BONE STATUS IN YOUNG WOMEN LIVING IN NOVA SCOTIA: SECONDARY ANALYSIS USING QUANTITATIVE ULTRASOUND DATA

by

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Abstract

Background: Low bone mass in young women is associated with a higher risk of developing osteoporosis and osteoporosis-related fractures in later life. **Objective:** This secondary data analysis aimed to assess the relationship between lifestyle factors (high-impact exercise, dairy intake, hormonal contraceptive use, and alcohol consumption) gathered by a questionnaire and calcaneal quantitative ultrasound measures. **Methods:** Healthy young women (n=207) aged 18-25 years completed a diet and physical activity questionnaire, and their bone status was determined by quantitative ultrasound. A novel method, the lifetime osteogenic exercise score (LOGES), which includes the duration, frequency, osteogenic effect of each physical activity, and pubertal stage, was used to quantify bone-related exercise histories. **Results:** Dairy or alcohol intake or hormonal contraceptive use were not correlated with BUA, SOS, and SI. However, participants with high LOGES had significantly higher BUA (dB/MHz) [$F(4, 206) = 2.84$, $P=0.025$], SOS (m/s) [$F(2, 206) = 10.0$, $P= 0.000$], and SI [$F (2, 206) = 7.62$, $p = 0.000$)]. **Conclusions:** High-impact exercise before, during, and after puberty appears to be crucial for women to have stronger bones, and the LOGES system differentiated bone status in young women.

List of Abbreviations Used

PBM	Peak Bone Mass
DXA	Dual-energy X-ray Absorptiometry
pQCT	Peripheral Quantitative Computed Tomography
HRpQCT	High-Resolution Peripheral Quantitative Computed Tomography
QUS	Quantitative Ultrasound
BMD	Bone Mineral Density
BMC	Bone Mineral Content
aBMD	Areal BMD
vBMD	Volumetric BMD
SOS	Speed of Sound
BUA	Broadband Ultrasound Attenuation
SI	Stiffness Index
HC	Hormonal Contraceptive
FFQ	Food Frequency Questionnaire
BMI	Body Mass Index
LOGES	Lifetime Osteogenic Exercise Score
ASBMR	American Society for Bone and Mineral Research
ACSM	American College of Sports Medicine
AR	Activity Rating
D	Duration of Activity
F	Frequency of Activity
P	Pubertal Stage

YS	Years Since
SPSS	Statistical Package for the Social Sciences
SD	Standard Deviation
ANOVA	Analysis of Variance
BPAQ	Bone-specific Physical Activity Questionnaire
MET	Metabolic Equivalents of Task
IPAQ	International Physical Activity Questionnaire
GRF	Ground Reaction Forces
MVPA	Moderate to Vigorous Physical Activity

Chapter 1: Introduction

Low bone mass in young adulthood is associated with a higher risk of developing osteoporosis and osteoporosis-related fractures in later life (1, 2). Bone is accrued rapidly during adolescence, with peak bone mass (PBM) occurring between ages 20 to 30 (3, 4). However, peak bone mass at some sites such as calcaneal has been achieved in young adults by the age of 19 (5, 6). This suggests that there is considerable opportunity to optimize bone accretion in early-stage development (7, 8). This may be particularly important for women who are often at a greater risk of low bone mass and osteoporosis-related fracture in later life (1).

Bone mass and structure in are often assessed by dual-energy X-ray absorptiometry (DXA) for clinical use. PQCT is used primarily in research and is mostly unavailable for clinical use (9). However, these methods are not commonly used in healthy women due to resource allocation issues and radiation risk. Quantitative ultrasound (QUS) is a lower-cost, effective, non-invasive method of estimating bone status and provides an alternative to radiation-based bone assessment (10).

Different factors are associated with bone accrual and loss of bone tissue. Some of these factors are considered non-modifiable, such as genetics, ethnicity, and chronic diseases, whereas others are considered modifiable, such as lifestyle behaviors, like physical activity, nutrition, consumption of alcohol, and hormonal contraceptive use (11). This study is a secondary analysis of data from the study “Does exercise mitigate the negative effects of oral contraceptives on bone in young women?”. This study aims to assess the relationship between lifestyle factors (high-impact exercise, dairy intake, hormonal

contraceptive use, and consumption of alcohol) and bone status (Stiffness Index, broadband ultrasound attenuation, and speed of sound) amongst healthy young adult women living in Nova Scotia.

1.1. Objectives of study

1.1.1. Study purpose

This secondary data analysis aims to assess the relationship between lifestyle factors (osteogenic exercise, dairy intake, contraception uses, and alcohol) and bone status in a cohort of young women (n=227) from Halifax, Nova Scotia. These data were originally collected for two studies with identical protocols, one that aimed to determine if high levels of physical activity during childhood and adolescence mitigated an adverse effect of hormonal contraceptives on bone status in young women, and a second study that investigated whether use of hormonal contraceptives immediately post-puberty has a negative effect on the bone status of young women.

1.1.2. Specific objectives

Primary objective:

1. To assess the relationship between high-impact exercise before, during, and after puberty, as assessed by the novel method, Lifetime OsteoGenic Exercise Score (LOGES), and bone status as assessed by quantitative ultrasound at the calcaneus in healthy young women.

Secondary objectives:

2. To assess the relationship between dairy intake (i.e., self-reported dairy product consumption) and bone status (i.e., Stiffness Index, Broadband Ultrasound Attenuation, and Speed of Sound assessed by quantitative ultrasound at the calcaneus) in healthy

young women.

3. To assess the relationship between hormonal contraceptive use via a questionnaire about current use, history of use, period of use, and bone status as assessed by quantitative ultrasound at the calcaneus in healthy young women.

4. To assess the relationship between alcohol consumption and bone status as assessed by quantitative ultrasound at the calcaneus in healthy young women.

Chapter 2: Literature review

2.1. Bone growth and development

Development of the human skeleton begins in the embryo, where cartilage formation is replaced by bone in the process of endochondral ossification (12). The bone grows in length and width. Bone mass accretion in both women and men dramatically increases before and during puberty (13). Long bone growth stops as the growth plates close at puberty. High estrogen levels at the end of puberty result in growth plate closure and thereby ending longitudinal bone growth (14). However, bone growth in width continues and causes bone shape and mechanical changes (15). Bone growth and development are made of complex interactions which depend on genetic and environmental factors (16).

2.1.1. Bone cells and structure

Bone is a mineralized tissue that includes osteoblasts, osteocytes, and osteoclasts (17, 18). Osteoblasts are responsible for new bone formation (19). They synthesize and secrete bone matrix and regulate the calcium and phosphate balance by participating in bone mineralization during bone development (20). Osteocytes, which comprise 90–95% of all bone cells, reside within the bone matrix (21). They descend from osteoblasts and are situated to respond to changes in physical forces on the bone (22). Osteoclasts cells are responsible for aged bone resorption by degrading bone and mediating bone loss in pathologic conditions by increasing their resorptive activity (23).

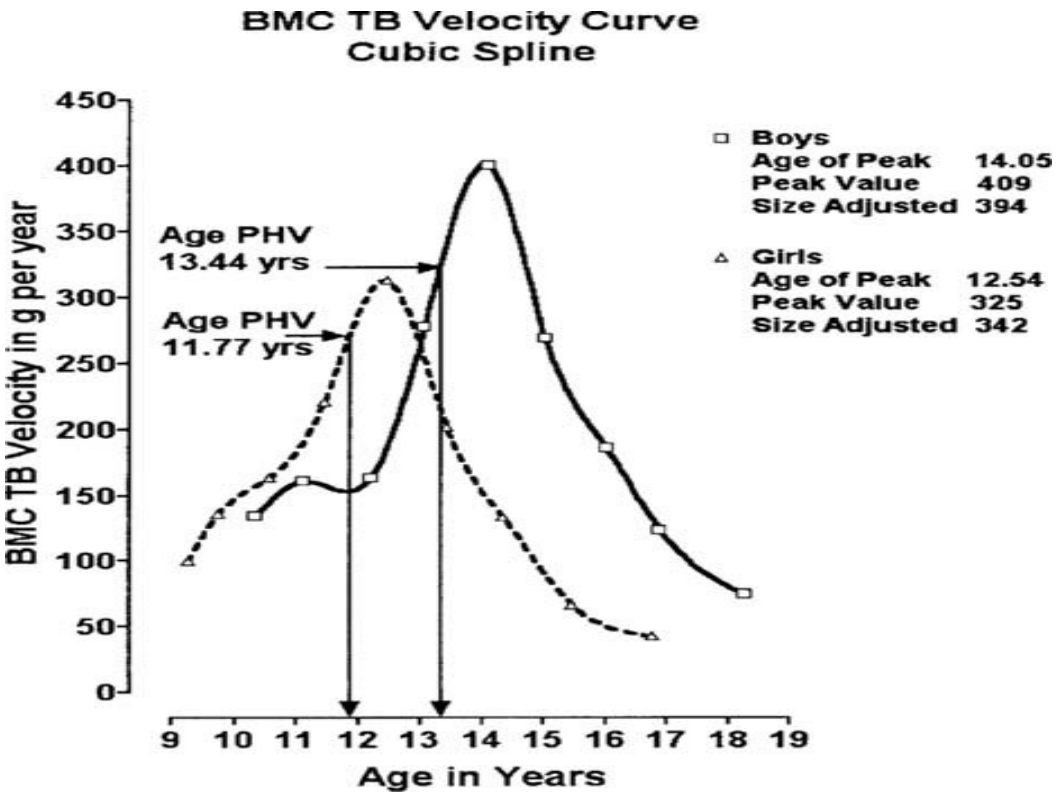
The bone has cortical and trabecular compartments. Cortical bone forms external parts of long bones and therefore constitutes the outer shell of trabecular bone (24). On the other hand, the trabecular bone can be found in the ends of long bones. It is more porous, and

the open weave of bone allows for active metabolic exchange (25).

2.1.2. Building bone during young adulthood

Before age 18, bone mineral content increases, applying mainly to bone size and cortical thickness (1, 26). After the age of 20 years old, most of an adult's bone mass, geometry, and microstructure will be achieved by the remodeling of endosteal surfaces (27). In turn, maximal bone mass, often termed peak bone mass, is usually reached between 20 and 30 years of age (27). Although peak bone mass is higher in men than women (1), studies showed it occurs earlier in women, related to differential timing in pubertal growth and closure of epiphyses, for linear length (1-3). Studies showed that childhood and adolescence are the most critical periods of skeletal mineralization (28). Also, the amount of bone accrued during the growing years is an important risk factor for fractures in later life (29). In the Saskatchewan Pediatric Bone Mineral Accrual Study, Bailey et al. showed that the amount of bone mineral accumulated during the adolescent years is substantial (Figure 1) (30). They demonstrated that the amount of bone gained in the 2 years preceding peak bone accrual velocity is comparable to the amount of bone mass lost throughout 30 years of adult life.

Figure 1: Total body (TB) peak BMC velocity (PBMCV) curve illustrating velocity at peak and ages at peak BMC and peak height velocities by chronological age for boys and girls. PHV = peak height velocity(30).



After achieving the peak bone mass, ongoing bone remodeling usually leads to cortical and trabecular bone loss over time in both sexes. However, this loss is varied in weight-bearing and non-weight-bearing bones (31-33). Although family history, genetic heritability, sex, and race are the main factors that affect peak bone mass, diet and physical activity are responsible for up to 25% of peak bone mass (34).

Nutrients and food components can potentially have a positive or negative impact on bone health (35). They may affect bone by various mechanisms, such as changing the bone structure, alteration of bone metabolism rate, and homeostasis of calcium and possibly of other bone-active mineral elements (36). These dietary factors include inorganic minerals such as calcium, magnesium, phosphorus, and some trace elements,

vitamins, and macronutrients (35). Calcium is an essential element for the body and is crucial in mineralizing the skeleton. Bones and teeth store more than 99% of the calcium in the body as calcium hydroxyapatite (37). Calcium plays an important function in normal heart rhythms, blood clotting, helping muscles contract, and nerve functions. Calcium in the bone also provides skeletal strength, acts as a reservoir of calcium to be released into the bloodstream, and helps these critical metabolic needs through bone remodeling (38).

2.1.4. Bone and mechanical loading

Many studies were conducted to figure out the response of bone to mechanical loading. Based on Wolff's Law, which Julius Wolff developed in the 19th century, our bones adapt to the loads placed upon them and become thicker and stronger over time. On the other hand, if there are no forces to act against, the bones become less dense and weaker due to the lack of the stimulus required for continued remodeling (39, 40). The relationship between bone remodeling and mechanical loading is usually assumed to follow the so-called Mechanostat Theory. The Mechanostat theory, first proposed by Harold Frost in 1987, proposes that a mechanism is present that monitors all the bone processes, such as the modeling and remodeling of the bone (41, 42). Therefore, when a bone receives a mechanical stimulus, it has the ability to detect if necessary thresholds of strain have been reached and, if so, the adaptation of the bone to increase bone strength will occur (43, 44). In 2008, Alexander Robling and coauthors published a set of experiments that elucidated the molecular mechanisms through which mechanical stimuli on bone translated to greater bone strength (45). The Wnt/Lrp5 pathway is essential to bone formation. However, the presence of sclerostin (Scl) in bone blocks Wnt from

combining with Lrp5, thereby blocking bone formation. When osteocytes in a bone sense a mechanical stimulus that is of sufficient magnitude, they reduce or stop producing sclerostin, and bone formation can therefore proceed. Robling et al. demonstrated that the intensity of stimuli differs in different parts of a long bone, which is directly reflected in the degree of Scl loss (45). Later research determined that the Wnt signaling pathway also plays an important role in the osteogenic differentiation of mesenchymal stem cells. Even in these initial stages of cell differentiation into bone cells, Wnt signaling activation leads to bone formation (46).

2.2. Bone measurement techniques

Different measurement techniques evaluate skeletal status, assess osteoporosis, and determine fracture risk (47, 48). However, non-invasive measurement of bone mineral content (BMC) (g) and bone mineral density (BMD) ($g*cm^{-2)}$) measured by dual-energy x-ray absorptiometry (DXA) remains the gold standard for clinical purposes recommended by the World Health Organization (WHO) and other guidelines for clinical use (49, 50). BMD is an independent predictor of fracture risk and is strongly associated with bone strength. BMD is influenced by many factors such as genetics, diet, exercise, and smoking which may affect the outcomes (51). Therefore, BMD and bone turnover markers are more commonly used as surrogate outcomes in studies on premenopausal women (52). Also, BMD testing is used to monitor bone treatment in a clinical setting. However, DXA has limitations, and there are several other non-invasive methods used to estimate skeletal strength and frequently used in a research setting.

2.2.1. A summary of common ways to assess human bone in vivo

Several techniques are available to estimate bone strength. These measurement techniques characterize different contributing factors to bone strength, such as BMD, areal BMD (aBMD*)) ($g*cm^{-2}$), volumetric BMD (vBMD**) ($g*cm^{-2}$), Speed of sound (SOS***) (m/s), broadband attenuation (BUA****) (dB/MHz), bone geometry, and, in some cases, microarchitecture (53). Of these methods, the most common are DXA, pQCT, and QUS, which will be described briefly in further sections.

2.2.2. Dual-energy X-ray absorptiometry

2.2.2.1 What is DXA?

DXA is the most commonly used method for clinically assessing BMD and the risk of osteoporosis (50). It is a two-dimensional imaging x-ray technique that assesses the attenuation of x-ray beams as they pass through tissues with different densities (53). It has excellent repeatability and, therefore, permits monitoring the effectiveness of bone therapy over time. It can evaluate BMD in the whole body or specific sections of it (50).

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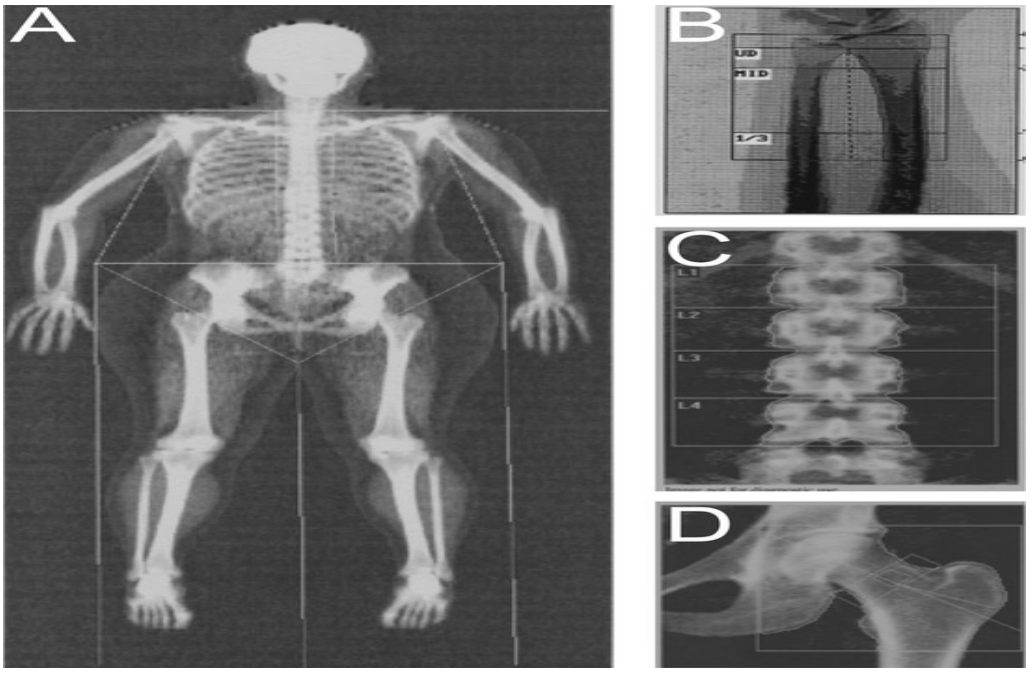
*aBMD: BMC divided by area, usually measured by DXA.

**vBMD: BMC per volume of bone, usually measured by CT or MRI.

***SOS: measured by QUS

****BUA: measured by QUS

Figure 2: DXA scan images (A: whole body, B: forearm, C: lumbar spine, D: hip) (54)



2.2.2.2 What does DXA measure?

DXA can be used to analyze the whole body to assess the mineral content of the entire skeleton (54). DXA can also look at site-specific bone and measures aBMD in the spine and hip, as these are considered clinically relevant sites due to being especially debilitating in osteoporosis patients (50). In addition, a software system called the hip structural analysis algorithm can be used to estimate the structural strength of the proximal femur (55). However, DXA cannot measure three-dimensional bone geometry and differentiate BMD between cortical and trabecular fractions (56).

2.2.2.3 How does DXA measure bone?

DXA emits two low-dose x-rays through the targeted tissue, which are absorbed differently by bones and soft tissues depending on the intensity of density and thickness of the tissue. Therefore, BMD is evaluated by analyzing the density profiles from these x-rays (54).

2.2.3. Peripheral quantitative computed tomography (pQCT)

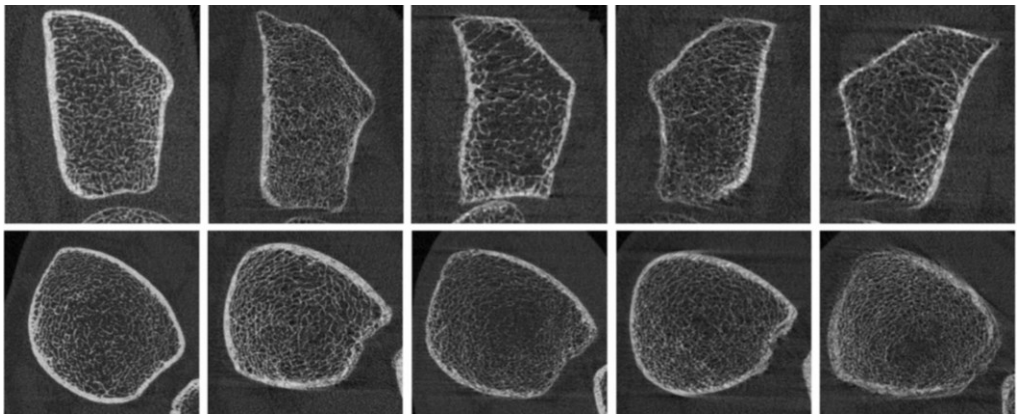
2.2.3.1. What is pQCT?

Peripheral QCT is a three-dimensional imaging technique that uses computed tomography equipment to construct three-dimensional bone images. These images provide information on bone geometry as well as a volumetric evaluation of cancellous and trabecular bone density because CT can differentiate between the cortical and trabecular bone fractions (53). This methodology is used primarily in research and is mostly unavailable for clinical use. High-resolution peripheral quantitative computed tomography (HRpQCT) is a 3-dimensional scanner that can perform a virtual bone biopsy and allows us to look at the bone structure in greater detail with superior sensitivity for bone changes and abnormalities (57).

2.2.3.2. What does pQCT measure?

pQCT measures vBMD of trabecular and cortical bone architecture and geometry. These measures include bone volume (mm^3), bone surface (mm^3), trabecular and cortical thickness (mm), cortical porosity (%), trabecular number (per mm), and trabecular and cortical BMD (mg $HA*cm^{-3}$) (57). From such data, the bone structural strength in compression, bending, and torsion can be estimated (58, 59)

Figure 3: pQCT scans of radius (top row) and tibia (bottom row) (283)



2.2.3.3. How does pQCT measure bone?

Since the density of cortical and trabecular bone compartments differs, pQCT analyzes x-ray beam differential attenuation to create bone images, allowing for the separation of trabecular and cortical measures and a better understanding of skeletal status (53, 58). Detailed information from pQCT on bone density, geometry, size, and strength, may better predict skeletal fragility than bone density alone (60-62).

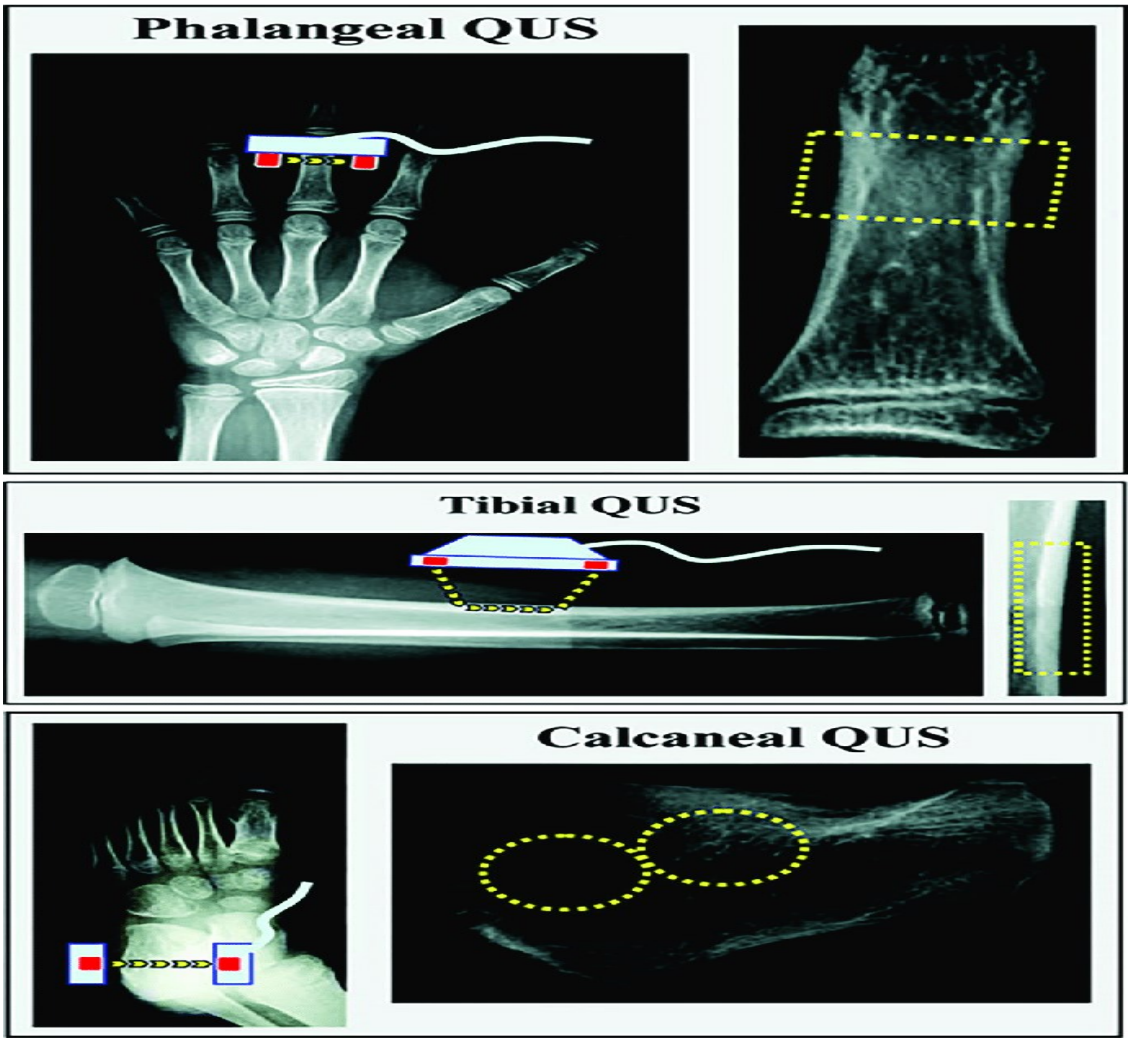
2.2.4. Quantitative ultrasound (QUS)

2.2.4.1. What is QUS?

QUS is a scanning technique that uses sound waves to assess the bone status and identify individuals at high risk for osteoporosis-related fractures (50, 63). QUS emits no radiation, requires relatively little training, and the instrument is substantially less expensive to purchase than those used for DXA or pQCT. In addition, it requires less physical space and is somewhat portable. Its lower cost makes it attractive to measure the amount of bone and its material and structural properties (64-67). Also, the low cost of QUS, not using ionizing radiation, and the ability of sound waves to incorporate several

bone properties such as BMD and bone elasticity have significantly increased its utilization (68).

Figure 4: QUS images (Phalangeal, tibia and calcaneal)



The elastic nature of ultrasonic waves in QUS appears to be useful in the examinations of bone tissue quality (69). QUS assesses bone mineral status in some peripheral skeletal sites such as calcaneus, phalanges of the hand, and tibia (70). QUS measurements of the calcaneus have been shown to be effective in predicting osteoporotic fractures with a similar performance to hip DXA (71-76). Also, the scan is quick, comfortable for

patients, and has a user-friendly design that makes it easy to operate (77).

The calcaneal site is the most common location to measure QUS and determine bone status. Reasons for the selection of the calcaneus include that there is a considerable body of data to which results can be compared because, compared to the other bone segments, more QUS research has been performed on the calcaneus (78). Secondly, 95% of the calcaneus is trabecular bone, which response to factors faster than cortical bone (79). Most QUS instruments are designed to be used on the calcaneus. The various iterations of the Achilles Insight instrument have been the most used QUS system worldwide. They measure BUA (dB/MHz), SOS (m/s), and a composite variable, resulting from the SOS and BUA combined, the Stiffness index (SI) (80). However, other QUS devices have been designed to be used on the tibia, radius, and/or finger (71). These devices also differ in coupling methods and parameter calculation algorithms (71). As the sound waves pass through the bone, QUS measures the SOS and any changes in BUA (81).

2.2.4.2. How does QUS measure bone?

QUS devices generate pulsed acoustic waves with a range of centre frequencies between 500 kHz and 1.25 MHz, depending on the manufacturer. While Calcaneal QUS devices use a thermally controlled water bath for transmission of the ultrasound waves, others use coupling gel (Phalangeal) or gel-free system using isopropyl or ethylic alcohol (70%).

In calcaneal QUS devices, unique piezoelectric probes are positioned on each tissue side to emit and receive sound waves that pass-through bone longitudinally or horizontally (81). QUS then measures ultrasound parameters in bone, including SOS and/or the frequency dependency of the attenuation of the ultrasound signal, or BUA (82). In

general, SOS and BUA are lower in osteoporotic bones with less trabecular mass than in non-osteoporotic bone; BUA is lower because there are fewer trabeculae in the calcaneus to attenuate the signal, and the SOS is lower because, with the loss of mineralized bone, the elastic modulus of the bone is decreased. SI, calculated from BUA and SOS, is the sum of the scaled and normalized BUA and SOS values. The SI combines BUA and SOS into a single measure that has a lower precision error than either variable alone (205). The Achilles instrument uses the following formula, SI = ((0.67*BUA + 0.28*SOS) – 420).

2.3. Factors that influence bone

Different factors are associated with bone mass accrual and can influence bone health during childhood and adolescence. Some of these factors are considered non-modifiable, such as genetics, ethnicity, and sex, whereas others are considered modifiable, such as lifestyle behaviors (e.g., physical activity, nutrition, smoking, caffeine, consumption of alcohol, and hormonal contraceptive use) (53).

2.3.1. Non-modifiable factors

Non-modifiable factors such as genetics, ethnicity, sex, and pubertal timing can significantly affect bone strength (83). Heritable factors could explain 60–80% of the variability in bone mass and osteoporosis risk (53). Racial differences are associated with various rates of BMD gain (84). For instant, cross-sectional studies on premenopausal and postmenopausal women showed white women have bigger bone sizes at the distal radius and distal tibia compared with Asian women. However, Asian women had thicker, denser cortices and thicker trabeculae (85). Moreover, men and women have differences in BMC and BMD, especially after puberty (84, 86, 87). Puberty is an important period in

life that is characterized by endocrine-initiated reproductive maturation and noticeable growth in the skeleton (88, 89). Although puberty timing is not modifiable (90, 91), studies showed that later puberty may be associated with lower BMD in adolescence and adulthood (92, 93) and a higher risk of osteoporosis later in life. However, the association between puberty timing and long-term bone accrual from early life up to adulthood is still unclear (94).

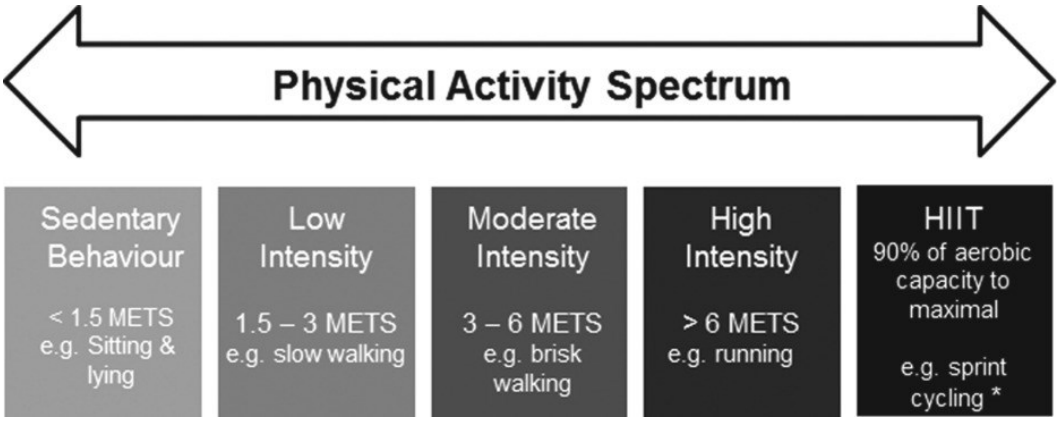
2.3.2. Modifiable factors

Previous studies suggested that modifiable factors are also associated with bone strength and the prevalence of osteoporosis (53, 95). Diet and physical activity are the main modifiable factors affecting bone health (96). Here, we review four main modifiable factors that contribute to bone strength in most healthy, non-smoking young women.

2.3.2.1. Physical activity and exercise

Physical activity includes any form of body movements such as occupational, sports, conditioning, household, or other activities resulting from using energy. On the other hand, exercise is a planned, structured, and subset of repetitive physical activity that uses extra energyy to improve or maintain physical fitness (97).

Figure 5: Physical activity spectrum (285)



Physical activity is a low-cost activity that is widely accessible and associated with bone health. It is a modifiable factor that contributes to peak bone mass and strength and is one of the most common recommendations as an effective intervention for improving bone health among women of all ages (98). Exercise causes mechanical signals to be sent to the skeleton. These signals may initiate a cascade of molecular responses, which can lead to greater bone apposition (99, 100). In the last few years, many clinical and laboratory studies have been conducted to address strategies for maximizing the osteogenic effects of exercise (53, 101). Among different kinds of exercises, the evidence for the high impact, such as jumping and running, is the most robust and recommended by International Osteoporosis Foundation and other agencies for osteoporosis prevention (99, 102, 103). In addition, investigations showed that weight-bearing activity in both sexes has the most significant positive associations with BMD (104). a cross-sectional study on more than 200 athletes confirmed the positive effect of high-impact, multi-directional, and repetitive low-impact exercise loading on bone thickness (105). Ground-based exercises that impart strain rapidly to the skeleton, such as vertical jumping, are also known to affect bone strength positively (105). It has been shown that

multidirectional loading exercises, such as ball sports, are associated with high-ground reaction forces that could lead to higher peak bone mass accrual, more fracture-resistant bones, and more strength in bone (106-108). Although common physical activities such as cycling and weighting have been shown to be beneficial to bone health and strength, they cannot rival in terms of apparent osteogenicity with multi-directional exercises such as racket games, step aerobics, speed skating, or soccer (109, 110). According to Wolff's Law, these results could be meaningful, suggesting that the greater bone loading forces result in stiffer and more fracture-resistant bones. On the other hand, activities that include many hours in a limited-strain environment, such as slow walking, provide little benefit to the skeleton.

Recently, more research, including systematic reviews, meta-analyses, and clinical trials, that focused on the effects of exercise programs on bone in girls, premenopausal women, and postmenopausal or older women has provided new insights. For example, a recent study showed that high-intensity resistance and impact training is associated with the higher proximal femur and lumbar spine density and geometry in postmenopausal women (111). Also, bone adaptation to mechanical loading is greater during growth than after maturity (112), and weight-bearing exercise has been shown to increase bone strength during adolescence (113). Moreover, a review indicated that high-impact exercise alongside weight-bearing and aerobic training might prevent a decline in bone mass with aging (114). Similarly, a meta-analysis that investigated the effect of exercise interventions for improving bone health, as measured by QUS of the calcaneum, stated that 4 to 36 months of exercise is associated with significant improvement in calcaneum BUA across the age spectrum (115).

Similarly, a meta-analysis and a review showed that high-impact, short-duration jumping (116), and high-impact short-bout exercise (117) could significantly improve femoral neck BMD and trochanter BMD. However, a systematic review that assessed the effects of exercise training on whole-bone strength found no significant overall effects of exercise on bone-strength-related parameters (118). Therefore, studies are needed to determine the amount of osteogenic physical activity required to improve the bone status and, if it occurs at certain times of development, whether it makes a difference to the final outcomes of young adult bone.

Only a few, mostly animal studies, have aimed to determine the duration, dose, and timing of the physical activity needed to maximize the development of bone strength (119-121). One meta-analysis defined the effectiveness of high-impact and short-duration two-legged jumping exercises in women (116). In this meta-analysis, Zhao and colleagues reported that skeletal response is highly sensitive and site-specific to jumping exercises. They also indicated that the bone response to mechanical stimuli might increase with short rest intervals between loading bouts during jumping exercises (116). Another study showed that high-intensity resistance and impact training is associated with the higher proximal femur and lumbar spine density and geometry in postmenopausal women (111). Interestingly, a 12-month randomized control trial investigated the relationship between quantities (both magnitude and rate) of strain and changes to the bone measured by HRpQCT in young women (122). Results showed that the mechanical strain rate and quantity, as well as the number of loading bouts all contributed to small but significant changes in bone.

Historically, researchers have tried to develop methods to predict what types of physical

activity could be recommended for developing a strong skeleton. They used different methods to code the physical activities by type and by intensity. There are different methods to assess physical activity such as self-report questionnaires, self-report activity diaries, direct observation, and using devices such as accelerometers, pedometers, and heart-rate monitors (123). Differences in the methods used limit the comparability of results across studies.

To facilitate research in this area, a list of physical activities needs to be comprehensive, flexible enough to meet the researchers' needs, and coded with a standardized system. Such a list can be applied to the physical activities done by any one participant, whether collected by diary recall or direct observation methods (124).

Metabolic equivalent of task (METs) is defined as the ratio of metabolic rate in specific physical activity compared to a reference which is the amount of energy used while sitting quietly (125). In METs, various types of activities can be grouped by purpose and intensity, which provides flexibility in calculating the energy cost of physical activities. Physical activity intensity can also be indicated using METs and translated into light, moderate, and vigorous intensities of exercise (124, 126). METs are usually used to categorize cardiorespiratory fitness.

Most vigorous exercise that affects the heart also affects the skeleton (127, 128), such as soccer and jumping. However, METs can not catch the main characteristics of osteogenesis during specific physical activities, specifically mechanical load magnitude and application rate (127). Also, water sports, such as swimming and rowing, can result in high METs values but exert insufficient strain and/or strain rates on the skeleton (129). Moreover, METs require a sustained elevated heart rate, but only a few repetitions of

sufficient mechanical strain are needed to improve bone. Although METs might not represent the loading of bone, they are still used in some bone and osteoporosis studies (130). In 2006, Dolan et al. showed that physical activity loading scores demonstrated more consistent positive associations with bone mass compared to physical activity measured in METs (131). While METs failed to record the key characteristics of force and loading rate associated with physical activities, Dolan et al. presented a bone-loading history questionnaire (BLHQ) alongside DXA measures to estimate loads on the hip and spine experienced during different life stages in premenopausal women. However, their key objective was to identify women at risk of osteoporosis early, not to determine which physical activity was most effective for developing a strong skeleton. Therefore, their focus was on clinically relevant sites for osteoporosis. However, the BLHQ is a time-consuming instrument and cannot provide load factors for physical activities from direct measures of force (132).

In another study, Weeks and Beck introduced a brief bone-specific physical activity questionnaire (BPAQ) to record both current and historical activity and applied loading values of ground reaction forces (GRF) (132). BPAQ records the frequency of current and historical activity, load intensity, and years of participation in physical activity. It is designed for both sexes and can predict variance in indices of bone strength at clinically relevant sites.

Weeks and Beck compared BPAQ with other common physical activity measures for its ability to predict parameters of bone strength in young adults. They showed that BPAQ is a better tool to predict bone strength at skeletal sites at risk of osteoporotic fracture than BLHQ and other traditional physical activity measures (132). Cross-sectional studies also

have found positive associations between BMD and bone-loading physical activities estimated by BPAQ scores (133-135). A study used the BPAQ method and examined historical self-reported bone in postmenopausal women, suggesting that self-reported osteogenic exercise affected bone even years later. In addition, it shows the benefits of bone-specific physical activity in early and later life for maintaining bone health in women (136). Similarly, a recent study investigated the differences in BMD based on alcohol consumption behaviors and bone-loading history using BPAQ and BMI and found a positive effect of bone-loading physical activity to increase bone mass before peak bone mass formation (137). However, BPAQ does not consider the duration of activity (132) and does not demonstrate acceptable validity against accelerometer data (138).

2.3.2.2. Dairy intake and bone

Nutritional factors contribute to the acquisition of bone mass during young adulthood (35, 139, 140). Studies have revealed that nutritional risk factors such as low calcium, vitamin D intake, and alcohol intake are associated with developing fragile bones (140). The health value of dairy foods is expressed in most food-based dietary guidelines (141). Dairy provides nutrients such as calcium, protein, and phosphorus, which are positively related to BMD and BMC (142). These nutrients can be poor in diets with limited or no dairy products, such as vegan or dairy-restrictive diets (143, 144). A study found that milk and milk plus calcium supplementation has a beneficial effect on the arm, spine, and whole body in young women (145). Also, a Canadian population-based cohort study showed positive associations between dairy intake and lumbar spine BMD (146). Another study reported that White girls aged 15–16 years showed an increase in trochanter BMC

and trochanter, spine, and femoral neck BMD after 2-years of supplementation with dairy products (147). A prospective cohort study showed that higher calcium intake from dairy products is associated with higher aBMD at various vertebral sites over a 7-year follow-up period in 10 to 18 years old girls (148). In another cohort study reported in 2008, after a 12-year follow-up period, daily consumption of more than 2 servings of dairy compared to weekly consumption of 2 servings of dairy had beneficial effects on BMC in the arms, trunk, ribs, and pelvis in adolescents aged 15- to17-years (149). Also, another cross-sectional study from 2007 found that calcium from milk consumption is associated with higher lumbar spine BMC and BMD compared to other dietary sources of calcium in postmenarcheal girls ages 12 to 22 years (150).

In contrast, some observational studies investigating the association between long-term dairy consumption and bone health and fractures have suggested that dairy consumption is associated with a greater risk of fractures (151, 152). However, longer follow-up and including dairy products other than milk may have affected these results. Also, other large prospective studies showed that higher milk consumption during adolescence or adulthood leads to greater hip fracture risk in both men and women (152, 153). As a result, the relevance of dairy products in reducing osteoporotic risk is still controversial.

Studies primarily assess milk intake in various quantities or servings to estimate the consumption of dairy products (154). However, many interventions used other dairy products (i.e., yogurt and cheese) along with milk (147, 155-158). Although yogurt and cheese have higher nutrient concentrations on a weight basis and smaller serving sizes than milk, calcium bioavailability among various dairy products and milk are mostly the same (159, 160). Some studies measuring dairy intake ignore the effect of combinations

of foods consumed in different dietary patterns (161, 162). Some interventions evaluated dairy consumption using a dietary history questionnaire (163, 164). The questionnaire could include items on dairy products or questions about servings per day/week (154, 165). There are also several dietary assessment instruments available to assess dairy consumption, including diet records, 24-hour dietary recalls, and Food Frequency Questionnaires (FFQ) (166).

2.3.2.3. Hormonal contraception and bone

Hormonal contraceptives have been used by many women since their first approval in 1960 (167). They mainly reduce estrogen and suppress progesterone, the two sex steroids produced by the ovaries, thereby decreasing the risk of unintended pregnancy (168). Oral hormonal contraceptives are also prescribed for such non-contraceptive benefits as reducing menstrual bleeding and lowering acne and may also reduce the risk of ovarian and endometrial cancers (169). Estrogen is an important hormonal factor associated with bone mass. During adolescence and young adulthood, estrogen has an important effect on achieving peak bone mass and the risk of osteoporosis later in life (170-173). High estrogen levels at the end of puberty result in growth plate closure (14). Also, it has been shown that estrogen inhibits bone remodeling activation and bone resorption (174).

Progesterone has also been shown to have a similar effect and act in partnership with estrogen to achieve optimal peak bone mass (175). The dosages present in hormonal contraceptive formulations determine the circulating level of sex steroids. If the sex steroid levels were insufficient due to hormonal contraceptive formations, they might negatively affect bone tissue metabolism (176). It has been suggested that hormonal contraceptives, when taken during puberty, may affect different aspects of bone

development, such as decreasing bone turnover within the cancellous bone, which increases bone density (177), or closure of the growth plates, which determines the length of the bones (178).

Most studies that evaluated the effects of HCs on bone, investigated the impacts on BMDs (179). Conducting a randomized controlled trial that truly assesses a fracture risk endpoint in young people is difficult, due to the decades and multiple life factors of free-living humans.

Data derived from different studies on young women who take HC are conflicting. A systematic review on young women showed that HC has no effect on BMD and fracture rates (180). Similarly, a prospective cohort study on 245 women 18-39 years old found no difference in percent changes in the spine, hip, or total body between HC users and controls after 36 months (181). Similarly, cross-sectional studies evaluating BMD in perimenopausal women also found no significant differences in bone outcomes between HC users and nonusers (182-184). In contrast, other studies in young women indicated that HC use in the first 3 years post-menarche is associated with lower BMD (185). Investigations revealed that taking a low dose (20 mcg Ethinyl estradiol) of HC could lead to lower rates of bone mineral accrual in teenagers (185). Similarly, a 12-month study on adolescent girls aged 12-18 found smaller BMD gains in teens taking a low-dose HC (n=79) compared to nonuser controls (n=107) (186). A Canadian observational study investigated the total hip BMD in 16-19 years old girls who had never used HC versus those who had. In this study, those who had never used hormonal contraceptives had significantly greater gains in total hip BMD compared with those who had taken hormonal contraceptives (187). There is no perfect method of assessing contraception

use. Direct observation, self-reports, clinic/pharmacy records, pill counts, and electronic Monitoring Devices are the common approaches to measuring hormonal contraceptive use. Self-reported data on contraception use is often the standard approach for contraception research and provides data comparable to previous studies. However, it is not so reliable and prone to social desirability bias (188).

2.3.2.4. Alcohol intake and bone

Alcohol is a non-essential nutrient and a unique, addictive substance many people consume. High consumption of it causes intoxication and can damage the brain, liver, muscles, and skeleton (189). Alcohol is usually used for cultural or entertainment purposes, and almost one-third of people are current drinkers worldwide (190). It can have adverse social effects on the user's family and society and may increase the chance of committing crimes and violations (191). Studies indicated that the rate of alcohol consumption is as important as the amount of drinking in producing intoxication and stimulation (192-194). Therefore, the consumption rate is an important indicator of alcohol use.

Heavy alcohol intake is associated with lower BMD and more fractures (195, 196). However, the effect of light-to-moderate alcohol intake on bone status is still controversial (195-199). One study reported that drinking two or more glasses of alcohol per day could lead to lower hip and spine BMD in men (200). Also, it has been shown that there is a significant risk for osteoporosis in young women after drinking 5-24 g of alcohol per day (201). Studies proposed a J-shaped association of alcohol intake (heavy to light or moderate) with fracture risk (198, 202). A recent meta-analysis assessed 46,916 individuals with BMD assessment and 240,871 individuals with a risk of fracture

(203). It indicated that compared to no alcohol consumption, three or more standard drinks of alcohol per day are associated with a higher risk of hip fractures. Also, higher alcohol consumption increased the risk for other osteoporosis-related fractures, but this observation was not statistically significantly (203). Moreover, experimental studies measuring osteocalcin level (a measure of bone formation) before and after periods of abstinence (7 days to 2 years) for heavy drinkers (6-17 drinks per day) revealed that osteocalcin increased significantly after abstinence from drinking (204-207) . It has been suggested that alcohol may disrupt the balance of calcium concentration in the bloodstream, which is required for the nerves and muscle proper functioning (208). Alcohol also affects the hormones that regulate calcium metabolism and the hormones that influence calcium metabolism, such as steroid reproductive hormones and growth hormones (209).

In contrast, cohort studies suggested that alcohol consumption is positively associated with femoral neck bone density (210-213). They found a linear relationship between each drink per day and increased femoral neck bone density. Furthermore, Dennison et al. (214), Baron et al. (215), and Rejnmark et al. (216) showed that women with greater alcohol consumption had lower bone density loss. A recent meta-analysis confirmed these findings and showed that up to two standard drinks of alcohol per day is associated with higher lumbar and femur neck BMD values than non-drinkers (203). Also, one standard drink of alcohol has been shown to be related to an increase in hip BMD compared to no alcohol consumption (203). Recently, the focus of research on alcohol consumption at the population level is on patterns of consumption. There are different ways to assess alcohol consumption (217). Each alcohol consumption collection data

technique has a different approach and set of assumptions to convert data on consumption to the volume of alcohol consumed (218). However, the most common way in studies is by using direct questions included in FFQs (203). Due to the mixed result in the literature, we wanted to determine the association of alcohol consumption with bone status.

2.3.2.4. Gaps in the literature

The evidence for the high impact exercise such as jumping and running and fast multi-directional ground-based exercises such as soccer, are the most robust and recommended by many agencies for osteoporosis prevention (98). However, the amount of osteogenic physical activity and whether it makes a difference to final outcomes if it occurs at certain times of development of young adult bone is still unclear. Also, a comprehensive method that could be used to code physical activities that includes information such as duration of activity and puberty stage is missing.

The relevance of dairy products for increasing BMD and reducing osteoporotic risk is still controversial. Some studies found beneficial effects on bone mineral content and bone mineral density (148-150); on the other hand, some observational studies have suggested that dairy consumption is associated with a greater risk of fractures (151, 152).

Data derived from different studies on young women using H.C. are conflicting. Some studies found no effect or benefits on skeletal health as assessed both BMD and fracture risk (180, 181, 219). In contrast, other studies in young women indicated that Hormonal contraceptive use is associated with lower bone minerals, especially in the first 3 years post-menarche (186, 220).

The effect of alcohol on bone is still controversial. Some studies found alcohol associated

with a higher risk of fractures and lower bone mineral density (200, 221). On the other hand, some studies found a positive or no effect of greater alcohol consumption on bone density loss (222, 223).

Chapter 3: Research questions and hypotheses:

The aim of my thesis is to assess the relationship between lifestyle factors (high-impact exercise, dairy intake, consumption of alcohol, and hormonal contraceptive use) gathered by a questionnaire and calcaneal quantitative ultrasound measures.

3.1. Research questions:

The research questions for this project are:

Primary question:

1. What is the relationship between osteogenic exercise history acquired retrospectively and bone status in young women?

Secondary questions:

2. What is the relationship between current dairy intake and bone status in young women?
3. What is the relationship between current and history of hormonal contraceptive use and bone status in young women?
4. What is the relationship between current alcohol consumption and bone status in young women?

3.2. Hypotheses

The hypotheses associated with the research questions above are:

1. Women with a more robust osteogenic exercise history have higher BUA, SOS, and SI.
2. Women with higher dairy intake have higher BUA, SOS, and SI.
3. Women with lower hormonal contraceptive use have higher BUA, SOS, and SI.
4. Women with lower alcohol consumption have higher BUA, SOS, and SI.

Chapter 4: Methods

4.1. Study design

4.1.1. Original study

This study was a secondary analysis of data from the original study "Does exercise mitigate the negative effects of oral contraceptives on bone in young women?". Secondary data analysis is a study in which researchers use the information collected by someone else for new purposes. Secondary data analyses usually answer a new research question or examine an alternative perspective on the original question of a previous study.

The original study sought to investigate whether the use of hormonal contraceptives at a young age has a negative effect on the bone status of young women and, if so, whether exercise could mitigate the negative effect on their bone status. They used an Achilles InSight bone ultrasonometer (224, 225) to determine SI, BUA (dB/MHz), and SOS (m/s) and a questionnaire to measure participants' basic information, diet and exercise history, age at menarche, and hormonal contraceptive history. Data collection occurred in three phases. The first phase was between January 21 to February 25, 2009; the second phase occurred from October 29 to November 30, 2009, and the third phase occurred on the 19th and 20th of January 2010. The consent forms and the inclusion and exclusion criteria were similar during all three data collection phases. However, the inclusion criteria were slightly different in the second data collection, where the upper limit for age was 24 years while the other two were 25 (see Appendix). These original studies recruited 226 young women, and records from 207 women were used for the current

analyses. Twelve records were excluded due to incomplete exercise histories, 6 others were excluded because the subjects did not meet the age requirements for this analyses, and one record was missing. The following table (Table 1) explains the inclusion and exclusion criteria.

Table 1: The inclusion and exclusion criteria for all phases.

Inclusion	**Exclusion**
- Women - Age 18-25 (phases 1&3) -Age18-24 (phase 2) - Non-smokers	- Are or have been pregnant - Are or have been post-menopausal through surgical, medically induced, or natural menopause - Have been diagnosed with osteoporosis - Having a specific underlying disease that could affect vitamin D absorption, bone metabolism, and/or bone density - Took medications that could adversely affect bone metabolism or vitamin D- absorption

4.1.2. Secondary data analyses study

In this secondary data analysis, we used the data from the original study, which had been collected through primary sources, to assess the relationship between four lifestyle factors (high-impact exercise, dairy intake, hormonal contraceptive use, and consumption of alcohol) and bone status (SI, BUA, SOS) amongst healthy young adult women living in Nova Scotia. We included all 207 participants with complete data sets from the original research with the same inclusion and exclusion criteria and the upper age of 25.

4.1.3. Ethics

The original study protocols were approved by the Research Ethics Committee of Capital Health (Ethics number: CDHA-RS/2009-263 & CDHA-RS/2008-034) in accordance with the Declaration of Helsinki. Before initiating this research work, informed consent forms were obtained from all the participants. Ethical approval was not needed for the secondary data analyses because it is covered by the original study protocols (see Appendix).

4.2. Measurements:

Initially, each eligible participant completed the informed consent form and clarified any study details. Dalhousie University Kinesiology students Nina Laroche, Britney MacMurter, Kendra Bertin, and Vanessa Slayter completed the informed consent with participants and did the data collection in three phases. We reported the results of each wave separately because there were no differences within and between each data collection as a total.

4.2.1. Anthropometry

Prior to a data collection session, each participant's height and weight were measured using a stadiometer (m) and standard scale (kg), respectively. All participants removed their outdoor shoes and jackets but otherwise remained fully clothed during the height and weight measurement. Body Mass Index (BMI) was calculated as mass (kg) divided by height squared (m2), or BMI= $kg*m^{-2}$.

4.2.2. Questionnaires

Each subject completed a questionnaire that included her age, race, education level,

exercise history, and diet history. This questionnaire was developed specifically for this study. The information on the lifestyle that the participants provided included dairy servings they consume daily (0-1, 2-3, 4 or more), calcium-fortified foods intake (everyday, at least once a week, at least once a month, no intake), calcium supplement (everyday, taking multivitamin, no intake), and vitamin D supplement (in multivitamin, separate, no intake), and their alcohol intake (no drinks, ≤ 2units/day, 0-1/week; ≤ 7 units/week, ≤ 2 days/wk; > 5 units at one time, ≥ once/wk). The questionnaire also asked about their fracture history in their lifetime, medications, age of menarche, past and current hormonal contraceptive (HC) use, age of starting, and duration of HC usage (<6 months, ≥6 months, ≤1 yr, 1-2 yr, 2-3 yr, >3 yr) and the type of HC used (see Appendix). The questionnaire was developed by Dr. Jo Welch (Bryant) specifically for this study.

4.2.2.1. The lifetime osteogenic exercise score (LOGES)

Participants were asked to list all past and/or current sports, types of training, or physical activity they participate in and/or have participated in, in their lifetime, approximate start and end dates, and hours per week they spent engaged in each activity (see Appendix). The Lifetime Osteogenic Exercise Score (LOGES) combined information from the questionnaire's exercise data with the pubertal stage at which each activity was performed. The LOGES equation was developed by Dr. Jo Welch (Bryant) and students Tara Dahn and Alex MacDonald to estimate the effect on the skeleton of a single entry in an exercise history. It has not been published yet; however, it was presented at the American Society for Bone and Mineral Research (ASBMR) conference in 2010 and the American College of Sports Medicine (ACSM) Annual Meeting in 2011.

$$LOGES = (AR^2*D*F*P)/ [(YS/2) +1]$$

AR = Activity rating: Each sport or exercise was assigned a ranking based on the effects of that sport or similar activity on bone, as reported in peer-reviewed papers (See Table 2).

Physical activity with greater amounts of osteogenic movement, including high loading rates, has been shown to be most effective in improving bone microarchitecture and strength (226).

D = Duration of activity: The time over which the activity took place.

F = Frequency of activity: The number of hours per week

Both time spent in a specific activity and the pattern of doing it might affect bone accretion during and after puberty (227).

P = Pubertal stage when activity was performed:

1= not during puberty (either before or after),

2= during puberty,

3= before and during puberty.

Puberty was defined as beginning 2 years before menarche and ending 2 years after menarche

Studies showed that physical activity during puberty is an important predictor of BMD and BMC at young ages (228). However, the effect on bone could be varied with normal to vigorous physical activity (229).

YS = Years since the activity was last practiced

Bone is living tissue; it changes in response to the forces placed upon it over time. Regular exercise helps the process of bone remodeling and, as a result, builds more bone tissue and creates a denser bone (230).

LOGES is calculated for each exercise activity, and then a sum of those scores provides a total score for each subject.

Table 2. Examples of exercise types assigned to each activity rating

Activity Rating	**Examples of exercise types assigned to each activity rating**	
5	Gymnastics	Sprinting
4	Soccer	Basketball
3	Running	Weight training
2	Skiing	Dance
1	Walking	Softball
0	Swimming	Kayaking

4.2.2.2. Reliability and validity of LOGES

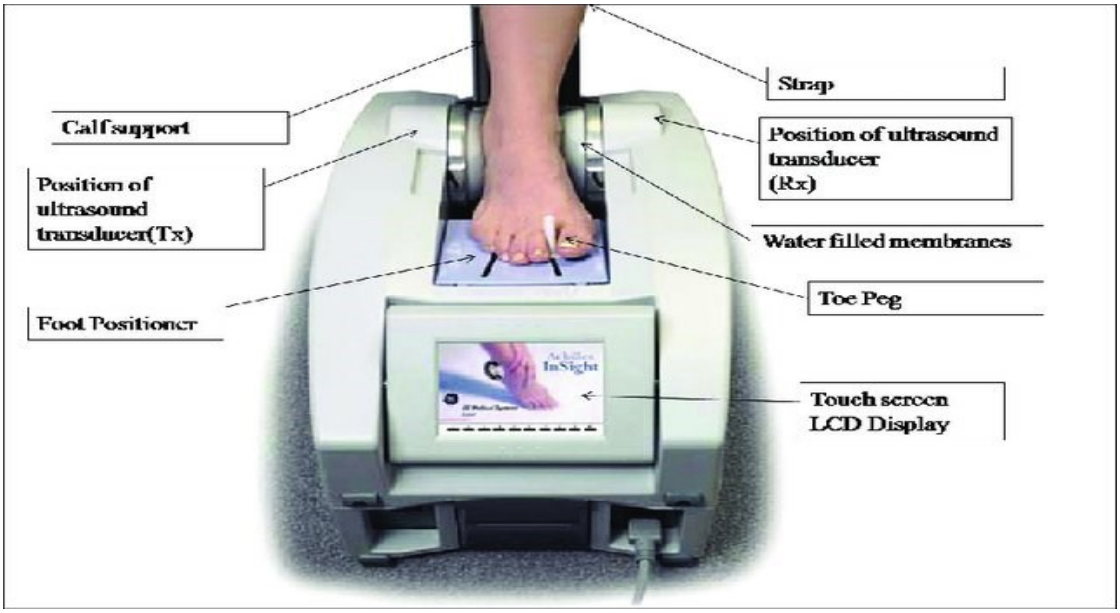
To test the reliability of the LOGES scoring system, 8 university undergraduate students who were unfamiliar with the field of exercise and bone health were provided with 5 minutes of verbal explanation and a short written description of the scoring system. Each novice rater then scored 5 randomly selected subjects from their initial questionnaires. Pearson's correlation coefficient for inter-rater reliability was $R = 0.996$.

4.2.3. Measuring bone status

An Achilles InSight Quantitative Ultrasonometer (QUS) (General Electric, WI, USA) was used to perform the calcaneal bone tests. The testing method was performed based on the recommendations of the manufacturer. Before each testing session, a calibration assessment was performed using a manufacturer-provided phantom to measure quality assurance. Scans were conducted from the right heel of each subject, SOS (m/s) and BUA (dB/MHz) were measured by the instrument, and then the SI was calculated. The heel was surrounded by warm water that was encapsulated between inflated membranes. The

water served to couple the sound between the transducer and the foot.

Figure 6: The working position and the components of the QUS device (231)



Achilles Insight measurements are performed with the person seated, with one foot placed on the Footplate. Water is the best medium to transmit the ultrasound. So warm water encapsulated between inflated membranes surrounded the heel. On one side of the heel, a transducer transforms an electrical signal into a sound wave. This wave passes through the water and the person's heel. On the opposite side of the heel, another transducer receives the sound wave and transforms it into an electrical signal that can be analyzed. The analysis includes measuring SOS, BUA, and SI. In general, SOS and BUA are lower in osteoporotic bones with less trabecular mass than in non-osteoporotic bone; BUA is lower because there are fewer trabeculae in the calcaneus to attenuate the signal, and the SOS is lower because, with the loss of mineralized bone, the elastic modulus of the bone is decreased. SI, calculated from BUA and SOS, is the sum of the scaled and normalized BUA and SOS values. The SI combines BUA and SOS into a single measure

that has a lower precision error than either variable alone (232). The Achilles instrument uses the following formula, SI = ((0.67*BUA + 0.28*SOS) – 420) (233).

4.2.4. Statistical analyses

All data were analyzed using a statistical package for the social sciences (SPSS) for Windows, version 26 (SPSS Inc., Chicago, IL, USA). Descriptive statistics (i.e., mean, standard deviation, maximum and minimum) were calculated for age, weight, height, and BMI. Frequency statistics were calculated for dairy intake, alcohol intake, and contraceptive use. The mean of accumulated LOGES for each person was also calculated. Continuous variables were presented as mean values ± SD, while categorical variables were presented as frequencies. The data distribution was assessed using the Kolmogorov–Smirnov test. For normally distributed data, the following test was used to answer each research question, as presented in Table 3.

Table 3: Tests that were used to answer each research question.

Research questions	Variables	Test
What is the relationship between osteogenic exercise history acquired retrospectively and bone status in young women?	**Independent:** dairy servings consumed daily **Dependent:** BUA, SOS, SI	One-way ANOVA
What is the relationship between current dairy intake and bone status in young women?	**Independent:** LOGES **Dependent:** BUA, SOS, SI	Pearson Correlation / Divided into 5 groups → One-way ANOVA
What is the relationship between current and history of hormonal contraceptive use and bone status in young women??	**Independent:** Length of HC, history, and current use. **Dependent:** BUA, SOS, SI	One-way ANOVA
What is the relationship between current alcohol consumption and bone status in young women?	**Independent:** Alcohol consumption **Dependent:** BUA, SOS, SI	One-way ANOVA

One-way ANOVA was used to test for the significant differences in bone status (BUA, SOS, SI as continuous variables) among dairy intake categories, hormonal contraceptive use categories, and alcohol intake categories (categorical variables). Pearson's correlation coefficient was used to measure the association between LOGES and bone status (BUA, SOS, and SI). ANOVA helps to find out whether the differences between groups of data are statistically significant. It works by analyzing the levels of variance within the groups through samples taken from each of them. Three ANOVA assumptions were met. The data were independent of each other and were normally distributed. Also, the population had the same variance tested by Levene Test. To compare the low and high physical activity, we tested the effects of dividing the participants into 3, 4, 5, and 6 categories of equal numbers of subjects based on their LOGES. We then used one-way ANOVA to determine the significant differences in BUA, SOS, and SI among categories. Less than 5 categories did not show significant differences, and higher than 5 had too few participants in each category. When 5 categories (quintiles) were tested, some differences emerged. Pairwise comparisons were then assessed using the Tuckey post hoc test to determine within-group differences. Bar charts were used to visualize the data. A P-value less than 0.05 was considered statistically significant for all statistical analyses.

Chapter 5: Results

The characteristics of the participants in this study are presented in Table 4. The frequencies of each categorical variable, in particular dairy and alcohol intake, and hormonal contraceptive use, are shown in Table 6. The data collection occurred from January 21 to February 25, 2009, October 29 to November 30, 2009, and 19 to January 20, 2010. There were no differences between each data collection wave in age, height, weight, and BMI ($p<0.05$). Since there were no differences between waves; they were combined for all subsequent analyses and will be referred to as one cohort for the rest of the thesis.

Table 4: Descriptive data for the study population (n = 207). Data are means ± SD. None of the means were statistically different (p ≤ 0.5). BMI = Body mass index.

	Wave1 (n=79)	**Wave2 (n=21)**	**Wave 3 (n=107)**	**Total (n=207)**
Age (year)	21.9 ± 1.7	20.9 ± 1.5	20.7 ± 3.6	21.2 ± 2.9
Height (cm)	167.2 ± 7.3	165.4 ± 5.3	165.3 ± 13.1	166.2 ± 10.6
Weight (kg)	63.9 ± 11.3	61.6 ± 7.7	64.8 ± 9.3	63.9 ± 10
BMI ($kg*m^{-2}$)	22.8 ± 3.5	22.5 ± 2.9	23.9 ± 4.6	23.3 ± 4.1

The mean for the LOGES values is shown in Table 5.

Table 5: Mean LOGES values for the study population (n = 207). Data is mean ± SD

	Total
LOGES	1137 ± 1589

We divided the participants into five equal groups based on their LOGES values (low, med-low, medium, med-high and high). An analysis of variance (ANOVA) yielded significant variation among the 5 groups on BUA [F(4, 206) = 2.84, P=0.025], SOS [F(2, 206) = 10.0, P= 0.000] and SI [F(2, 206) = 7.62, p = 0.000)].

A post hoc Tukey test showed that participants in the high LOGES group had significantly greater values than those in the other 4 quintiles in BUA, SOS, and SI at $p < 0.05$ (Figures 9,10, and 11). There was no significant difference among other groups ($p>0.05$).

Figure 7: Broadband ultrasound attenuation (BUA) means in LOGES quintiles. The highest LOGES quintile was significantly greater than the other 4 quintiles (p < 0.05).

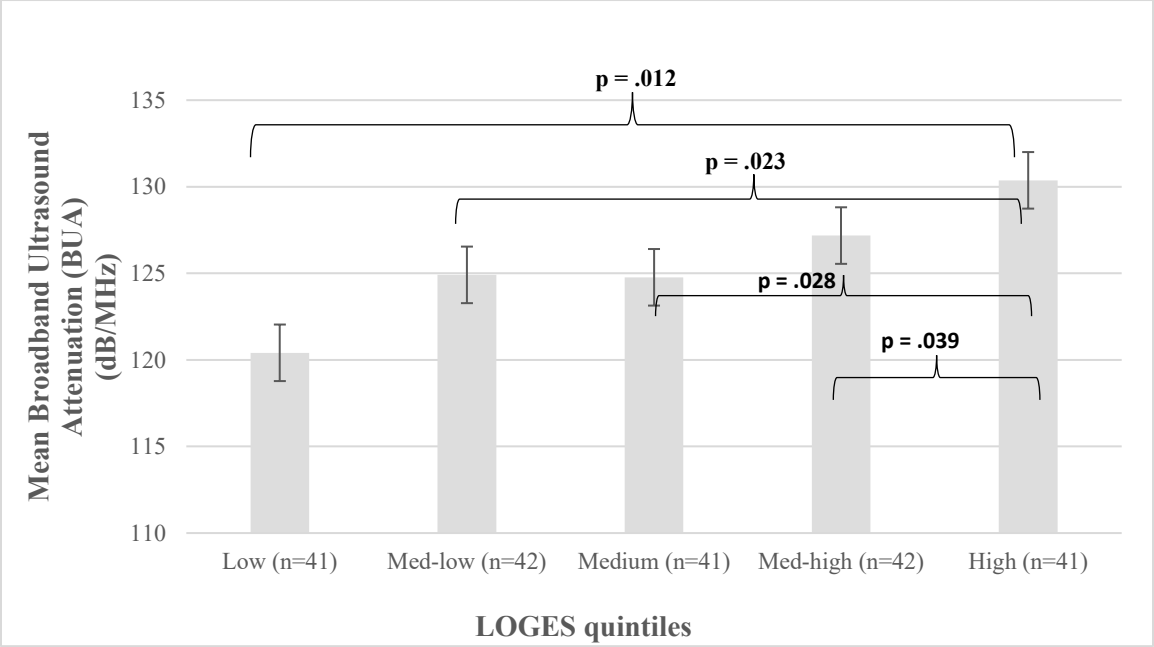


Figure 8: Speed of sound (SOS) means in LOGES quintiles. The highest LOGES quintile was significantly higher than the other 4 quintiles at p < 0.05.

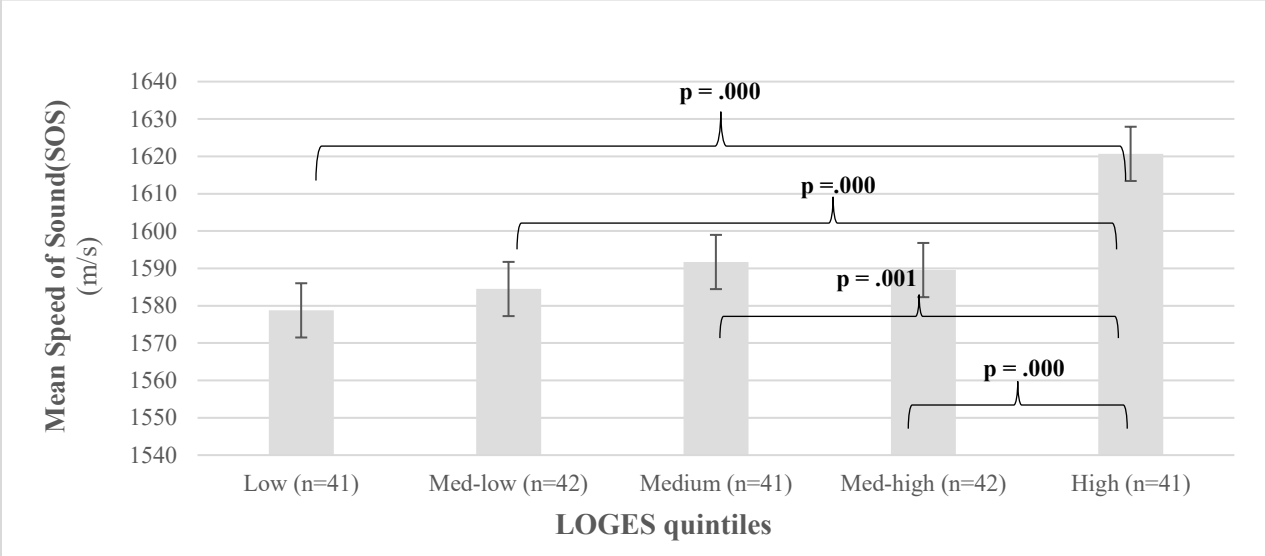


Figure 9: Stiffness index (SI) mean in LOGES quintiles. The highest LOGES quintile was significantly higher than the other 4 quintiles at p < 0.05

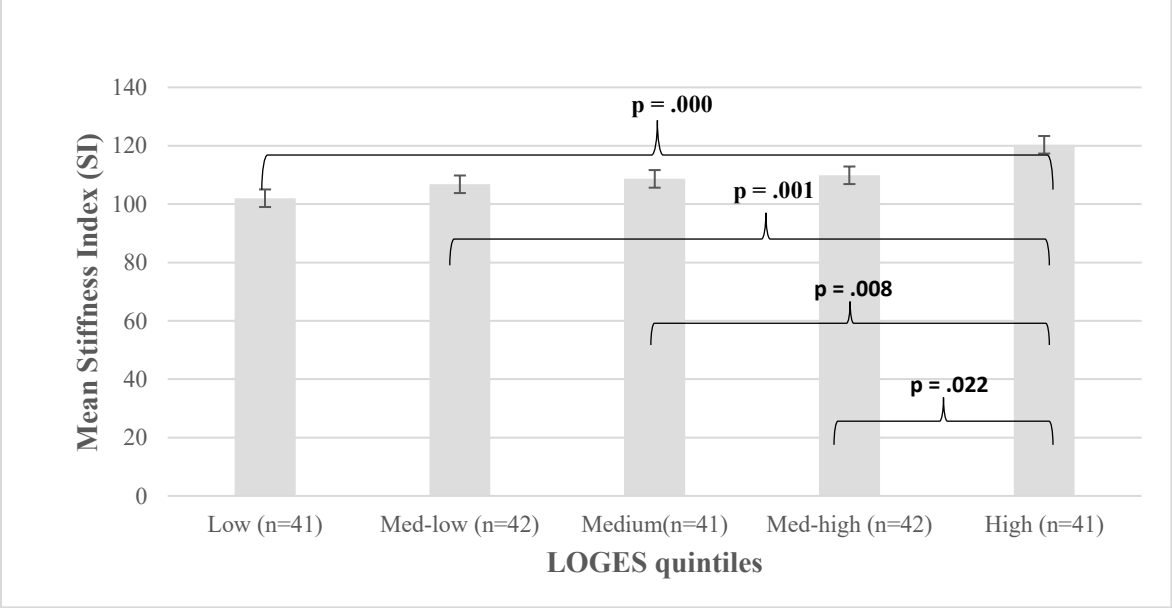


Table 6: Frequency data for the study population (n = 207).

	Categories	Total n (%)
DAIRY INTAKE (servings/d)	0-1	44 (21.2)
	2-3	144 (69.5)
	≥4	19 (9.1)
ALCOHOL (Units)	No alcohol	17 (8.2)
	<2/day, ≤1d/wk	111 (53.6)
	≥7/wk, ≤2/day	26 (12.5)
	≥5 units/bout, ≥1/week	53 (25.6)
CURRENTLY CONTRACEPTIVE USE	yes	125 (60.3)
	no	82 (39.6)
HISTORY OF CONTRACEPTIVE USE	no	40 (19.3)
	yes	167 (80.7)
LENGTH OF CONTRACEPTIVE USE	<6 months or never	48 (23.1)
	≥6 months, ≤1 yr	12 (5.7)
	1-2 yr	13 (6.2)
	2-3 yr	37 (17.8)
	>3 yr	97 (46.8)

For dairy intake, there were no significant differences among the three dairy intake categories in BUA [F(2,204) = 0.18, *p=0.8*], SOS [F(2,204) = 2.32, *p=0.1*] and SI [F(2,204) = 0.59, *p= 0.5*]. For alcohol intake, there were no significant differences among the four intake categories in BUA [F(3,202) = 0.85, *p= 0.5*], SOS [F(3,202) = 0.32, *p= 0.8*] and SI [F(3,202) = 0.67, *p=0.6*].

There was no relationship between the length of contraceptive use and BUA [F(4,205)= 1.5, p=0.2], SOS [F(4,205)= 1.0, *p=0.4*], and SI [F(4,205)=1.8, *p=0.1*]. Also, there was no relationship between the history of contraceptive use and BUA[F(2,205)= 0.4, *p=0.6*], SOS [F(2,205)= 0.23, *p=0.8*], and SI [F(2,205)=0.18, *p=0.8*]. There was no relationship between current contraceptive use and BUA [F(2,205)= 0.42, *p=0.3*], SOS [F(2,205)= 0.23, *p=0.2*] and SI [F(2,205)=0.18, *p=0.2*]

Chapter 6: Discussion

This study assessed the relationship between four lifestyle factors (high impact exercise (LOGES score), dairy intake, hormonal contraceptive use, and consumption of alcohol) and bone status (SI, BUA, and SOS), using QUS technology amongst healthy young adults women living in Nova Scotia. We found a significant difference between participants in the high LOGES score group and each of the other 4 groups in BUA, SOS, and SI. Also, there were no significant differences between BUA, SOS, and SI for dairy intake, HC, and alcohol intake.

6.1. Physical activity and bone

Participants in the highest quintile of LOGES had significantly higher BUA, SOS, and SI values than did women in each of the other four quintiles of LOGES. This is similar to the results reported in many other studies, that high-impact exercise has a positive effect on bone in young women (30, 99, 234-236).

Many researchers have tried to develop methods to predict what types of physical activity could be recommended for developing a strong skeleton. Different methods have been used to code physical activities by type and intensity. However, differences in methods limit the comparability of results across studies. A list of physical activities should be comprehensive, flexible enough to meet the researchers' needs, and coded with a standardized system in order to facilitate research in this area. This list of physical activities done by any one participant can be collected by diary, recall, or direct observation methods (203). METs can group various types of activities by purpose and intensity (124). However, METs cannot catch the main characteristics of osteogenesis

during specific physical activities, specifically mechanical load magnitude and application rate (127). BLHQ was developed to estimate loads on the hip and spine experienced during different life stages in premenopausal women. However, it is a time-consuming instrument, and its focus is on clinically relevant sites for osteoporosis, so it cannot provide load factors for physical activities from direct measures of force (132). BPAQ was a better tool to predict bone strength at skeletal sites at risk of osteoporotic fracture than BLHQ and other traditional physical activity measures (132). It records the frequency of current and historical activity, load intensity, and years of participation in physical activity. It is designed for both sexes and can predict variance in indices of bone strength at clinically relevant sites (132).

Our study used a novel approach to estimate bone loading by enhancing the BPAQ methods to a more comprehensive equation. While BPAQ includes frequency of current and historical activity, load intensity, and years of participation in physical activity, LOGES includes duration, frequency, years of participation, and rate of physical activity alongside the puberty stage on bone status. In our study, the significant difference of the participants in the highest quintile compared to the other groups strongly suggested that high-intensity exercise performed before and during puberty is important for bone strength. The results also indicated that to have a stronger skeleton, osteogenic physical activity should be continued after puberty.

6.2. Dairy intake on bone

We found no significant relationship between dairy intake and bone values. This finding contrasts with several previous studies that found some benefits of dairy products on bone mass accrual (155, 158, 237-239). Cohort studies showed a positive effect of dairy

intake on young women's bones (145, 147-149). Also, review studies found that calcium from milk consumption was associated with higher BMC and BMD (240, 241). A recent systematic review revealed that consuming 16 months of dairy products during childhood and adolescence in various quantities leads to 8% greater gain of BMD (242). Although there is evidence that dairy intake during childhood and adolescence could increase BMD during growth and adulthood (243, 244), its association with fracture risk in adulthood is still controversial. That may be mostly due to the large inaccuracy of food intake recorded years later (243). Short-term clinical trials showed that increased cow milk intake resulted in changes in bone turnover markers in favor of bone retention (243). In a meta-analysis, vegans who avoid dairy products had a lower lumbar spine and femoral neck BMD compared to omnivores and vegetarians who avoid meat and fish but consume dairy products (245). Another meta-analysis showed a dairy-rich diet is associated with a 41% lower prevalence of low BMD (246). A study on 3251 women aged 20–49 years showed that consuming less than one portion of milk weekly during childhood is associated with 5.6% lower BMC in adulthood compared with those who had consumed more than one portion per day (247).

Torres-Costoso et al. investigated the relationship between milk consumption and BMD in young adults. They revealed that lower dairy intake was associated with higher BMI and adipose tissue percentage. They also showed that the effect of milk consumption on bone development is indirect and is fully mediated by body composition variables in young adults (248). These findings are in contrast with our study, which found no relationship between dairy intake and bone values.

Similar to our results, some studies have reported no benefit of a higher dairy intake to

the skeleton. A cross-sectional study on 1522 men and 1104 women aged 32 to 81 found no association between higher intake of dairy products (milk, yogurt, and cheese) with higher trabecular and vBMD in women (249). Additionally, some evidence shows that dairy products' positive impact on BMD may depend on adequate serum vitamin D levels (250). Also, a meta-analysis found no association between the group with high milk consumption and the risk of osteoporotic fracture and hip fracture (251). Some other observational studies on the association between long-term dairy consumption and bone health and fractures suggested that dairy consumption is associated with a greater risk of fractures (151, 152). However, longer follow-up and including dairy products other than milk may have affected these results. Also, some large prospective studies showed that higher milk consumption during adolescence or adulthood might be associated with greater hip fracture risk in both men and women (152, 153). Our study seems to be in agreement with these findings, which stated that dairy use did not significantly affect bone values.

6.3. Hormonal contraceptive use and bone

We found no significant relationship between hormonal contraceptive use and quantitative ultrasound measures. Our results are similar to those of some other studies yet different from that of others because data from different studies on the effect of HC on young women's bones are conflicting (252). Some studies report positive effects (253), some report no effect (254), and others report adverse effects (255-257). Many of these studies were done in a much older population and considered a fracture endpoint.

One large cohort study found that the risk of fracture incidents among women who had

ever-used HC was significantly greater than women who had never used it (258). Another large cohort study found that increased duration of HC use was associated with a 30% increased risk of all fractures (259). The Canadian Multicentre Osteoporosis Study is a population-based osteoporosis study that used extensive questionnaires to assess the relationship between hormonal contraceptive use and BMD in women aged 25–45. From the results, Prior et al. reported lower BMD values for the trochanter and spine in those who had used oral contraceptives for more than 3 months compared with those who had never used them (< 3 months). However, 87% of their participants were on hormonal contraceptives, a higher proportion than in our study (60.3%). Also, this difference in results might be explained by the age of participants, which was higher in their study (25–45 years of age) (260).

A systematic review showed that there is not enough evidence for any significant detrimental or protective effect of HC use on BMD in the general population (180). Similarly, several cross-sectional studies on perimenopausal women also found no significant difference in BMD between HC users and nonusers (182-184, 261). Therefore, most previous research aligns with our study's findings that HC use did not affect bone values.

6.4. Alcohol intake and bone

We found no significant differences in quantitative ultrasound measures for alcohol intake. Alcohol consumption can be divided and analyzed according to the drinking pattern and means of alcohol consumption. However, it is challenging to define light, moderate, or heavy alcohol consumption (262). Recent studies categorized alcohol consumption into three groups: light, only occasional consumption; heavy, chronic

alcohol consumption; and binge drinking (195, 263). Evidence suggests light alcohol intake could positively affect bone density (203, 210-213, 264). A review paper showed that light alcohol consumption in women (one drink per day) does not adversely affect bone tissue, whereas higher consumption levels (2–4 drinks per day) can be deleterious for bone tissue (195). It also indicated that the effects of alcohol consumption on bone are related to the dosage, duration of consumption, and age of the consumer. Similarly, other studies reported improved bone mass with light alcohol consumption (265-268).

Evidence on moderate alcohol consumption is contradictory. The effect of 2-3 drinks per day on bone depends on age, sex, hormonal status, and the type of beverage (195). Moderate alcohol consumption in older people, especially postmenopausal women, is often associated with higher BMD and lower fracture risk (222, 269-271). However, it is not beneficial for premenopausal women (195) because 5–24 g/day of beer or liquor consumption could increase their risk of fracture (272).

Heavy alcohol consumption was not associated with lower BMC as well as BMD in women (273). A cross-sectional study examining 57 heavy alcohol users aged 27 to 50 showed a lower BMD in men but not in women (273). In contrast, a review study reported a linear association between heavy alcohol consumption and both higher bone density and lower bone density loss over time (201). Also, it suggested a J-shaped relationship between alcohol consumption and hip fracture risk. However, there were more heavy drinkers in studies that investigated fracture risk than those which evaluated bone density (201).

Different mechanisms may be responsible for the effects of alcohol on bone. A possible mechanism could be that alcohol decreases bone resorption (274). A review paper

postulated that phenolic compounds such as resveratrol, flavonols, and anthocyanins, which are found in alcoholic beverages could inhibit bone resorption and increase bone formation (275). Also, alcohol may disrupt the balance of calcium concentration in the bloodstream, which is required for the nerves and muscles' proper functioning (208). Alcohol also affects the hormones that regulate calcium metabolism and the hormones that influence calcium metabolism, such as steroid reproductive hormones and growth hormones (209). Moreover, it seems that the various type of alcoholic beverages has different effects on bone metabolism (195). This may be because of the different constituents present in wine and beer (265). In our study, 25.6% of participants drank more than 5 units at one time, at least once a week, and were categorized as heavy drinkers. However, our participants were relatively young (18 to 25), and the effect of alcohol on bone is a long-term effect. Therefore, it is likely that the effect of alcohol intake did not apply yet.

6.5. Implication

This study adds further credence to Canadian (276), World Health Organization (276), and American (277) recommendations for children and youth that they should engage in substantial moderate to vigorous physical activity. Although the common recommendation of 60 minutes of daily moderate to vigorous physical activity (MVPA) does not specifically address bone-specific activity, this study demonstrates that those who participate in considerable MVPA throughout childhood and adolescence are much more likely to also acquire a robust skeleton. Our findings also provide more evidence into the previous studies (278, 279) about the importance of multidirectional loading sports such as soccer in young women in improving bone health compared with those

who participated in repetitive lower-impact sports such as distance running.

Research conducted from 2001 to 2006 reported that levels of MVPA measured by accelerometers of young Nova Scotians aged 8, 12, and 17 years declined rather drastically with age such that a very low proportion of 17 year olds met the PA requirements (280). More recently, a survey conducted across Canada in October 2020 of parent-reported MVPA of youth aged 5 to 17 indicated very low levels of MVPA (281). Given that self-reported MVPA levels are routinely inflated over those collected by accelerometers (282), the fraction of youth who are receiving the benefits of MVPA must be extremely low. Therefore, our study, which reports the relationship of high levels of ground-based vigorous PA during and soon after growth on good bone status as determined by LOGES, adds additional evidence to the urgency that more MVPA needs to be incorporated into the daily lives of children and youth.

6.6. Strengths and limitations

This study has many strengths, including its utilization of data from a somewhat large sample which provides a comprehensive and representative sample of the young university women population in Nova Scotia. We combined two studies and three data collection waves with a similar population to gather large data samples. Also, we used the LOGES system, which combined physical activity type, duration, frequency, and puberty stage. It is possible that this system may result in a better interpretation of the physical activity status of each participant than does the more commonly used PBAQ method. Bone values were measured by QUS, a safe, inexpensive, easy-to-use, and without-radiation device.

There were some limitations to this study. Primarily, the exercise histories obtained from

participants may not be accurate because remembering what exercise they did, and its duration can be difficult. Also, the collection of bone measures entailed several operators for the QUS devices. Therefore, slight differences in positioning a person's foot could result in different results. Additionally, the device might have fluctuated during the study, although quality assurance tests were performed daily using the supplied phantom. Classification of each woman's type of sport and PA was challenging and a likely source of inaccuracy. For example, a girl could play volleyball with no jumping or play it with significant vertical impacts if she regularly jumped to block the ball. The selection of classification of some physical activities was therefore based on how seriously the sport was being played. For example, a varsity-level volleyball player was awarded a high score, whereas a girl of 10 who played once a week was presumed recreational and was given a lower score for that PA.

We used a lifestyle questionnaire that asked the participants about the servings of dairy products per day which may not be accurate because quantifying amounts from memory is very difficult. Also, we considered dairy intake, a total intake level, and ignored the effect of combinations of foods consumed by our participants. The questionnaire used asked only for servings of dairy but did not specify which foods. This could make a difference because, for example, the calcium content of milk differs from cheese. A big difference between our results and the literature is the age of the participants. Most of the studies were on children or older adults, while our participants were young adults.

Regarding hormonal contraceptive use, many women could not remember what type of contraceptives they had used, especially if several different types had been prescribed.

The alcohol intake reports obtained from participants may not be accurate because

remembering the exact amount of alcohol they consumed can be difficult. This might cause misclassification toward higher or lower alcohol intake categories. Also, we did not differentiate between various types of alcoholic beverages, and it seems that the various type of alcoholic beverages has different effects on bone metabolism (195).

Future studies are needed to further test and validate the LOGES. For instance, testing a data set using both LOGES and BPAQ should be taken into consideration in order to compare the two systems. Also, testing the relationship between LOGES and bone status using pQCT and comparing it with QUS data is suggested. Furthermore, the LOGES system should be tested not only in women but also in men with different ages and races. For instance, examining the LOGES system in women over 35 years old would be interesting as they have been shown to have more fracture incidence than younger women.

6.7. Conclusion

This study has presented the relationship between 4 lifestyle factors (high-impact exercise, dairy intake, hormonal contraceptive use, and alcohol consumption) and quantitative ultrasound measures amongst healthy young adult women living in Nova Scotia. We introduced the LOGES method, which differs from previous methods such as BLHQ and BPAQ. Only LOGES values were correlated with all three quantitative ultrasound measures (BUS, SOS, and SI). Participants in the highest quintile of LOGES values had significantly higher BUA, SOS, and SI than women in all other quintiles of LOGES. This study suggests that high-intensity, ground-based exercise before, during, and after puberty is crucial for women to have stronger bones. Also, in physical activity,

not only duration and frequency but also the rate of physical activity alongside the puberty stage could affect bone status. We believe that the LOGES system could be a useful avenue for further explorations in bone research. Also, the more intense and continuous physical activity is throughout the lifetime, the more likely a young woman will develop a stronger skeleton.

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APPENDICES

Questionnaire

Subject Number: _____ Date: _____

Please fill out the following questionnaire to the best of your ability. If you have questions, do not hesitate to ask for clarification. Circle the letter corresponding to your answer or fill in blanks where necessary.

Hormonal Birth Control History

1. Are you currently taking birth control?
   a. Yes
   b. No

2. Have you ever taken birth control in the past?
   a. Yes
   b. No

3. If you answered yes to either of the 2 previous questions, at what age did you begin taking birth control? _____ years

4. How long have you been taking/did you take birth control (cumulative)?
   a. Less than 6 months
   b. 6 months to 1 year
   c. 1 year to 2 years
   d. 2-3 years
   e. 3 years or more

5. Which type(s) of hormonal birth control do you/have you used? Circle all that apply.
   a. Low dose
   b. Combination
   c. Depo-Provara
   d. Progesterone Only
   e. Not sure
   f. Other; name: _____

6. Age of menarche (age of first menstrual period)
   a. 8-10 years
   b. 11-12 years
   c. 13-14 years
   d. over 14 years

Subject Number: _____ Date: _____

Exercise Participation

7. Starting most recently, please list all past and/or current sports, types of training, or physical activities you participate in and/or have participated in, in your lifetime, using the following table. Include corresponding approximate start and end dates (indicate "current" as an end date if you are presently participating in an activity to his date), and how many hours per week are/were spent engaged in each activity. Use back of page if you require more space.

Sport/Activity	Start Date	End Date	Hours/Week

Lifestyle

8. How many servings of dairy products do you consume per day?
   a. 0-1
   b. 2-3
   c. 4 or more

9. Do you eat calcium fortified foods (orange juice, waffles, etc)?
   a. Yes, every day
   b. Yes, at least once a week
   c. Yes, at least once a month
   d. No

Subject Number: _____ Date: _____

10. Do you take a calcium supplement?
    a. Yes, every day
    b. Yes, I take a multivitamin
    c. No

11. Do you take a vitamin D supplement?
    a. Yes, in a multivitamin
    b. Yes, as a separate supplement
    c. If "yes", how much? _____
    d. No

12. In the summer months (April through to September), are you able to get outside between the hours of 10am and 3pm?
    a. Never
    b. Sometimes
    c. Often

13. If this has changed over the years, please describe: _____
    _____

14. When you are outside do you use sunscreen?
    a. Never
    b. Sometimes

15. Alcohol Intake
    a. I do not drink alcohol
    b. I drink occasionally (no more than 2 units of alcohol per day, 0-1 days per week)
    c. I drink 7 or less units of alcohol per week, with no more than 2 units per day
    d. I drink more than 5 units of alcohol at one time, at least once per week

16. Do you drink caffeinated beverages? Yes_____ No_____

17. If yes, how many cups per day? _____

Subject Number: _____ Date: _____

18. Have you ever fractured a bone? Yes_____ No_____

19. If yes, please list which bone was broken, what your age was at the time, and what was the cause of the break.

Bone_____ Age _____ Reason _____

Bone_____ Age _____ Reason _____

Bone_____ Age _____ Reason _____

Bone_____ Age _____ Reason _____

20. Are you currently taking any medications? Yes_____ No_____

21. If yes, what are these medications? _____

_____

Demographics

22. What is your age? _____

23. Are you:
    a. Caucasian
    b. African Canadian
    c. Asian
    d. Hispanic
    e. Native
    f. Other _____

24. What is your highest level of Education?
    a. Some high school
    b. High school graduate
    c. Some university/college
    d. Undergraduate degree/diploma
    e. Post-graduate studies

25. What is your current occupation? _____

Consent Forms

Consent Form
Category B

CONSENT TO TAKE PART IN A RESEARCH STUDY
Participant Information

STUDY TITLE: **Does the use of oral contraceptives at a young age have a negative effect on the bone status of young women?**

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PART A
RESEARCH STUDIES – GENERAL INFORMATION

1. INTRODUCTION

You have been invited to take part in a research study. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.

Please read this carefully. Take as much time as you like. If you like, take it home to think about it for a while. Mark anything you don't understand, or want explained better. After you have read it, please ask questions about anything that is not clear.

The researchers will:

- Discuss the study with you
- Answer your questions
- Keep confidential any information which could identify you personally
- Be available during the study to deal with problems and answer questions

We do not know if taking part in this study will help you. You may feel better. On the other hand it might not help you at all. It might even make you feel worse. We cannot always predict these things. We will always give you the best possible care no matter what happens.

If you decide not to take part or if you leave the study early, your usual health care will not be affected.

PART B
EXPLAINING THIS STUDY

2. WHY IS THIS STUDY BEING DONE?

Some research has shown that oral contraceptives taken by young women have a small but damaging effect on their skeletons. We think that stage of maturity in which oral contraceptives are taken might be important. A lot of mineral is packed into the skeletons of girls when they go through puberty so oral contraceptives taken during puberty might prevent some of this mineral from packing into the bones. Therefore, we are doing this study to determine if birth control pills prescribed to girls under the age of 15 might have a negative effect on their skeletons. We are also asking questions about other factors such as exercise and diet because they might help us understand the relationship between oral contraceptive use and skeletal health in young women.

3. WHY AM I BEING ASKED TO JOIN THE STUDY?

You have been invited to join in this research study because you are a non-smoking female

between the ages of 18-24 years who has expressed an interest and has contacted us after seeing our advertisement.

4. HOW LONG WILL I BE IN THE STUDY?

The study will take approximately one (1) hour of your time, and involves only one (1) study visit.

5. HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

This study is taking place only in Halifax, Nova Scotia. Approximately 60 participants will take part. We are seeking 20 women who began taking oral contraceptives before the age of 15, 20 women who have taken oral contraceptives but not until the age of 16 or older, and 20 women who have never taken oral contraceptives. We expect that the study will be completed in the winter of 2009.

6. HOW IS THE STUDY BEING DONE?

Women who are interested in this study will contact the researcher. The study coordinator will ask you a few "screening" questions to determine if you are eligible. This will take less than 5 minutes. You will be told if you are, or are not, eligible. If you are eligible for this study, you will be asked to come to a testing location. At this meeting, the study coordinator will explain the study to you. If you decide to join the study, you will be asked to sign this consent form. You will only be asked to meet with the researcher once for approximately 40 minutes. During this time you will be asked to give informed consent, complete a questionnaire and have two ultrasound measurements performed on your right heel. You will be given your bone test results to keep and to share with your family physician.

7. WHAT WILL HAPPEN IF I TAKE PART IN THIS STUDY?

SCREENING

If you want to be in this study, the study coordinator will asked you a few questions to see if you are eligible. This is called screening and will take about five (5) minutes. The questions will include your age, whether you have used oral contraceptives, and if so, when you first began taking them. You will also be asked if you have had certain medical problems or take certain medications that might prevent you from being eligible for this study. It is possible that your answers to the screening questions will show that you cannot be in the study.

STUDY

We will do the following as part of the study:
If the screening process shows that you are eligible for the study and you indicate that you still want to participate, then you will be scheduled for one appointment with the research coordinator. At that appointment the following processes will take place:
You will be asked to read and sign a consent form to participate in the study. Typically, this takes 5-10 minutes.

You will be asked to complete a questionnaire regarding your use of oral contraceptives, exercise history, and other lifestyle factors that might affect bone status. The questionnaire will take approximately 10 minutes to complete.

Your bone status will then be assessed by ultrasound. This test will require you to remove the shoe and sock from your right foot. Your heel will be scrubbed with rubbing alcohol and placed in the heel cup of a bone ultrasound machine while you are seated in a chair. The test takes about 10 seconds and two tests will be performed. You will not feel the ultrasound. To help us understand the data, we will also measure your height, weight, and heel width.

8. ARE THERE RISKS TO THE STUDY?

There are risks with this, or any study. To give you the most complete information available, we have listed many *possible* risks, which may appear alarming. We do not want to alarm you but we do want to make sure that if you decide to participate in this study, you have had a chance to think about the risks carefully. Please be aware that there may be risks that we do not yet know about.

QUESTIONNAIRES

Although unlikely, you may find the telephone screening questions and the questionnaire distressing. You may not like all of the questions that you will be asked. You do not have to answer those questions you find distressing.

ULTRASOUND

There are no risks associated with the heel ultrasound test. There is no ionizing radiation as only sound waves are used to penetrate the heel. The machine is disinfected between each use, and each participant will have their testing heel disinfected with rubbing alcohol before placing it in the ultrasound device.

RESULTS OF THE TEST

You might find out that you have poor bone status, which might alarm you. If this happens, we will encourage you to ask your physician for another kind of bone test to confirm this result or to see if the same result is true for other bones in your body. Our machine can only test heels.

9. WHAT HAPPENS AT THE END OF THE STUDY?

You will be given a copy of the final publication when the study is finished if you wish to receive it.

10. WHAT ARE MY RESPONSIBILITIES?

As a study participant you will be expected to:

- Follow the directions of the Principle Investigator or study coordinator
- Answer all questions to the best of your ability

11. CAN I BE TAKEN OUT OF THE STUDY WITHOUT MY CONSENT?

Yes. You may be taken out of the study at any time, if:

- There is new information that shows that being in this study is not in your best interests.
- There is new information that makes you ineligible to participate in this study.
- You do not follow the directions of the Principal Investigator
- The Capital Health Research Ethics Board or the Principal Investigator decides to stop the study.

If for any reason, your participation is no longer required or you no longer meet the inclusion criteria, you will be given the reason why.

12. WHAT ABOUT NEW INFORMATION?

It is possible (but unlikely) that new information may become available while you are in the study that might affect your health, welfare, or willingness to stay in the study. If this happens, you will be informed in a timely manner and you will be asked whether you wish to continue taking part in the study or not.

13. WILL IT COST ME ANYTHING?

Compensation

There is no cost to participate in this study and you will not be paid to be a participant in the study. However, you will have the opportunity to learn about your bone health as we will share your result with you.

Research Related Injury:
If you become ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no additional cost to you. Your signature on this form only indicates that you have understood to your satisfaction the information regarding your participation in the study and agree to participate as a subject. In no way does this waive your legal rights nor release the Principal Investigator, the research staff, the study sponsor or involved institutions from their legal and professional responsibilities.

14. WHAT ABOUT MY RIGHT TO PRIVACY?

Protecting your privacy is an important part of this study.
When you sign this consent form you give us permission to collect information from you.

Access to records
Members of the research team will see your questionnaire and test results that identify you by name. Some other people or groups may need to review your study records to make sure all of the information is correct. All of these people have a professional responsibility to protect your privacy. These groups are:

- The Capital District Health Authority Research Ethics Board which is responsible for the protection of people in research
- Quality Assurance staff including the auditor for the Capital Health Research Ethics Review Board who ensure that the study is being conducted properly.

The information they check may include your bone status test and questionnaire results. You

may also be contacted personally by the Capital Health research auditors for quality assurance purposes.

Use of records.
The research team will collect and use only the information they need to complete the study. This information will only be used for the purposes of this study.
This information will include your:

- date of birth
- sex, height, weight
- the results of the bone test that you had during the study
- information from the questionnaire

Your personal information will be kept strictly confidential during this study. Although your name may be used in study records, no identifying information will be related to your data will be released outside of Capital Health or Dalhousie University. We will use subject numbers to track data. If the results of this study are presented at a meeting or in publication, individual names or anything else that would identify your individual participation in the study will not be released. Your records will be stored in a secure area in a locked file cabinet in an office or laboratory. Only research staff will have access to these files. After the study ends, files will be kept for 7 years in a secure storage area owned or leased by Capital Health or Dalhousie University. The Principal Investigator is the person responsible for keeping your records secure. If you decide to withdraw from the study, the information collected up to that time will continue to be used by the research team. It may not be removed.

Your access to records
You will be given a copy of your bone tests results. You may also receive a copy of your questionnaire if you request it.

15. WHAT IF I WANT TO QUIT THE STUDY?

If you choose to participate and later decide to change your mind, you can stop your participation at any time. You will not be penalized for a decision to stop participating in the study. You will be required to leave the data collected on you to date in your file.

16. DECLARATION OF FINANCIAL INTEREST

There is no sponsor for this study. The Principal Investigator has no financial interests in conducting this research study.

17. WHAT ABOUT QUESTIONS OR PROBLEMS?

For further information about the study call **Dr. Jo Welch**. Dr. Welch is in charge of this study at Dalhousie University (She is the "Principal Investigator"). Dr. Welch's work phone number is **(902) 494-2475.** If you can't reach the Principal Investigator, please refer to the attached Research Team Contact Page for a full list of the people you can contact for further information about the study.

The Principal Investigator is Dr. Jo Welch.
Telephone: (902) 494-2475

Your Research Coordinator is Ms. Vanessa Slayter.
Telephone: (902) 494-1144

The Associate Investigator is Dr. Stephanie Kaiser.
Telephone: (902) 473-3728

18. WHAT ARE MY RIGHTS?

After you have signed this consent form you will be given a copy.

In the next section, you will be asked if you agree (consent) to join this study. If the answer is "yes", you will need to sign the form.

PART C:

19. CONSENT FORM SIGNATURE PAGE

I have reviewed all of the information in this consent form related to the study called:

Does the use of oral contraceptives at a young age have a negative effect on the bone status of young women?

I have been given the opportunity to discuss my participation in this study. All of my questions have been answered to my satisfaction.
My signature on this consent form means that I agree to take part in this study. I understand that I am free to withdraw at any time.

_____ Signature of Participant	_____ Name (Printed)	_____ / _____ / _____ Year Month Day*
_____ Witness to Participant's Signature	_____ Name (Printed)	_____ / _____ / _____ Year Month Day*
_____ Signature of Investigator	_____ Name (Printed)	_____ / _____ / _____ Year Month Day*
_____ Signature of Person Conducting Consent Discussion	_____ Name (Printed)	_____ / _____ / _____ Year Month Day*

If the consent discussion has been conducted in a language other than English, please indicate language: _____

_____ Signature of Translator	_____ Name (Printed)	_____ / _____ / _____ Year Month Day*

Note: Please fill in the dates personally.

I will be given a signed copy of this consent form.

Thank you for your time and patience!!

Research Proposal: Does the use of oral contraceptives at a young age have a negative effect on the bone status of young women?

Principle Investigator: **Jo Welch, PhD**
Dalhousie University
School of Health and Human Performance
6230 South Street, Halifax NS, B3H 3J5

Co-Investigators: **Stephanie Kaiser, MD**
Vanessa Slayter, B.Sc. Hons Kin (Candidate)

Part A: BACKGROUND AND RATIONAL

Skeletal fractures in women over 50 years of age are a cause of substantial morbidity and mortality in Canada. About 20% of women die within one year of a hip fracture and a further 40% are negatively affected on a long term basis (1). However, the root cause of bone fragility may lie decades earlier during growth and maturation. Failure to develop strong bones during growth may predispose women to bone fragility (2). Medications such as glucocorticoids can interfere with bone development and be responsible for bone fragility in children with serious illness (3). However, less is known about the effects on the skeleton of medications such as hormonal contraceptives when they are prescribed to healthy girls who have not reached skeletal maturation. Although hormonal contraceptives are typically prescribed to prevent ovulation and thus fertilization, they are also often prescribed to adolescents for complaints such as acne (4) and dysmenorrhea (5).

Some cross-sectional studies on women who used oral contraceptives (OCs) reported that this medication was associated with compromised bone health (6-10) but others reported no apparent correlation (11, 12). However, the age at which hormonal contraceptives are taken may affect subsequent bone mass because these medications may blunt the accretion of mineral into the skeleton at puberty. For girls, the velocity of bone growth doubles during adolescence

with longitudinal bone growth diminishing around 14 years of age (13). Mean peak bone growth occurs at age 11.8 years and is followed by mineralization, which occurs rapidly (14). Excess mineral is "packed into" the skeletons of girls, presumably for future fetal and lactation needs (15). The peak age of calcium accretion in Saskatchewan girls is 10.5 to 14.6 years, with a mean age of 12.5 years (14). Limited research suggests that the use of OCs during pubertal mineralization may prevent young women from accumulating maximal bone mass (7, 16), which may put them at an increased risk of fracture particularly later in life. Women who initiated OC use within three years of menarche demonstrated lower bone status (7); similarly, age at first use of OCs was the best predictor of low vertebral bone mineral density (BMD) in female endurance athletes (16). However, girls first using OCs at the age of 15-19 did not demonstrate a difference from non-users in areal BMD measures (17), which suggests that very early use of OCs may be more harmful to bone mineral accrual than somewhat later use.

Oral contraceptives are reported to be the most popular method of birth control among young women (18) and it is therefore important to understand their impact on the bone health of young women. When OCs are taken by girls during their peak bone accretion period, OCs may compromise bone health (7). Given the prevalence of OC use, surprisingly few studies have examined the effects of OCs on bone and even fewer have considered the effects of OCs on bone in pre- and early teenage girls. Furthermore, to our knowledge, the proposed pilot study will be the first examination of the effects of early use of oral contraceptives in Canada.

Research question: Does the use of oral contraceptives at a young age (≤14 yr) have a negative effect on bone status in females aged 18-24 years?

This study will compare the bone status of women aged 18-24 who began taking oral contraceptives at ≤14 years of age to women who began using oral contraceptives at 16 years of age or older (≥ 16 yr) and to non-users of oral contraceptives.

Research hypothesis: We hypothesize that women 18-24 years of age who began taking oral contraceptives at ≤14 years of age will have lower bone status than both oral contraceptive non-users and oral contraceptive users who began using oral contraceptives at ≥ 16 years of age.

Part B – METHODOLOGY

Subject Selection

Inclusion Criteria: Subjects will be included if they meet the following criteria:

- Female
- Aged 18-24 years
- OC users who began taking OCs at ≤ 14 years of age and used them for 6 months or longer, or
- OC users who began taking OCs at ≥ 16 years of age and have used then for 6 months or longer, or
- OC non-users, including those who have taken OCs for less than 6 months after the age of 16.

The study will only recruit female participants because males do not use OCs. Subjects will be limited to young women 18-24 years old because peak bone mass at the calcaneal site has been achieved in most women by that age (19) and it allows enough time to investigate a possible difference in bone status for women who began using oral contraceptives ≤ 14 years of age.

Exclusion criteria: Subjects will be excluded if they meet any of the following criteria:

- smokers, currently or previously
- are unable to give informed consent
- have a history of other medical disorders that could affect vitamin D absorption, such as celiac, colitis, or Crohn's disease
- pregnant
- taking medications that could adversely affect bone metabolism or vitamin D absorption, including steroidal treatments

Pregnant women will be excluded due to temporary changes in bone metabolism during pregnancy. A pilot study conducted in our lab at Dalhousie University in 2007 determined that including smokers interferes with the ability to accurately understand the relationship between OCs and bone. Other researchers have concluded that smoking is negatively associated with BMD (10, 20). Findings indicated that currently moderate smokers had lower BMD and increased vertebral deformities due to increased bone resorption accompanied by decreased bone formation (21). Therefore, smokers will be excluded from the proposed study.

Sample size calculation: The number of subjects needed to find a difference in bone status between those who used OCs at ≤ 14 years of age and those who did not was estimated from studies that used similar populations. The closest study was a German investigation by Hartard and colleagues (7) who compared peripheral quantitative computed tomographic (pQCT) measures of the tibia in women who first used OCs at an average age of 15.7 years, with women who had not used OCs. The age of the women at the time of measurement was the same as for our proposed study: 18-24 years. Based on their data from a population size of 54

in the early use and 47 in the non-use groups, only 3-4 subjects would be required to detect a difference at an alpha level of 0.05 and a power of 80%. However, we have conducted two studies regarding the effects of OCs on bone in women 18-24 years; one was an initial pilot study and the other is an ongoing larger study (CDHA-RS/2008-034) from which interim results from 80 women have been calculated. Both of our studies assessed the effects of exercise on bone in oral contraceptive users and few of the participants (5 and 6, respectively) were early OC users. Our studies demonstrated lower bone measures in the early users, with a $p = 0.08$ in the pilot study and no statistical trend in the ongoing study. Therefore we intend to recruit 20 women who took OCs for more than 6 months at $\leq$ 14 years of age, 20 women who began taking OCs at $\geq$ 16 years of age and have taken then for 6 months or longer, and 20 women who are OC non-users, including those who have taken OCs for less than 6 months after the age of 16.

Recruitment of subjects

Posters will be placed in popular areas around the Dalhousie University campus, such as the Life Sciences Center (LSC), Kellogg and Killam Libraries, Student Union Building (SUB), Tupper Medical Building and Dalplex. Other locations on Dalhousie University and Capital Health property may also be used. All willing participants will respond to the posted advertisements by phoning the Bone Lab number located on the advertisement or emailing Vanessa Slayter, the research coordinator. All subjects will be required to read and sign an informed consent document that has been approved by the Research Ethics Board of Capital Health before participating. The site location will be the Bone Lab, Rm 218, Kinesiology Suite, Dalplex, Dalhousie University.

Screening procedures

Subjects will be screened via telephone according to the inclusion and exclusion criteria. Those who meet the criteria for this study will be invited to take part. If they wish to join the study, a date for them to receive a full explanation of the study, complete the informed consent process, and complete the study will be booked. Those who do not meet the criteria will be informed as to why they do not.

Research Plan

The design of the proposed study is a cross-sectional survey so randomization and blinding are not components of this study. Participants who meet the inclusion criteria and wish to become a subject in this study will be invited to an appointment at the Bone Lab, Rm 218, Kinesiology Suite, Dalplex, Dalhousie University. They will then receive a full explanation of the study. If they choose to sign the informed consent document, the questionnaire will be administered, their standing height, body mass and heel width will be measured, and the bone measurements will take place. Bone testing will be done using an Achilles InSight Ultrasonometer which uses ultrasound to determine 3 surrogate measures of bone strength at the calcaneus (heel bone). The questionnaire takes approximately 10 minutes, the height and weight take about 5 minutes, and the bone tests take a further 10 minutes, for an estimated total time of 25 minutes per subject.

Data Collection

Demographic Data: We will use a questionnaire to collect demographic information from each participant. Information collected will include age, OC use, exercise history, dietary habits, and past medical history.

Outcome Measures:

Subjects will be categorized into one of three groups:

A. OC users who began taking OCs at ≤ 14 years of age and used them for 6 months or longer

B. OC users who began taking OCs ≥16 years of age and have used then for 6 months or longer, or

C. OC non-users, including those who have taken OCs for less than 6 months after the age of 16.

Bone status will be assessed by quantitative ultrasound of the calcaneus, in duplicate from the sitting position. Parameters measured will be broadband ultrasound attenuation (BUA) and speed of sound (SOS) and, from these measures, Stiffness Index (SI) will be calculated.

We have chosen to use quantitative ultrasound (QUS) technology because it is a noninvasive technology without radiation and provides a well validated method of predicting fracture risk(22-24). The latest model of Achilles QUS, the Achilles InSight will be used as it has recently been reported to clearly differentiate the majority of patients who have fractures from those who do not (25, 26). QUS has the added benefits of portability, speed of use and is administered with the patient in sitting position. Duplicate tests for each patient will be performed and averaged, as this has been shown to improve precision (27). The precision of the Achilles InSight instrument in our lab in 2008, using the same machine that will be used in the proposed study, was calculated from duplicate measures from 80 women aged 18-24 years. This precision calculation, expressed as coefficient of variation (%CV) is as follows: SI = 1.2%, SOS = 0.4%, and BUA = 2.2%.

Explanation of test results

Subjects will receive copies and explanations of their bone test results. If a bone test suggests a bone deficit, then the subject will be advised to discuss this result with her primary care physician who might suggest further tests and/or treatment.

Statistical Analysis of Data

Statistical analysis will be performed with SPSS version 15.0. Data will be checked for normality and an ANOVA test will be used to determine if the bone status (BUA, SOS, SI) of women who began OCs at ≤ 14 years of age, began OCs at > 16 years of age, or did not take OCs is statistically different at an α-level of 0.05. A post hoc test, the least significant difference (LSD) test, will be used to test differences between any two groups.

Confidentiality

All of the data collected throughout this study will be electronically protected by password. All data-related documents will be kept in a locked filing cabinet in the Bone Lab, Room 218G, Dalplex at Dalhousie University for a minimum of 7 years, according to Capital Health policy. The only persons who will have access to any data collected will be Dr. Jo Welch, Vanessa Slayter, and other members of Dr. Welch's research staff.

Possible Risks

The QUS is a noninvasive measure of screening. There is no radiation emitted from the ultrasound. The ultrasound machine will be disinfected using isopropyl alcohol after each subject is tested, which will minimize transmission of foot-borne pathogens. The questionnaire

may contain questions that a participant may not feel comfortable answering. Subjects will be informed that may choose to skip any question(s) that they do not feel comfortable answering without penalty. Subjects will not be asked why they did or did not take OCs as this information may be uncomfortable for the subjects and is not relevant to the study.

Possible Benefits

This study may help to clarify the relationship between using OCs at a young age and bone strength. If early use of OCs is shown to be correlated with reduced bone status, then physicians may choose to take into account this information when considering whether or not to prescribe OCs versus other treatments to adolescent girls. Additionally, early indication of marginal or poor bone status in the subjects in this study could lead to their treatment or improved exercise and dietary practices that, in turn, could prevent future fractures.

Disclosure of Any Financial Compensation

Subjects will not receive reimbursement for their participation in this study. Neither the principal investigator nor the co-investigators have any financial interests related to the study.

Translation of knowledge

We intend to bring the findings of this study to multiple groups of interest via presentations and publication in a peer review journal. We also intend to communicate our findings to appropriate health care groups in Nova Scotia.

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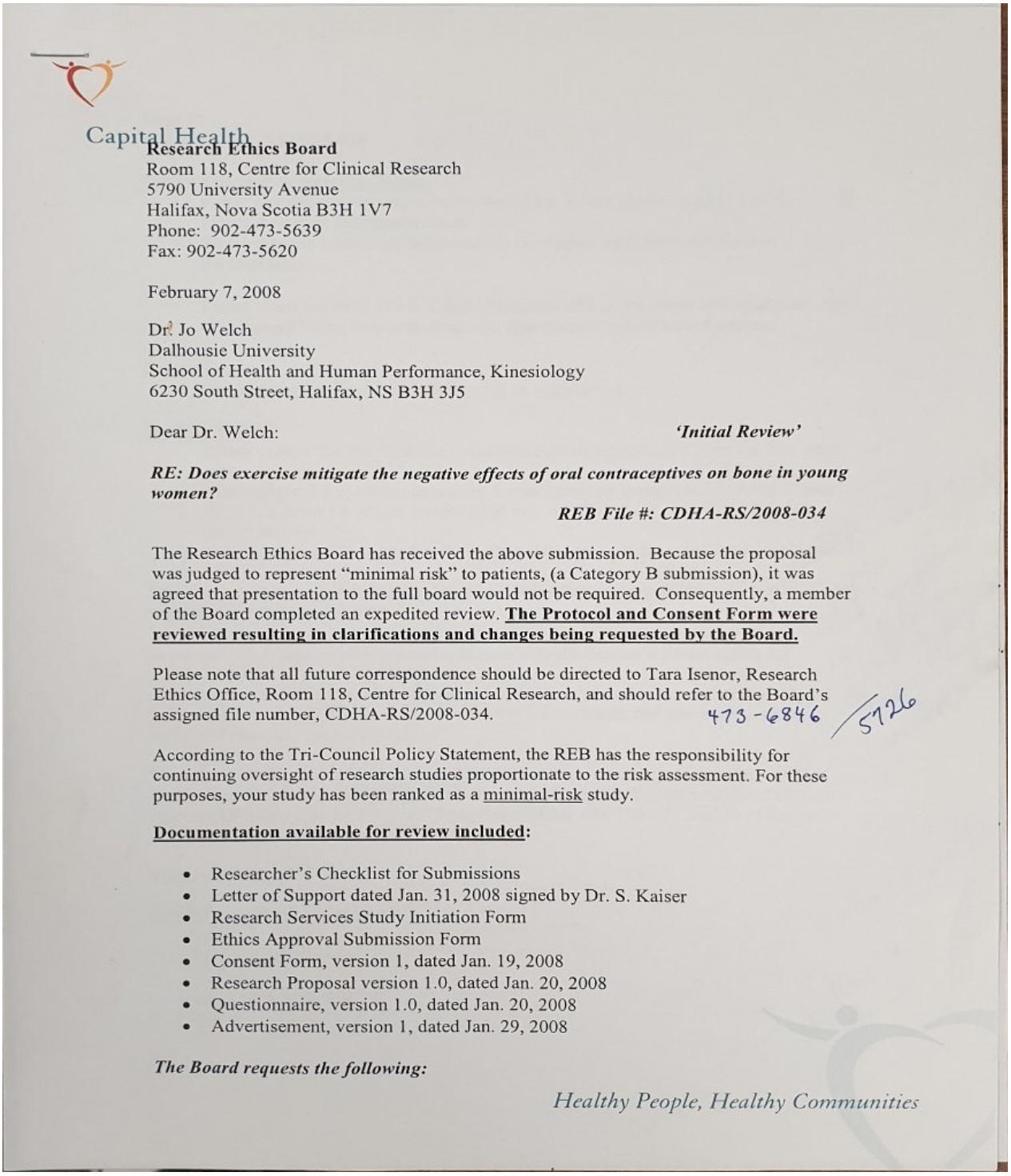
Ethics Forms



Capital Health

Research Ethics Board
Room 118, Centre for Clinical Research
5790 University Avenue
Halifax, Nova Scotia B3H 1V7
Phone: 902-473-5639
Fax: 902-473-5620

February 7, 2008

Dr. Jo Welch
Dalhousie University
School of Health and Human Performance, Kinesiology
6230 South Street, Halifax, NS B3H 3J5

Dear Dr. Welch: ***'Initial Review'***

RE: Does exercise mitigate the negative effects of oral contraceptives on bone in young women?

REB File #: CDHA-RS/2008-034

The Research Ethics Board has received the above submission. Because the proposal was judged to represent "minimal risk" to patients, (a Category B submission), it was agreed that presentation to the full board would not be required. Consequently, a member of the Board completed an expedited review. **The Protocol and Consent Form were reviewed resulting in clarifications and changes being requested by the Board.**

Please note that all future correspondence should be directed to Tara Isenor, Research Ethics Office, Room 118, Centre for Clinical Research, and should refer to the Board's assigned file number, CDHA-RS/2008-034. 473-6846 5726

According to the Tri-Council Policy Statement, the REB has the responsibility for continuing oversight of research studies proportionate to the risk assessment. For these purposes, your study has been ranked as a minimal-risk study.

Documentation available for review included:

- Researcher's Checklist for Submissions
- Letter of Support dated Jan. 31, 2008 signed by Dr. S. Kaiser
- Research Services Study Initiation Form
- Ethics Approval Submission Form
- Consent Form, version 1, dated Jan. 19, 2008
- Research Proposal version 1.0, dated Jan. 20, 2008
- Questionnaire, version 1.0, dated Jan. 20, 2008
- Advertisement, version 1, dated Jan. 29, 2008

The Board requests the following:

Healthy People, Healthy Communities

Ethics Approval Submission Form:

1. Please provide the signatures of the Associate Investigators on page 1 of the Ethics Approval Submission form.
2. Please provide a Letter of Support from the School of Health and Human Performance.

- Please place the REB File #: CDHA-RS/2008-034 in the lower left-hand corner of the Consent Form, Research Proposal, Questionnaire, and Advertisement.

Protocol:

- Please discuss how confidentiality will be maintained.

Consent Form:

1. Please ensure that the REB file # and version/date information is on the first page too.
2. Please place the contact information for the associate investigators on the 1st page.
3. Page 5, section 18, please use the REB new wording for the Research Related Injury section.
4. Page 7, section 22, please list the contact info again for the associate investigators.

Please remember to update the version # and date of all revised documents before resubmitting them to the REB.

Please submit 1 copy of all requested information to the Research Ethics office for review. The Board would remind you that submissions that have not received correspondence within **3 months** of the initial response from the Research Ethics Board will be marked closed. Please note that once the file is closed, and you wish to reapply, the Board requires a new submission.

The Research Ethics Board for the Capital District Health Authority complies with the Tri-Council Policy Statement, the ICH Harmonized Tripartite Guidelines: Good Clinical Practice, Division 5 of the Food and Drug Regulations, and Titles 21 and 45 of the code of Federal Regulations of the United States.

Yours very truly,

RESEARCH ETHICS BOARD

[redacted]

Dr. Sarah Kirby, MD, FRCPC
Co-Chair

/tji

New wording says to only include if symptoms + side effects are applicable to your study. They are not, so omit.

Centre Clinical/Res.
Rm 118

Capital Health

Letter of Support
From Department/Division Head

To: Research Ethics Board
Room 118, Centre for Clinical Research

Date: December 10, 2008

Re: **Submission to the Research Ethics Board entitled:**
Does the use of oral contraceptives at a young age have a negative effect on the bone status of young women?

Principal Investigator: Jo M. Welch

The above research proposal has been reviewed within the Department/Division of

School of Health and Human Performance, Dalhousie University

I confirm that I am aware of this research study and that:

1. **Qualifications –** The investigator is qualified by education, training and experience to assume responsibility for the proper conduct of this research.

2. **Competing studies –** I am unaware of any on-going studies that would unduly compete for this target population. It is reasonable to expect that the number of patients can be readily recruited in the time allowed.

   Signature: [redacted]
   (Pr

3. **Unexpended research funds –** The arrangements that have been made for the disposition of any unexpended funds at the conclusion of the protocol are acceptable and in accordance with generally accepted practice.

   Signature: [redacted]
   (Dep

   Print name: [redacted]
   (Department/Division Head)

Version #1
December 3, 2008

Capital Health

Research Ethics Board
5790 University Avenue
Room 118, Centre for Clinical Research
Halifax, NS B3H 1V7
Phone: 473-5726
Fax: 473-5620

January 13, 2009

Jo. M. Welch
Dalhousie University
School of Health and Human Performance, Kinesiology
6230 South Street
Halifax, NS B3H 3J5

Attention: ***Vanessa Slayter***

Dear Dr. Welch: ***Final Approval Letter***
(January 13, 2009- January 13, 2010)

RE: Does the use of oral contraceptives at a young age have a negative effect on the bone status of young women? ***REB FILE #: CDHA-RS/2009-263***

Thank you for your response (received November 26, 2008) regarding your proposed study.

Documents resubmitted for review included:

- Email Correspondence- To: Orshy Torok- From: Venessa Slayter – RE: REB File # CDHA-RS/2009-263 (dated January 9, 2009 2:45 PM)
- Email Correspondence- To: Orshy Torok- From: Venessa Slayter – RE: REB File # CDHA-RS/2009-263 (dated January 9, 2009 2:24 PM)
- Email Correspondence- To: Jo Welch- From: Orshy Torok- RE: REB File # CDHA-RS/ 2009-263 (dated January 9, 2009 11:15 am)
- Revised Research Protocol (Version 1.1 dated 1/7/2009)
- Revised Consent To Take Part In A Research Study- Participant Information (Version 1.1 dated January 7, 2008)

I have reviewed your amended protocol, consent form and supporting documents on behalf of the Board and note that all requested changes have been incorporated.

I am now pleased to confirm the Board's full approval for this research study, effective today. This includes review and approval / favourable opinion for:

- Researcher's Checklist For Submission (signed and dated by Dr. Jo Welch on 2008/12/10)
- Letter of Support- From Supporting CDHA Acting Division Head, Endocrinology (signed by Dr. Stephanie Kaiser on December 3, 2008)
- Letter of Support- From Department/Division Head (signed by Merv Ungurain on December 10, 2008)
- Research Services Study Initiation Form (REB Version #1 dated December 3, 2008)
- Ethics Approval Submission Form (Version 1.0 dated December 10, 2008)
- Screening Questionnaire: Young Oral Contraceptives and Bone (Version 1.0 dated December 10, 2008)

- Questionnaire: Oral Contraceptive and Bone (Version 1.0 dated December 3, 2008)
- Advertisement: 'How Strong Are Your Bones? Female Volunteers Needed! (Version 1.0 dated December 3, 2008)
  - Email Correspondence- To: Orshy Torok- From: Venessa Slayter – RE: REB File # CDHA-RS/2009-263 (dated January 9, 2009 2:45 PM)
  - Email Correspondence- To: Orshy Torok- From: Venessa Slayter – RE: REB File # CDHA-RS/2009-263 (dated January 9, 2009 2:24 PM)
  - Email Correspondence- To: Jo Welch- From: Orshy Torok- RE: REB File # CDHA-RS/ 2009-263 (dated January 9, 2009 11:15 am)
  - Revised Research Protocol (Version 1.1 dated 1/7/2009)
  - Revised Consent To Take Part In A Research Study- Participant Information (Version 1.1 dated January 7, 2008)

Please note that the Board's approval for this study will expire on the anniversary date *(January 13, 2009)*. To ensure continuing approval, submit a Request for Annual Approval to the Board at least 2 weeks prior to this date. If the study is not reapproved prior to the anniversary date, the Board will close your file and you must cease all study activities immediately. To reactivate a study, principal investigators must resubmit the Initial Submission to the Board (together with the usual fee) and await notice of review and reapproval.

Please also be sure to notify the Board of any:

- Proposed changes to the initial submission (e.g., amendments to the protocol or informed consent forms)
- Material designed for advertisement or publication with a view to attracting subjects,
- Written information to be provided to subjects,
- Payments and compensation available to subjects,
- Unanticipated problems involving risks to subjects or others,
- Serious adverse events experienced by local subjects,
- Sponsor-provided safety information (e.g., reports of serious unexpected adverse reactions, changes to the investigator's brochure / product monograph, DSMB reports)
- Upcoming audits / inspections by a sponsor or regulatory authority (see research policies RS 02-001 and RS 02-002),
- Closure of the study (within 90 days of the event).

Approved studies may be subject to internal audit. Should your research be selected for audit, the Board will advise you and indicate any other requests at that time.

For administrative purposes, please specify the Board's assigned file number (CDHA-RS/2009-202) on all future correspondence concerning this project.

Yours very truly,
Research Ethics Board

[redacted]

Chris MacKnight, MD, FRCPC
Co-Chair

This statement is in lieu of Health Canada's Research Ethics Board Attestation:
The Research Ethics Board for the Capital District Health Authority operates in accordance with:

- *Food and Drug Regulations, Division 5 "Drugs for Clinical Trials Involving Human Subjects"*
- *Natural Health Products Regulations, Part 4 "Clinical Trials Involving Human Subjects"*
- *ICH Good Clinical Practice: Consolidated Guideline (ICH-E6)*
- *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans*
- *Titles 21 and 45, U.S. Code of Federal Regulations.*

/ot