrual cycles. Though, OC use has been reported to be associated with improvements in BMD, particularly at the lumbar spine, femoral neck, and hip in amenorrheic and/or oligomenorrehic women (140). However, nutrition therapy should still be encouraged for addressing low energy availability and bone health treatment in amenorrheic athletes (141). Regarding the menstrual irregularities our figure skating athletes reported, it is alarming that almost half of them stated their periods have stopped for 3 consecutive months or longer, and some of them still facing that issue. It was recently reported that young athletes whose menstruation has stopped as a result of intense activity, typically have lower BMD at the lumbar spine site in particular than their normally menstruating counterparts (13-16, 72). These findings coincide with the low BMD values we found in our figure skating athletes, which average Z-scores at the lumbar spine site, were of – 0.2. We speculate that one of the key factors influencing the menstrual irregularities reported by our athletes is lower than required energy intake, which may also increase their risk of RED-S (76).

Strengths and limitations. To our knowledge, there are no studies currently published that have examined BMD, energy, vitamin D and calcium intake in young female Canadian student athletes. Another notable strength of this study was the implementation of a short version of the FFQ, which complemented the 3-day Food Log analyzed with the Food Processor Software. This helped to extract more details on the total energy, vitamin D and calcium intake from each participant. However, we found that the FFQ (short version) was not the most accurate instrument to measure calcium and vitamin D intake in processed foods, due to a lack of specificity on the exact types of foods consumed (e.g., type of yogurt, milk, cheese, which have different amounts of calcium and/or vitamin D).

We would like to acknowledge limitations for this study. First our sample size may limit the interpretation of the data. Secondly, we acknowledge the limitations of self-reporting on measures of physical activity, energy intake and menstrual function.

Clinical Implications. The current findings demonstrate that female varsity athletes participating in high impact and odd impact sports have higher BMD values compared to their sedentary counterparts. In contrast, female skaters and synchronized swimmers had similar bone mass as the sedentary students, These data suggest that strength and conditioning specialists working with female athletes who participate in lower impact sports should incorporate percussive activities into dry-land training to optimize bone health. We did not find any relationship among BMD and energy, vitamin D, and calcium intake. However, it was found that energy intake was below the recommended amounts for all of the participating athletes. Moreover, most of the participants reported a low intake of Vitamin D and calcium, with or without supplement consumption. This suggests that female varsity athletes would benefit from consultations with a Sports Dietician; to encourage healthy eating that will ultimately enhance bone health.

In conclusion, we have shown that female athletes who participate in high impact activities enjoy better bone health compared to low-impact athlete and sedentary women. We also found that most female varsity athletes report consuming significantly less than recommended energy intake. In the future, longitudinal studies are need to better track changes in bone health during competitive years and after graduation. Finally, it is clear that strength and conditioning specialists and sports dieticians should be included as part of multidisciplinary teams work with female varsity athletes to optimize and promote both well-being and bone health.

Table 1. Participant Anthropometric and Menstrual Characteristics.

Variable	Basketball (B)	Volleyball (V)	Figure Skating (FS)	Synchronized Swimming (SS)	Sedentary (S)	Total
	n = 13	n = 11	n =13	n = 7	n = 29	N = 73
Age (yr)	20.5 ± 1.8	21.2 ± 1.6	19.6 ± 1.3	20.4 ± 2.0	21.3 ± 1.96	20.8 ± 1.9
Ethnicity *n* (%)						
Caucasian	6 (8)	11 (15)	12 (16)	7 (10)	12 (16)	48 (66)
Asian	0	0	0	0	9 (12)	9 (12)
Black	6 (8)	0	0	0	2 (3)	8 (11)
Other	1 (1)	0	0	0	6 (8)	8 (11)
Height (cm)	174.4 ± 5.8*	177.6 ± 3.97*	164.3 ± 5.0	164.8 ± 6.2	162.5 ± 7.9	167.4 ± 8.8
Weight (kg)	69.8 ± 9.5*	67.7 ± 4.8†	58.8 ± 6.1	59.5 ± 7.5	59 ± 9.3	62.3 ± 9.2
BMI (kg/m^2)	22.8 ± 2.2	21.4 ± 1.2	21.7 ± 1.8	21.8 ± 2.9	23 ± 5.2	22.5 ± 3.6
Fat Mass (%)	26.9 ± 4.9*	26.7 ± 3.8*	28.1 ± 5.1*	28.3 ± 4.9†	35 ± 5.5	30.4 ± 6.2
Lean Mass (kg)	48.2 ± 4.9*	47.4 ± 3.4*	40.3 ± 3.1	40.8 ± 5.4	36.5 ± 5.0	41.3 ± 6.6
PA MET/min/wk	8387 ± 4144*	7680 ± 3021*	5991 ± 3775*	6505 ± 2232*	957 ± 385	4722 ± 4129
Menstrual Function (%)						
Oral contraceptive use	10	8	11	8	30	67
Use for regulating	15	12	12	8	37	85

menstrual cycles						
Period stopping more than 3 consecutive months or longer	1.4	3	7	3	14	27

* $p < .001$ significantly different from sedentary group.
† $p < .05$ significantly different from sedentary group.

Table 2. Participant Nutritional Characteristics

Variable	Basketball (B)	Volleyball (V)	Figure Skating (FS)	Synchronized Swimming (SS)	Sedentary (S)
	n = 13	n = 11	n =13	n = 7	n = 29
Energy Intake (kcal/day)	2107 ± 533	2290 ± 572	2152 ± 677	1833 ± 657	1746 ± 441
Delta Energy Intake (kcal/day)	933 ± 685*	712 ± 615	641 ± 660	978 ± 746*	186 ± 476
[a] Vitamin D intake (IU/day)					
NO Supplement	125 ± 46	106 ± 94	195 ± 105	113 ± 123	117 ± 81
+ Supplement	196 ± 302	204 ± 312	377 ± 400	174 ± 486	291 ± 417
[a] Calcium intake (mg/day)					
NO Supplement	628 ± 243	710 ± 357	702 ± 197	866 ± 414	582 ± 209
+ Supplement	628 ± 243	727 ± 370	733 ± 250	866 ± 414	688 ± 429

[a] Micronutrient intakes expressed with the average intake between Food Processor and Food Frequency Questionnaire analyses.
* $p < .001$ significantly different from sedentary group

Table 3. Total Body, Femoral Neck and Lumbar Spine BMD Z-score of all participants

BMD (Z-score)	Basketball (B)	Volleyball (V)	Figure Skating (FS)	Synchronized Swimming (SS)	Sedentary (S)
	n = 13	n = 11	n =13	n = 7	n = 29
Total Body	2.32 ± 1.20*	1.86 ± 1.32†	0.65 ± 0.84	0.93 ± 0.85	0.44 ± 0.86
Femoral Neck	1.68 ± 1.13*	1.70 ± 1.66†	0.56 ± 1.10	1.10 ± 1.20	0.06 ± 1.04
Lumbar Spine	1.65 ± 1.53*	1.53 ± 1.54†	-0.18 ± 0.83	0.01 ± 1.14	-0.32 ± 1.19

* $p < .001$ significantly different from sedentary group.
† $p < .05$ significantly different from sedentary group.

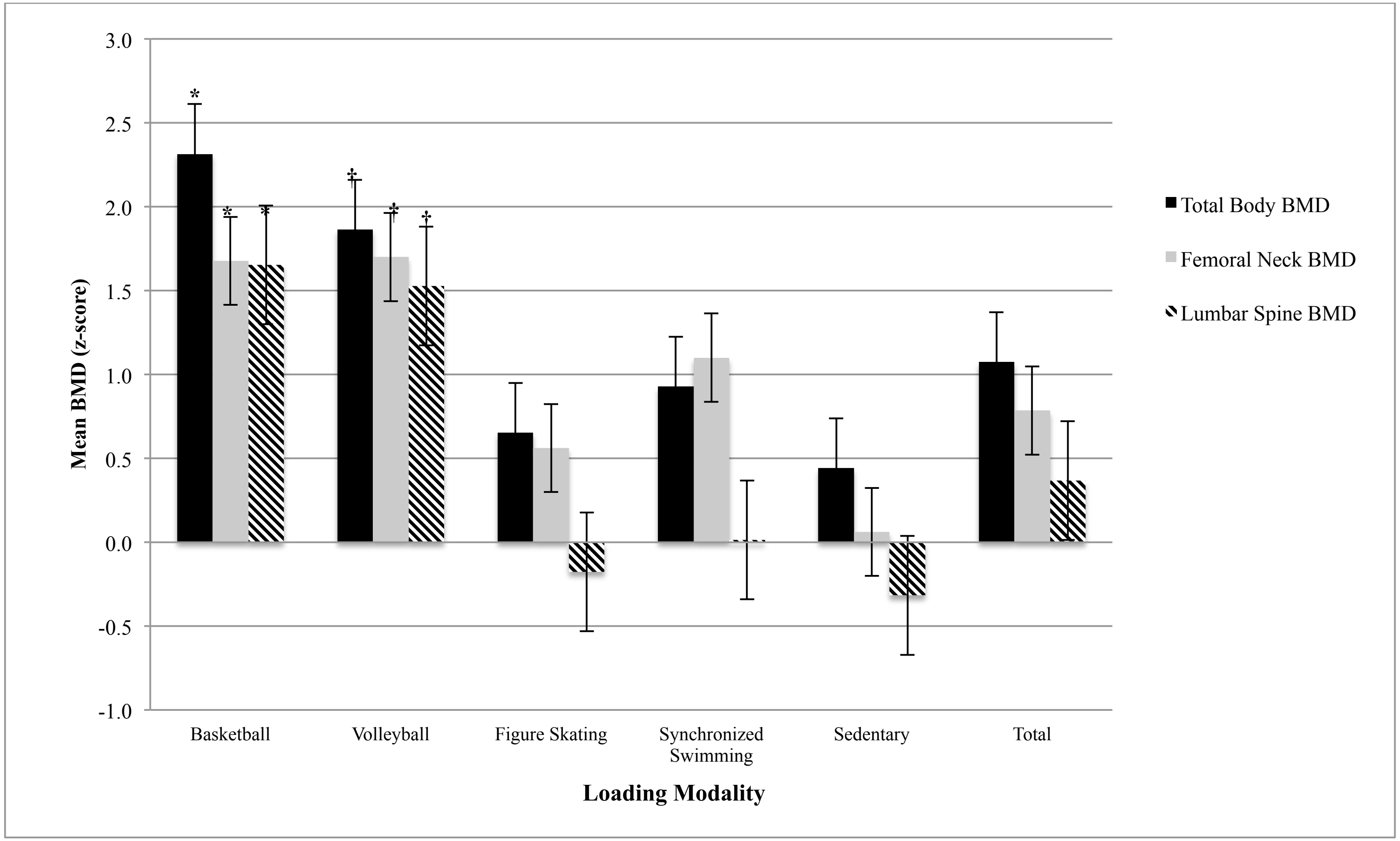

Figure 3. Mean (± SE) BMD values (Z-score) of total body, femoral neck and lumbar spine across different type of sports compared to a sedentary group.
*** *p* < .001 significantly different from sedentary group.**
† *p* < .05 significantly different from sedentary group.

3.0 GENERAL DISCUSSION

3.1 Findings

The principal aim of this study was to determine the influence of the type of sport on regional and total BMD, and its association with dietary energy, vitamin D, and calcium intake. It was hypothesized that: 1) there would be significant differences in bone health among the three different impact sports and the sedentary group on regional and total body BMD; and that 2) there would be a significant association among energy availability, dietary vitamin D and calcium intake with regional and total body BMD in varsity athletes and sedentary students.

One of the main findings of this cross-sectional study suggests that high impact and odd impact sports were positively associated with a higher BMD in female varsity athletes. Basketball and volleyball athletes had higher BMD compared to the sedentary students. These results confirm the previous work conducted by Bagur-Calafat and colleagues (21) who followed 16 elite basketball adolescent girls (age 14-18) over a 3-year period. They showed that basketball players had a significantly greater increase in BMD (specially at the lumbar spine and proximal femur) compared to an aged matched control group ($P = 0.014$) at each follow up period (21).

In contrast, athletes involved in synchronized swimming (non-impact sport) revealed a lower BMD compared to the athletes in other sports, as well as the sedentary group. This suggests that, the coaches and sports nutritionists working with synchronized swimmers need to be actively seeking strategies to revise dryland training and meal planning to improve bone health in these young women.

Standforth et al. (20), conducted a longitudinal and cross-sectional study comparing BMD in athletes across five different sports (basketball, volleyball, soccer, track sprinters and jumpers and swimming) with sedentary participants. Swimmers (non-impact sport) also showed lower values of BMD compared to the athletes in other sports (20). More recently, Tenforde and colleagues (22) evaluated 16 NCAA Division I sports teams, and found that non-impact and low impact sports such as swimming/diving, cross-country running, and synchronized swimming were significantly associated with lower BMD ($P < 0.05$) compared to high-impact and odd-impact sports such as gymnastics, volleyball, basketball, and softball (22).

In our study, athletes participating in figure skating (singles and ice dancers) did not show any difference in their BMD compared to the sedentary group. The figure skaters' Z-scores at the lumbar spine site were negative. Although the nature of the single discipline involves a substantial amount of jumping during training, both disciplines are considered aesthetic sports, where athletes tend to restrict their energy intake to maintain a lean appearance (133). According to all figure skaters' food records, their energy intake was significantly lower than the recommended amounts for this high level of physical activity (reported in the IPAQ), which also may have impacted negatively their bone health.

Additionally, figure skaters reported the highest frequency of menstrual irregularities (4 athletes using oral contraceptives to regulate their menstrual cycles and 5 having an absence of their period for more than 3 consecutive months) (Data available in Table 1). Prelack and colleagues (133), also conducted a study assessing BMD, controlling for dietary intake of energy, calcium, and vitamin D in 36 nationally ranked female adolescent skaters, among 3 disciplines (single, pairs, and dancers) and showed negative BMD Z-scores in the lumbar spine site, as well. They reported that average dietary intake was also below the recommended amounts for their reference population (500 kcals below standard intakes). Vitamin D intake was also below the recommended DRI, and 33% of the participants had calcium intake levels below their recommended intake (133).

One of the most alarming findings in this investigation was that a large proportion of the female athletes consumed significantly less overall energy than the recommended amounts for their level of activity. We found a significant difference between the recommended intake minus actual intake (Delta Intake). The basketball and synchronized swimming athletes had the greatest delta intakes, which were both higher than the sedentary control group (p = .003 and p = .020, respectively). Energy deficiencies are associated with a decline in performance, a higher risk of injury, as well as a decline in BMD, which can lead to stress fractures (64, 65).

Schaal and colleagues (67), recently conducted a research on 9 female synchronized swimmers (mean age 20 yrs) on the French national team, in order to evaluate their energy availability (taking into account energy expenditure from their training sessions, as well as their dietary energy intake) over a 4 week high-intensity training period. They found their energy availability at baseline was already significantly lower than the 30 kcal.kg/LBM/day, which was required to be in energy balance. They also reported that the athletes did not compensate enough for the increase in intense training loads (67). Once again, these findings are consistent with our data.

The low vitamin D intake reported by our participants also coincides with results obtained from previous studies. Halliday et al. (37), assessed dietary vitamin D intake in 41 athletes (18 men and 23 women, mean age = 20 yrs) from the NCAA Division I from the University of Wyoming. The results demonstrated that a very small percentage of athletes (5% in the fall, 6% in the winter, and 4% in the spring) consumed the current RDA of 600 IU from food alone. This increased to 27% in the fall, 18% in the winter, and 20% in the spring, when supplements were included (37).

There are very few sufficient dietary sources of vitamin D, and according to what our participants communicated during their evaluation, many of them were lacking in knowledge regarding which food sources could provide vitamin D. Also, many of them mentioned being unfamiliar with supplementation. Some of them were unsure if they needed it, while others were fearful of intoxication due to improper use. These findings once again highlight the importance of high quality nutrition education and counselling for university athletes.

Other factors such as lack of sun exposure during the long winter, characteristic in northern countries, and ethnicity, can accentuate the low levels of vitamin D, especially among Canadian varsity athletes. It has also been demonstrated that black populations may be at a much higher risk for vitamin D deficiencies compared to white athletes due to racial differences that may be related to genetic variations in Vitamin D-binding protein (VDBP), the primary vitamin D carrier (134, 135).

Calcium intake was not as low as vitamin D proportionally (739 mg/day of calcium intake representing 74% of the DRI, compared to 238 IU/day of vitamin D intake representing 40% of the RDA), since calcium food sources are broader and were easier to identify by our participants. The lowest Calcium intake was found among basketball players (average 628 mg/day). Leachman et al. (90), conducted a study performed in 59 American female collegiate athletes (age 18-23 yrs) from the NCAA Division 1-A. They also revealed that calcium intake was below RDA levels (898 mg/d) (90). More recently, Anderson (136) evaluated the dietary intake of 15 female NCAA Division II volleyball (mean age = 20 yrs) players. He found that 50% of the athletes' calcium intake was below the RDA at baseline assessment, according to their 3 day food records analyzed using the Food Processor Software™ (136).

Public health guidelines suggest that; < 1,000 MET/min/week or < 150 min of moderate to intense physical activity would be the cut point for classifying an individual as "sedentary" (119). We realized that despite of having some sedentary participants that according to their PA level, were considered not that sedentary (PA level in MET/min/week very close or even slightly higher than 1,000), their BMD values (particularly at the lumbar spine site) resulted to be very close to the limit or in the limit to be considered with an osteopenia risk according to their Z-score. Self-report questionnaires may overestimate levels of activity in some individuals. Therefore, future studies should also include objectively measured physical activity with accelerometry to better track their sedentary behaviours and low, moderate and vigorous activity throughout the day.

Information about menstrual status can be a reflection of adequate estrogen levels, which may help identify women at risk for low BMD (72). Current menstrual status is likely to be a good marker for bone density, as demonstrated by studies of amenorrheic athletes (64, 73, 74, 142). Ackerman et al., (75) in 2011, also reported that amenorrheic adolescent athletes had lower BMD than eumenorrheic athletes and non-athletes. They assessed lumbar spine, femoral neck and hip BMD in 16 amenorrheic athletes, 18 eumenorrheic athletes, and 16 non-athletes aged 15-21 yrs. They found that the BMD Z-scores for the lumbar spine site was lower in amenorrheic athletes compared to eumenorrheic athletes and the non-athletic group ($p = 0.007$). Also, Z-scores at the femoral neck and hip sites were significantly lower in amenorrheic athletes compared to eumenorrheic athletes ($p = 0.02$ and $p = 0.0004$, respectively) (75).

In general, menstrual function results assessed by applying the LEAF-Q, revealed that 67% of our participants had their first period between the age of 12-14 years old. These findings correspond with Canadian national data reported by Al-Sahab and colleagues, from the National Longitudinal Survey of Children & Youth in Canada. They estimated that the mean age at menarche was 12.72 years (143). Rotermann and colleagues (144), analysed data form Statistics Canada’s 2007-2009 and 2009-2011 Canadian Health Measures Survey (CHMS), and the estimated that approximately 1.3 million women, aged 15 to 49 yrs were taking OC's in the past month (144). Moreover, 56% of our participants reported using oral contraceptives (OC), and 44% have been using them for 1 year or more.

There has been controversy around the use of OC's. DiVista & Gordon suggested that OC's may not be advice for young hypoestrogenic women with osteopenia or osteoporosis since their mechanism of action conserve the hypoestrogenic status preventing any improvements on BMD (145) . Oral contraceptives may inhibit the hepatic production of insulin-like growth factor (IGF) in young women among other important hormones, which are fundamental elements for bone growth and development during puberty and bone health throughout life (145). Several reports have suggested that OC use in amenorrheic young women can enhance BMD values. Ackerman and colleagues conducted a randomized trial evaluating 121 normal-weight oligo-amenorrhoeic athletes (age 14-25 yrs) engaged in weight-bearing activities to determine the effect of OC (pill) versus transdermal oestogen administration (patch) on bone. BMD was assessed at the lumbar spine, femoral neck, total hip and total body using DXA at baseline, 6 and 12 months. They found that over the 12 months, the patch group improved BMD Z-scores (precisely at the lumbar spine, femoral neck site and hip) better than the pill group ($p = 0.011$, $p = 0.021$, and $p = 0.018$, respectively (140). The type and dose of contraception (which we do not have any details from our participants), lifestyle factors, eating habits (consumption of Vitamin D and calcium), and age at first use may affect the impact of OC use on bone health (146). Still, nutrition therapy should be the first option for addressing low energy availability and bone health problems (141, 147).

In our research, we found a large proportion of our participants reported using OC's for regulating their menstrual cycles. This was concerning given that many of the figure skaters reported that their periods had stopped for 3 consecutive months or longer. According to previous research, young athletes who stop menstruating as a result of strenuous activity consistently have lower bone density, particularly at the lumbar spine, compared to eumenorrheic competitors (13-16, 72). Consistent with the low BMD Z-score values we found in our figure skating participants (-0.2 at the lumbar spine site). Those menstrual irregularities might be very much related to our participants' lower energy intake than their recommendations, which can also increase the risk of RED-S.

Unhealthy habits such as smoking and drinking alcoholic beverages were not taken into account as predictors since only 2.7% of only sedentary participants reported smoking occasionally, and a total of 13.7% reported drinking alcoholic beverages 4 to 6 times per week (6.8% figure skating, 4.1% volleyball, 1.4% sedentary, and 1.4% synchronized swimming participants). Thus, these habits likely not interfere with BMD since few women reported them.

3.2 Strengths and Limitations

To our knowledge, there are no studies available assessing BMD including energy, vitamin D and calcium intake in Canadian female varsity athlete, and taking into account some factors that might influence bone health such as ethnicity, menstrual function, and unhealthy habits (smoking and drinking alcohol).

Implementing a short version of the Food Frequency Questionnaire complemented the 3-day food log analyzed with the Food Processor Software. This approach helped to have more detailed information on the total energy, vitamin D and calcium intakes. Precisely, distinguishing some types of foods participants were consuming in case they did not reported detailed description of each product (i.g. if they recorded eating bread and did not specify which type, the FFQ asks how much of white, dark, whole wheat or high fiber bread they consumed separately).

We also acknowledge the sample size of this study may limit the interpretation of the findings. In addition, we recognize the problems associated with self-reporting energy intake, menstrual history and physical activity. For example, the IPAQ has been found to overestimate activity in women (129, 148). Finally, self-reported recollection of menstrual cycle dates might be less reliable due to any possible gaps in memory, compromising real menstrual function in our participants.

3.3 Conclusions and future directions

In conclusion, we demonstrated that BMD values were highest in volleyball and basketball athletes, which are considered high and odd impact sports, respectively. In addition we did not find any differences in BMD values between the synchronized swimmers, figure skaters and the sedentary control group. No association was observed between BMD and energy intake, vitamin D, or calcium intake. Alarmingly, energy intake records in all our athletes resulted in consuming less than their daily recommendation. Moreover, most of our participants reported a low intake of Vitamin D and calcium, with or without supplement consumption. These data suggest that female varsity athletes should work closely with sports dieticians to promote healthier eating and optimize bone health and possibly performance.

This study may allow researchers to have a better understanding of the important role that weight-bearing activities as well as energy, vitamin D and calcium intake have on bone mineral density and overall bone health in Canadian collegiate female students. This may offer health professionals important insights on how to better optimize bone health in young women and ultimately lower the risk of fractures later in life.

In the future, larger-scale longitudinal studies may provide more detailed insight on how high-impact and odd-impact loading sports are associated with gains in BMD.

Additionally, long-term intervention studies evaluating BMD in conjunction with dietary behaviours might contribute to a better understanding of bone health in female athletes. Finally, this study suggests that Sports Dieticians need to become part of the multidisciplinary team that train and take care of female varsity athletes.

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