A longitudinal perspective of oral contraceptive use on bone mineral content in adolescents and young adulthood

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ABSTRACT

In females, peak bone mineral velocity is attained at approximately 12 years of age, with bone mass accrual plateauing at around age 18 years. Optimizing bone accrual during growth is believed to prevent osteoporosis and related fracture risk later in life. A number of lifestyle factors such as physical activity and diet are known to influence bone accrual. In addition, estrogen plays a key role and is a main component affected by oral contraceptives (OC). OC are becoming commonly prescribed for females from 12 years of age. Currently, research on the impact of OC use at this age on bone development is equivocal. Therefore, the purpose of this study was to use a longitudinal study to compare bone mass between OC users and non-users during adolescence and young adulthood.

One hundred and twenty-one female participants were drawn from the University of Saskatchewan's Bone Mineral Accrual Study (BMAS). Participants were grouped based on the initiation and duration of OC use. Bone mineral content (BMC) and areal bone mineral density (aBMD) were assessed by Dual Energy X-Ray absorpitometry. Questionnaires were used to ascertain OC use. BMC and aBMD were assessed between groups at each biological age (BA) using ANCOVA (covariates: height, lean mass, physical activity, vitamin D and calcium). BA is the years from peak height velocity (PHV).

Individuals who had initiated OC use after 18 years of age were shown to have, at 30 years of age, significantly less total body (TB) BMC than those individuals that had never used OC. In contrast, persons who initiated OC use between the years of 12 and 18 did not have significantly different TB BMC, between the ages of 20 to 30 when compared to non-users and users who initiated after 18 years of age. OC usage between 12 to 18 years significantly improved lumbar (LS) spine BMC 7 years post PHV. It was found aBMD was not significantly influenced by duration of OC use.

When OC use began during adolescence there did not appear to be a detrimental effect on TB bone accrual at 30 years. However, it was found that LS accrual was enhanced at approximately 19 years of age, a difference that was no longer evident by 30 years.

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DEDICATION

This thesis is dedicated to my wonderful family and my beautiful dog Bozley for their never-ending enthusiasm in the adventures I pursue. Whether it means travelling to visit, or to meet up to share Bozley, everyone in my family (you know who you are) has been on board and an incredible support- Thank you.

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aBMD	areal BMD
BA	Biological Age Relative to Peak Height Velocity
BMAS	Pediatric Bone Mineral Accrual Study
BMC	Bone Mineral Content
BMD	Bone Mineral Density
Ca	Calcium
DEPO	Depot medroxyprogesterone acetate
DXA	Dual Energy X-Ray Absorptiometry
FN	Femoral Neck
FSH	Follicular Stimulating Hormone
g	Grams
g/cm^2	Grams per centimeter squared
HC	Hormonal Contraceptive
IUD	Intrauterine device
LH	Luteinizing Hormone
LS	Lumbar Spine
OC	Oral Contraceptive
PA	Physical Activity
PHV	Peak Height Velocity
RCT	Randomized Control Trial
SD	Standard Deviations
TB	Total Body
24-HFR	24-hour Food recall

1.0 INTRODUCTION

1.1 Osteoporosis

Osteoporosis is a disease typically affecting the elderly although its onset can occur at any age. The prevalence of osteoporosis is growing in Canada, with two million Canadians currently diagnosed (Osteoporosis Canada, 2010). It is a disease that affects 1 in 4 women, in comparison to 1 in 8 men.

Osteoporosis is characterized by low bone mineral density, deterioration of microarchitectural bone tissue and increased fracture risk (Kanis, Melton, Christiansen, Johnston, & Khaltaev, 2009). Osteoporosis is defined as an areal bone mineral density (aBMD) T score less than 2.5 standard deviations (SD) from the norm, based on BMD as a healthy young adult (Kanis et al., 1994). The majority of fractures occur at the hip and spine, which increases the risk of mortality in the year following the fracture (Busen, 2004). It would appear prudent therefore to better understand the process of bone accrual and the factors affecting bone accrual in order to reduce the risk of osteoporosis and death.

1.2 Bone Accrual

Bone accrues during childhood and adolescence through the action of continuous resorption and formation of bone cells. While 25% of the adult bone mass is accrued during the 2 years spanning the adolescent growth spurt (approximately 12 years of age in girls and 14 years in boys) (Baxter-Jones, Faulkner, Forwood, Mirwald, & Bailey, 2011; Bailey, McKay, Mirwald, Crocker & Faulkner, 1999), a further 50% increase in total bone mass accrual occurs between the ages of 12-18 years in females (Cromer, Bonny, Stager, Lazebnik, & Secic, 2008). This is important to note since bone mass peaks at approximately 18 years and starts to decline at the end of the third decade of life (Frost, 1997; Busen, 2004; Baxter-Jones et al., 2011), with the largest drop off of bone mass occurring in concurrence with menopause. Therefore, if enough bone is not laid down during the growth period, with the age-related decline in bone mass,

women are at higher risk for osteoporosis. As such, this would suggest that the antecedents of bone disease are in childhood. Previous cross-sectional (Ferretti, Capozza, Cointry, Garcia, Plotkin et al., 1998), short-term (Theintz, Buchs, Rizzoli, Slosman, Clavien, Sizonenko et al., 1992) and longer-term longitudinal studies (Maynard, Guo, Chumlea, Roche & Wisemandle, et al., 1998), suggest that bone mass, represented by total body (TB) bone mineral content (BMC) accrual values, are similar **(**for girls**)** before puberty (9 to 11 years of age). At puberty there is a marked acceleration in both linear height and bone mineral accrual with peak height velocity (PHV) being reached on average 8 months before peak bone mineral content velocity (Bailey et al., 1999). However, the timing (chronological age at peak) and magnitude of these maturational events vary systematically and markedly between individuals (Bachrach, Hastie, Wang, Narasimhan, & Marcus, 1999). In the longitudinal Saskatchewan Pediatric Bone Mineral Accrual Study it was found that the timing of peak BMC accrual occurred on average 1.5 years earlier in girls than in boys, and coincided in girls with the timing of menarche (McKay, Bailey, Mirwald, Davison & Faulkner, 1998). Longitudinal studies of human somatic growth have clearly demonstrated that although the pattern of linear growth is consistent within the human species, the timing and magnitude of growth is a highly individualized process. It has also been demonstrated that this is true for the pattern, magnitude and timing of bone mineral accrual in the human skeleton (Bailey et al., 1999). To adequately distinguish individual differences during childhood in the pattern of bone mineral accrual individuals must be studied at comparable biological rather than chronological ages (Molgaard, Thomsen, & Michaelsen, 1999). One way to do this is to identify individual growth trajectories; something that can only be achieved using longitudinally gathered data. The uniqueness of this approach is that it accounts for the wide variation shown amongst children's growth parameters at any given age, and in the velocity of these parameters from one age to the next (Tanner, 1962). Thus, researchers are able to assess bone growth in the time before and after PHV. This time of accelerated bone growth is a period

when influential environmental factors such as physical activity, diet and oral contraception usage may have a significant impact on bone accrual**.**

1.3 Environmental Factors

Genetics account for 60 to 80 % of bone mass accrual, with environmental factors and lifestyle accounting for a further 20-40% (Ruffing, Nieves, Zion, Tendy, Garrett, et al., 2007). Although there are many factors that influence bone, probably the most influential affecting bone growth and bone maintenance are physical activity (PA), diet and the hormonal milieu.

Physical Activity has been shown to be beneficial to the accrual of BMC during puberty (Bailey et al., 1999). High impact loading activities such as tennis, running and gymnastics are among the activities that are beneficial to effectively accruing bone (Bailey et al., 1999; McKay, Petit, Schutz, Jerilynn, Barr et al., 2000). As the skeleton matures, PA intensifies the amount of bone accrued (Bailey et al., 1999); therefore, it is important to consider PA levels and their implications on bone when assessing changes in BMC during growth.

Calcium (Ca) supplementation has also been shown to facilitate adequate bone mineral deposition during growth, especially around the time of PHV (Bailey et al., 1999). Intake of calcium, (1000 mg/d for girls), is necessary to increase bone accrual during the adolescent growth period (Vantanparast, Bailey, Baxter-Jones & Whiting, 2009). Vitamin D is also an essential nutrient for bone development. This nutrient can be obtained through sun exposure and dietary intake. Vitamin D is necessary for proper tissue formation and the achievement of full stature potential (Malina, Bouchard & Bar Or, 2004). Vitamin D deficiency is associated with osteoporotic fractures and it is suggested that 10 μg/d is necessary to improve or maintain bone health as well as increase bone mass of the growing skeleton (Institute of Medicine, 2010; Vantanparast, Calvo, Green, & Whiting, 2010). Since Vitamin D and Ca each have a high impact on bone accrual it is important to control for diet when assessing bone parameters.

The hormonal milieu has also been shown to be an important factor influencing bone accrual. Estrogen levels increase during puberty (Busen, 2004) due to the onset of the menstrual cycle, while the cessation of menses at menopause, results in a decrease in the circulating level of estrogen. Estrogen is secreted throughout the menstrual cycle to aid in the growth of a dominant follicle, which in turn grows and secretes more estrogen. High levels of estrogen lead to optimal peak levels of bone growth and bone maintenance since estrogen suppresses cell apoptosis of osteoblasts cells, which are essential in bone modeling and remodeling (Corwin, 2008). Estrogen also aids in the fusing of the epiphyseal plates at skeletal maturation in order to complete linear growth (Cutler, 1997). With a deficiency of estrogen, bone resorption increases, without an equivocal rise in bone formation (ESHRE Capri Workshop Group, 2010) leading to a potential decline in bone mass. It is known that hormones are affected by a number of factors with pharmacological agents often used as manipulative factors. Hormonal contraceptives (HC) are pharmacological agents that affect the level of circulating estrogen in the body. Progesterone in HC suppresses the levels of estrogen; therefore preventing the growth of estrogen-induced follicles, which in turn reduces peak amounts of estrogen during the menstrual cycle, ultimately resulting in decreasing circulating estrogen (Reed, Scholes, LaCroix, Ichikawa, Barlow, Ott et al., 2003). Although the question of whether lower doses of estrogen in HC suppress bone formation has been studied (Cromer et al., 2008), the effects of HC on bone mineral accrual remain equivocal, as research to date has demonstrated both a positive and negative impact. Oral contraceptive (OC) use is now more common during puberty and is continuing for longer periods of time. The question remains whether use of OC has a detrimental effect on bone health and more specifically, whether use during adolescence and young adulthood affects accrual of bone mineral content in the immediate and long-term future.

1.4 Purpose

The purpose of this study was to assess the long-term effects of OC use during adolescence and young adulthood on subsequent bone mineral accrual for the Total Body (TB), and at the Lumbar Spine (LS) and Femoral Neck (FN).

1.5 Hypothesis

1. It was hypothesized that non-users of OC will have greater TB, LS and FN BMC in comparison to OC users.
2. It was hypothesized that those individuals who use OC for a longer duration will have significantly lower bone mineral content than those that have used for a lesser time and non-users.
3. It was also hypothesized that those individuals that initiated OC during adolescence would have a greater decrease in bone mineral accrual than those individuals that initiated use in young adulthood.

2.0 LITERATURE REVIEW

Given the focus of this study, this review of literature will be limited to a review of musculoskeletal health during growth. While the interplay between endocrinology and bone health will be discussed it is not the focus of this review, and will be restricted to only those research studies directly applicable to the proposed research study.

2.1 Osteoporosis

Osteoporosis is a disease characterized by low bone mineral density (BMD), microarchitectural tissue deterioration and increased fracture risk (Osteoporosis Canada, 2011; Consensus Development Conference, 2000). It currently affects over 2 million Canadians (Osteoporosis Canada, 2011). Osteoporosis occurs without symptoms and is difficult for people

to know they have it (Osteoporosis Canada, 2011; Ioannidis, Papaioannou, Hopman, Akhtar-Danesh, & Anastassiades, 2009). However, tools are available to assess risk of osteoporotic levels and preventative strategies, as well as medications (Osteoporosis Canada, 2011; Ioannidis et al., 2009).

Osteoporosis is defined as aBMD T scores below 2.5 SD from the norm, based on BMD as healthy young adult (Kanis, Melton, Christiansen, Johnston & Khaltaev, 1994). Bone is comprised of both cortical and trabecular compartments. While cortical bone is the dense calcified bone that is found on the exterior compartment of bone, trabecular bone is a porous structure typically located in the vertebrae and at the proximal and distal ends of long bones (Khan, McKay, Kannus, Bailey, Wark et al., 2001). Osteoporosis is most evident in the trabecular bone and primarily affects areas of the body such as the lumbar spine, femoral neck, and radius (Dennison, Mohamed & Cooper, 2006; Rickelund, Carlstrom, Ekblom, Brismar, Schoutlz, et al., 2004). These high-risk areas are prone to fracture especially when unexpected falls occur. Once a fragility fracture of the hip or spine occurs, death often occurs within 5 years (Ioannidis et al., 2009). In fact the rise in risk factor increases up to 3 times for fractures as the aBMD moves 1 negative SD away from the norm (Kanis et al., 1994).

Women are typically more affected, with its occurrence as high as one in four after the age of 50; in comparison males show a ratio of one in eight (Osteoporosis Canada, 2011). It is thought women are more commonly affected due to the lower estrogen amounts at the onset of menopause in comparison to that which is evident through the years of menstruation (Wei, Winzenberg, Laslett, Venn & Jones, 2010). Also, prolonged irregular menstruation and other estrogen deficient ailments during the menstrual years can lead to osteoporosis (Wei et al., 2010; Rickelund et al., 2004). This is important as estrogen protects bone-building cells from cell death (ESHRE Capri Workshop Group, 2010), which is essential to maintaining bone homeostasis. Osteoporosis related fractures have an economic burden to Canadian health care costs of over 1.9 billion dollars (Osteoporosis Canada, 2011). The lengthy recovery time from

osteoporotic fractures is detrimental to the individual's health and drives up the cost of health care annually. Of each fracture occurring in persons over the age of 50, over 80% are due to osteoporosis (Osteoporosis Canada, 2011; Osteoporosis Canada-White Paper, 2011) In 1993, 25,000 people had a osteoporotic related hip fracture (Osteoporosis, 2011), that number has now grown to 30,000 per year (Osteoporosis Canada-White Paper, 2011).

Osteoporosis is a disease that affects adults, however, it is believed that its antecedents are in childhood; therefore, the childhood environment is critical for long-term bone health. PA and diet play an important role in accruing and maintaining bone health (Kannus, 1999) and in order to prevent osteoporosis it is essential that enough bone be laid down during the adolescent years, a critical growth period. The timing of bone accrual is important as growth stops at the end of the second decade of life and maintenance occurs between 20 and 50 years of age (Baxter-Jones et al., 2011). It is therefore important to look at how bone accrues and the factors that affect bone accrual in order to prevent this disease.

2.2 Bone Development

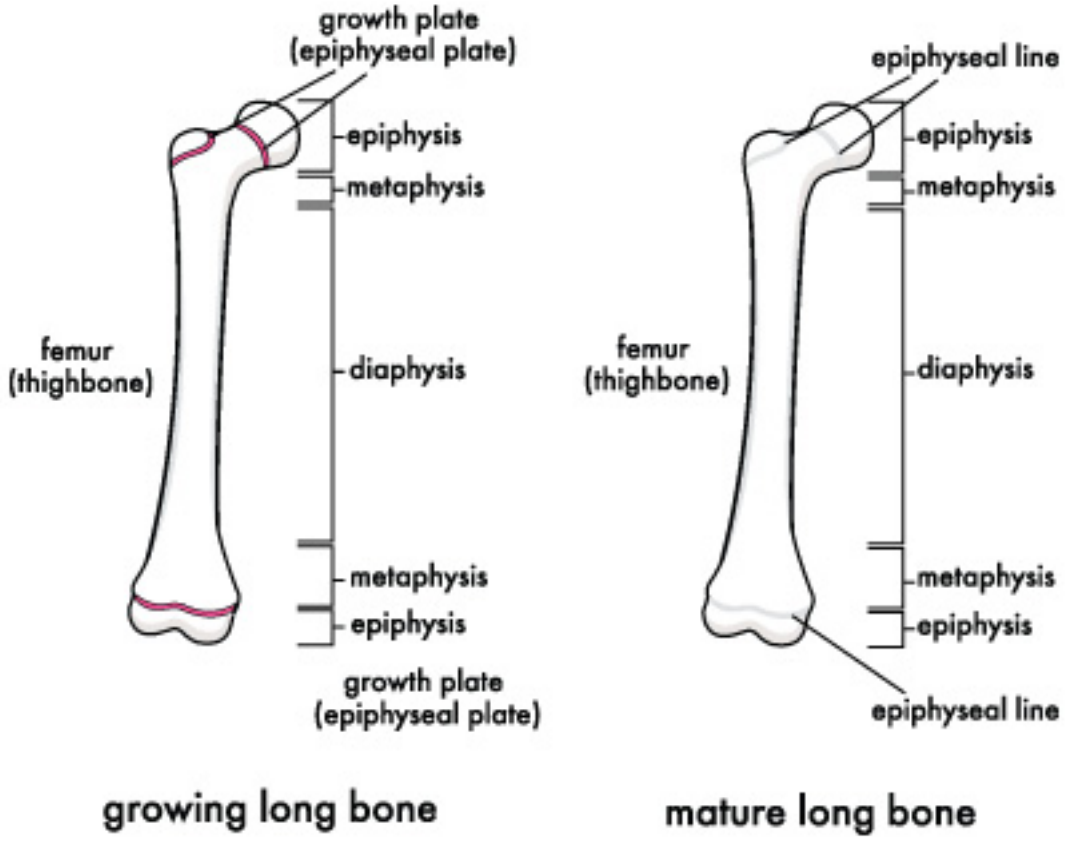
2.2.1 Bone Physiology

Bone is the platform of the skeleton that provides a basic structure (Drake, Vogl & Mitchell, 2005). It also produces red blood cells and stores minerals for the human body (Malina et al., 2004). Bone structure is a lifelong process of accrual and maintenance. Bones are in a constant dynamic process of modeling and remodeling (Malina et al., 2004). Bone modeling and remodeling occur during the original process of forming the adult skeleton, and remodeling, the constant recycling and rebuilding of bone, maintains the mature skeleton once bone modeling has ceased. During childhood and adolescent years bones are being modeled to acquire the mature adult skeleton. Bone is compromised of a bone matrix, which is a manifest action of collagen fibers and calcium and phosphorus minerals (Khan et al., 2001). Bone cells: osteocytes, osteoclasts and osteoblasts are responsible for the growth and maintenance of bone. Primarily the

osteoclasts begin resorption within the matrix in order to keep shape to the bone that is growing in width. Once osteoclasts have finished resorption they manifest into osteoblasts. The osteoblast deposit bone on the outer layers of the bone through the production of calcium. Osteoblasts are then trapped in the matrix and converted into osteoclasts, which mineralize the collagen fibers with calcium and phosphorus minerals.

Both a growth in length and width occur during the remodeling stage. Growth in length takes place at the growth plate of each bone (Rauch, 2005), which separates the epiphysis and metaphysis (Figure 2.1).

Figure 2.1 – Long Bone Diagram



(Dayton Children's, 2011)

Figure Description: the lines separating the epiphysis and metaphysis depict the growth plate.

Lengthening is due to modeling at the growth plates that are located at the ends of the long bones. Once full maturity has been reached, the growth plates fuse between the epiphysis and diaphysis and desist any further growth in length.

Bone width is achieved by the process of deposition on the outer surface of the periosteal layer (Khan et al., 2001); as the bone is increased on the outer surface, osteoclasts absorb the matrix from within the bone on the endocortical area creating a constant cycle of increase in width (Khan et al., 2001). Shape is maintained through compression and tension on the bone surface in order to inhibit and enhance bone growth (Rauch, 2005). Mild tension is believed to increase bone growth, and when small deformities occur, negative feedback from daily mechanical forces repair bone deformity (Rauch, 2005).

2.2.2 Bone Accrual in Adolescents

The process of bone modeling occurs throughout childhood and adolescence with a higher rate of bone deposition than bone resorption until full skeletal maturity has been reached. During the adult years bone is in the state of maintenance where there is a delicate balance of resorption and formation. Females continue to lay down new bone at a high rate until they are 18 years of age; this growth tends to be complete at approximately 20 years of age (Baxter-Jones et al., 2011). From this point bone remodeling takes over which is the same process of new bone being formed by osteoblast cells and old bone being resorbed by osteoclast cells as with modeling; however, a balance of the two processes exists. Even though bone loss is slight during these years (Frost, 1997) it is not until older age, typically after menopause in women, where resorption is at its highest in comparison to deposition, tilting the scales towards bone loss and osteoporotic levels (Cromer et al., 2008; Seeman, 2003).

2.3 Growth and Development

2.3.1 Timing of Maturation

Humans have similar skeletal growth patterns; however the timing of when the pattern occurs varies in accordance to each individual's timing and tempo of maturation (Baxter-Jones, Mirwald, McKay, & Bailey, 2003). In order to document and compare growth, statural growth charts are derived from a series of annual or bi-annual measurements over a long term to indicate the pattern of growth for each individual, typically from a pre-mature to post-mature age. These patterns are made of several small curves of velocity of change (tempo), spanning a distance of growth (timing) (Tanner, 1978). Several small velocity curves exist which are unique to each person, yet there is a significant increase in velocity, deemed the adolescent growth spurt, occurring between the ages of 10 and 12 for females and 13 and 15 for males (Khan, et al., 2001). This velocity of growth indicates the speed of growth in which the child increases in stature at a specific time point and magnitude unique to them.

From the time of birth, growth rate steadily declines until the adolescent growth spurt. Thus the growth spurt can be utilized as a point of maturation; maturity is reached once adult stature is fully attained. In some individuals there is also a mini growth spurt occurring between the ages of 6 and 8 years (Tanner, 1978). It is important to emphasize that these velocities of growth are different from individual to individual and that each child will have his or her growth spurt at a different time point relative to chronological age. Thus, chronological age during the adolescent growth spurt is a poor reflection of an individual's maturity. An alternative; therefore, is to align individuals using a measure of biological or maturational age.

In order to compare individuals, a curve must be fitted to each child's growth in stature (Tanner, 1978). This combines the several minute velocities of growth in individuals and leaves the distinct projection of change in speed of growth, the velocity curve. Once this curve is fitted, growth curve patterns appear as an "S" shape (Tanner, 1978). The rapid acceleration of growth is evident and can further be assessed on velocity charts where centimeters per year are plotted.

Once velocity charts are plotted, comparison between individuals are not reliable unless lined up by time of PHV. PHV is the peak of the velocity curve and is considered an index of biological age (BA). Aligning by BA discounts the difference in timing of PHV and compares individuals based on their maturational status. Therefore it is pertinent to align by biological age rather than chronological age to make comparisons between individuals during adolescence (Malina, 1978).

2.3.2 Maturational Measures

Somatic growth is a measurement of maturity that utilizes the assessment of the body's growth. This is the most important and widely used indicator of maturation in longitudinal growth studies. Velocity of growth is determined by a series of standing height measurements (cm/yr) (Bailey et al., 1999). Plotting velocity charts, the magnitude of growth will indicate an age where peak height is reached. At the age of this peak height, maturational age named biological age (BA) is determined. BA is set to zero, and accumulated there forth. One year after PHV, peak BMC is attained (Bailey et al., 1999). PHV allows for a constant in measurement and alignment amongst individuals in order to compare growth patterns, while controlling for maturational differences. Controlling for height is important, as an individual who is taller will have more BMC, yet may not yield a higher bone density. Also, when comparing between sexes it is important to note that in females skeletal maturation is achieved between the ages of 10 and 12 (Malina, 1978), while in males it occurs approximately two years later. Alignment by PHV allows researchers to assess and compare growth between individuals prior to and after PHV (Malina, 1978). Regardless of maturational measurement used, the marker must be used throughout the data collection series of the longitudinal study for reliability.

2.4 Hormonal Milieu

2.4.1 Hormones

Hormones such as estrogen play a key role in bone accrual (Cromer et al., 2008). Estrogen's role is to suppress cell apoptosis of osteoblasts, which are essential in bone remodeling (ESHRE Capri Workshop, 2010); therefore, allowing the osteoblasts to continue the production of bone. In individuals with a deficiency in circulating estrogen, such as oligiomenorrhea or menopause, bone resorption activity increases, without a subsequent increase in bone formation, leading to bone loss (ESHRE Capri Workshop Group, 2010).

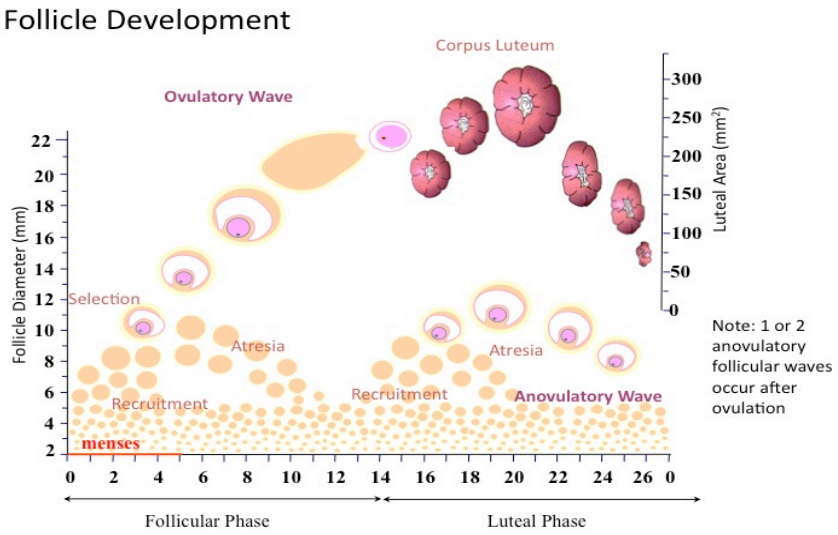
During adolescence the balance between bone resorption and formation is imperative for bone health, particularly during the onset of the menstrual cycle when levels of estrogens are increased. Due to lifestyle factors, such as OC use, the menstrual cycle and associated hormone balance may be altered and the delicate balance of bone maintenance shifted.

2.4.2 Menstrual Cycle

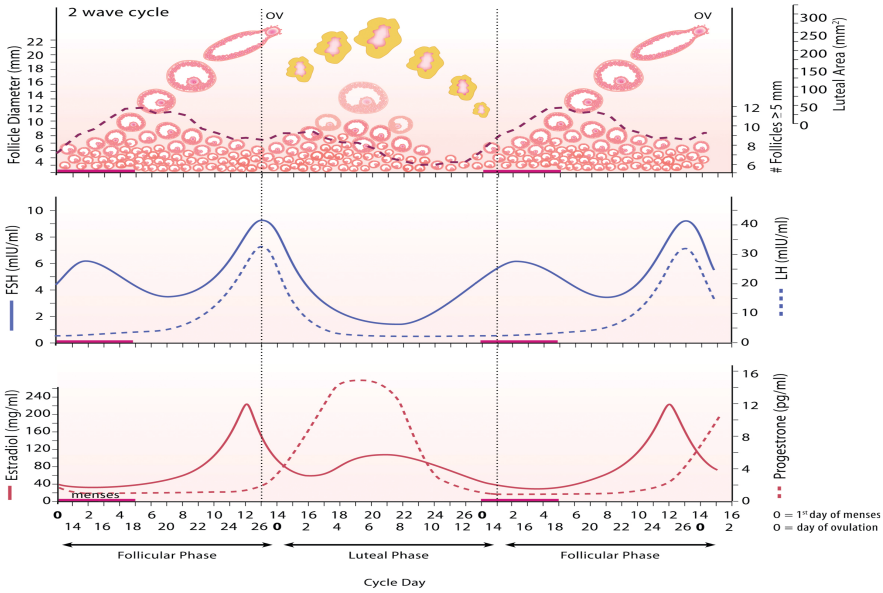
The onset of menstruation (menarche) occurs approximately 1 year after PHV (McKay et al., 1998). Hormones increase at the age of puberty with the onset of the menstrual cycle. The menstrual cycle is controlled by the hypothalamus, which secretes gonadotropin-releasing hormone into the hypothalmo-pituitary portal (Molina, 2009). Each cycle's function is to produce an egg (Molina, 2009). The menstrual cycle is divided into 2 phases: follicular and luteal. Each phase lasts 14 days and involves 5 main reproductive hormones: follicular stimulating hormone (FSH), luteinizing hormone (LH), estrogen, progesterone, and inhibin. Estrogen levels rise at the onset of the 28-day cycle and signal the secretion of FSH and luteinizing hormone LH from the pituitary gland (Molina, 2009). FSH and LH act upon the follicle located in the ovaries to foster the growth and production of an egg (See Figure 2.4 a and b).

Figure 2.4 Oral Contraceptive Effects on the Menstrual Cycle

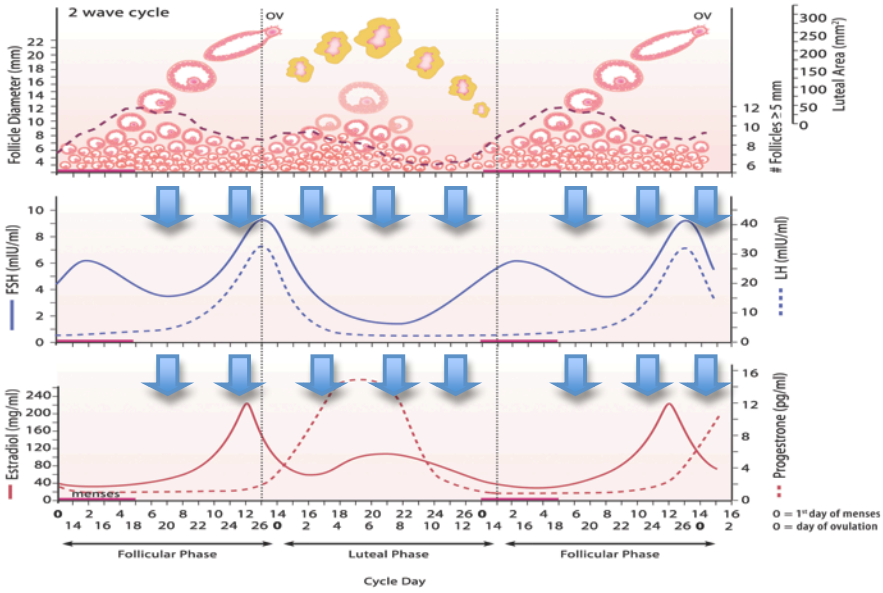
a)



b)



c)



(a) Baerwald, Adams & Pierson, 2011; b) Baerwald & Pierson 2003,
c) Adapted from Baerwald & Pierson, 2003)

Figure Description: a) Regular Menstrual Cycle. Days 1-7 (Menses), Days 1-14 (Follicular Phase). Day 14 LH Surge Days 15-28 (Luteinizing Phase). b) Regular Menstrual Cycle: Estrogen levels peaking at Day 14, which allows for growth of a follicle and LH surge at Day 14. Progesterone levels peak during Days 18-22 to decrease levels of estrogen and corpus luteum. c) Menstrual Cycle under the influence of OC - Arrows indicates OC influence on hormone levels from Day 7-28. Progesterone suppresses estrogen levels and minimizes the growth of a follicle.

The follicular phase occurs from days 1-14, where the first 7 days is the onset of menses. During the first week, gonadotropin hormones, FSH and LH are secreted and act upon the follicle to stimulate growth. Specifically FSH stimulates the granulosa cells of the follicle to grow and LH stimulates the theca cells to hypertrophy. Granulosa cells are the part of the follicle that produces and secretes estrogen and theca cells are responsible for producing androgens that convert into estrogen in the granulosa. Estrogen works as a feedback mechanism for the gonadotropins, with a small amount of estrogen the FSH and LH are reduced in secretion through a positive feedback mechanism; however, as the estrogen continues to be produced from

the granulosa cells, FSH and LH rise due to a negative feedback mechanism. Inhibin is also secreted from the granulosa and inhibits the rise in FSH and as estrogen starts to peak, its feedback on LH produces a larger rise and surge of LH mid cycle.

On day 14 the LH surge causes ovulation and the egg is released. The luteal phase is when the granulosa and theca cells of the follicle evolve into the corpus luteum. As LH begins to drop it acts upon the corpus luteum to produce and secrete more progesterone, estrogen and inhibin. Increased progesterone levels suppress the secretion of FSH and LH. The corpus luteum begins degeneration due to lower levels of LH; therefore, production of progesterone and estrogen also decrease. FSH and LH begin to secrete and the cycle begins once again. Natural occurrence of this cycle has not been show to be detrimental to bone health; however control of this cycle through OC use could be detrimental since circulating levels of estrogen are decreased which are important to bone maintenance (ESHRE Capri Workshop Group, 2010).

2.4.3 Hormonal Contraceptives and Oral Contraceptives

The term hormonal contraceptive encompasses a multitude of birth control types including the patch, intrauterine devices (IUD), depot medroxyprogesterone acetate (DEPO), and OC. Hormone releasing IUD is placed in the uterus and releases progesterone into the body. Both the patch and OC provide a combination of estrogen and progesterone. The patch is placed on and absorbed through the skin and OC is an orally ingested tablet.

2.5 Environmental Influences on Children's Bone Accrual

Early environmental factors such as PA and diet have been shown to have significant effects on bone later in life (Bailey et al., 1999) as has OC use (ESHRE Capri Workshop, 2010). Genetics accounts for large percent of bone mass while environmental lifestyle also contributes to bone mass accrual and reduction in osteoporotic diagnoses (Teegarden, Legowski, Gunther, McCabe &Peacock et al., 2005).

2.5.1 Physical Activity

PA has been proven to have a beneficial effect on the growing and mature skeleton (Bailey et al., 1999). Studies have demonstrated that high impact loading of the bone increases bone mineral accrual over and above accrual that is associated with normal habitual activities (McKay et al., 2000). Impact loading activities such as running, jumping, gymnastics, and tennis put a muscular load on the skeleton creating a strain on the bone that increases bone development. Putting strain on the bone via the skeletal muscles through high impact movement actualizes the overload principle. The dynamic workload allows deformation of the bone and the strain signals for repair by bone cells (Frost, 1997). Osteocytes within the cell matrix are engineered to detect the strain in the bone tissue and signal to adapt the bone tissue to increased levels of strain (Lanyon, 1996) through the process of modeling and remodeling. The mechanostat theory by Frost (1997), explains that throughout normal life, from everyday movement of the skeleton by skeletal muscles, there is an increase in tension and strain on the skeleton that results in benefits to bone mass.

Frost (1997), also points out that estrogen plays a role in Ca storage in the bones and that low levels of estrogen and disuse of the muscles after menopause may be responsible for changes in bone maintenance. During the menstrual years levels of estrogen regulate the storage of calcium needed for breast-feeding. These higher levels of estrogen, in comparison to after menopause, promote a high level of bone modeling and remodeling. If physical activity were to stay consistent, when estrogen is lowered during menopause, the remodeling and modeling thresholds are lowered and start to reduce the amount of bone mass maintained, even with the same amount of strain from exercise (Frost, 1997; Lanyon, 1996). In order to maintain optimal bone mass, it is thought that the strain from physical activity would need to increase. It is believed that estrogen plays a key role in adaptation to mechanical loads (Lanyon, 1996). Lanyon (1996) indicates that bone losses from disuse and estrogen decreases are similar and that a higher load may be needed to maintain optimal levels of bone mass.

Bailey et al., (1999), in their 8-year longitudinal pediatric bone mineral accrual study, analyzed PA at bone accrual during adolescence. Dual Energy X-Ray Absorpitometry (DXA) was utilized to address bone parameters of the TB, LS and FN BMC. Other measurements were taken of anthropometry, diet and PA. Data was collected bi-annually over the 8 years. PA was assessed by questionnaire. Participants were divided and analyzed by levels of activity (active, average, inactive). Participants in the active groups had a higher peak bone mineral accrual, which took place in the 2 years surrounding PHV, in comparison to the inactive group. Specifically the active female group had a 17% greater TB BMC than those in the inactive group. This difference was maintained in adulthood when it was found that active group had greater BMC ten years after PHV (Baxter-Jones, Kontulainen, Faulkner & Bailey, 2008).

PA is known for its benefits on the skeleton and it has been recently shown that PA doses increase BMC. A longitudinal study by Baxter-Jones et al., (2008) suggests that females that are physically active during their adolescence will have 9-10% more BMC in adulthood than those of the less active groups. During adolescence where peak amounts of growth are occurring, PA is essential to increasing BMC and to aid in gaining peak BMC levels. PA during adolescence and young adulthood has been shown to increase bone mass (Baxter-Jones et al., 2008; Kannus, 1999). High impact activities such as weightlifting and gymnastics are most beneficial to increasing BMC during adolescence (Bailey et al., 1999). PA attribution to bone mineral accrual and peak bone mass is important to consider when analyzing adolescents as the difference in activity levels will have a confounding effect on the outcomes. It is essential for adolescents to increase their PA in order to gain the reward of increased bone mass and decrease the risk of osteoporosis.

McKay, et al., (2000) conducted an intervention study on 144 school-aged children. The participants were divided into control and exercise groups. The exercise group was involved in an 8-month exercise program that used loading and movement exercises 3 times a week for a minimum of 10 minutes. DXA scans were taken of the TB, LS and FN at baseline and

conclusion of study. Results of this study indicated that children aged 7 to 11 who are involved in jump training will have a greater increase in femoral trochanter aBMD than those who do not participate in such activities.

MacKelvie, Khan, Petit, Janssen and McKay (2003) performed a 2-year intervention study with 179 female participants aged 8 -12 years old. The schools were randomized into exercise and control groups. The exercise group participated in a circuit incorporating plyometrics and jumping obstacles as the physical activity intervention. The exercise group had a significantly higher LS and FN BMC after 2 years of the high impact exercise program in comparison to the control group, from baseline to completion of the trial.

Fuchs, Bauer and Snow (2001) utilized activities of jumping from a box in order to give a ground reaction force of 8 times the body mass, to define safety and plausibility of this activity for young children and its potential benefits to bone health. Eighty-nine children participated in the intervention study and were randomized into jumping and control groups. The jumping group performed 100 jumps, three times a week from a 2 -foot tall box, while the control group performed light stretching exercises. After 7 months, the exercise group had a significantly higher LS and FN BMC than the control group. The exercise group also reported a significantly higher LS aBMD than the control group. Eight years after cessation of the intervention, the treatment group still had greater BMC (Gunter, Baxter-Jones, Mirwald, Almstedt, Fush et al., 2008).

2.5.2 *Diet*

2.5.2.1 Calcium

Dietary factors such as Ca are important to the growth and maintenance of bone. Ca is an essential component to build bone and is required during the adolescent growth phase of bone growth. Adequate Ca is needed from dietary sources in order to complement the demand by the cells in the bone (Bailey, 1997). Vantanparast et al., (2009) completed a longitudinal study on

113 girls ages 8 to 20 years, looking at Ca intake on bone mineral accrual and maintenance. This study demonstrated that girls from age 9 to 13 years should have a daily intake of 1116 mg of Ca, while those from 14 to 18 years should have 961 mg per day (Vantanparast et al., 2009.). The higher intake of Ca is needed in the years surrounding PHV, where there is a substantial increase in timing and magnitude of growth.

Ca is a mineral that plays a primary role in growth and maintenance of bone and is the basis of the skeletal structure. By the age of 20, an adult female will have approximately 2200g of BMC; Ca comprises 32.2% of this BMC (Ellis, Shypailo, Hergenroeder, Perez & Abrams, 1996). The recommended estimated average daily intake is 1,100 mg/d of Ca for females from 9-18 years old and 800 mg/d of Ca for females 19-50 years of age to meet the requirements of 50% of the healthy population (Institute of Medicine, 2010). Essential to maintaining optimum levels of Ca is bone remodeling. The role of calcium in bone growth is the deposition into the osteoid cell, where its mineralization occurs around the osteoblasts cells and turns active osteoblasts into their least active form of osteocytes (Khan et al., 2001). Once resorption occurs, the Ca is removed from the matrix of which it is formed. The process of formation and absorption is directly reliant on Ca stores and is a dynamic process that takes place throughout growth and the maintenance periods.

While it is known that adequate Ca intake is important to bone accrual, the question of whether Ca intake can conserve bone with the implementation of OC has also been addressed. A study by Teegarden et al., (2005) suggested that an increase in Ca had positive impacts on total hip BMD and BMC and was a protective agent in OC users. With the high impact of calcium intake on bone, it must be considered as a confounder when assessing bone accrual, as it is unknown whether the decrease in BMC is due to a change in Ca exposure or whether the OC are negatively impacting BMC levels.

2.5.2.2 *Vitamin D*

Vitamin D is essential in order to absorb the levels of calcium needed to maintain Ca homeostasis. Vitamin D plays a role in regulating levels of calcium (Khan et al., 2001). Vitamin D can be obtained from either sunlight or diet (Vantanparast et al., 2010) and is a regulatory factor in the dispersal of Ca from the bone, as well as a factor in the small intestine's absorption of Ca (Khan et al., 2001).

Since the primary source of Vitamin D is through sunlight exposure synthesis in the skin (Vitamin D_2) (Vantanparast et al., 2010), it is essential to supplement with dietary vitamin D (Vitamin D_2 and D_3) during the winter months in geographical areas with reduced levels of sunlight (Vieth, Cole, Hawker, Trang & Rubin, 2001). With an insufficient intake of Vitamin D, ailments such as osteomalacia and rickets, or softening of the bone, will occur (Vantanparast et al., 2010). The recommended (estimated) average daily intake is 10 μg/d of vitamin D to meet the requirements of 50 % of the healthy population (Institute of Medicine, 2010).

2.6 Hormonal Contraceptives and Oral Contraceptives Relationship to Bone and Fracture Risk

Although DEPO is a widely used contraceptive, and it is one of the top three choices of birth control, it has been found to negatively impact bone health, (Busen, 2004; Lopez, Grimes, Shulz and Curtis, 2011). A study by Berenson, Rahman, Breitkopf and Bi (2008) found participants who used DEPO for 2 years had an approximately 6% BMD loss at the hip and spine. The US Food and Drug Commission have identified a 2-year limit as it is warning for women using DEPO as contraceptive (Berenson et al., 2008). While DEPO has been found to result in bone loss, the effect of other forms of OC use during adolescence and young adulthood remains unclear.

OC are the most widely used type of birth control and is the number one choice amongst young women under 30 years of age in the United States (Scholes, Ichikawa, LaCroix, Spangler, Beasley et al., 2010). OC, which are primarily designed to prevent pregnancy, have a wide

variety of uses. For example, in adolescence and adulthood OC are prescribed as an acne medication. OC are also prescribed to regulate menstruation and to increase estrogen in women with oligio-amenorrhea or polycystic ovarian syndrome. It is important to understand the effect of OC use on the menstrual cycle in order to investigate its effect on bone, so to prevent usage that may be a cause of low bone density and osteoporotic conditions.

OC are available in both combinations of estrogen and progesterone, and progesterone only. OC deliver a synthetic form of estrogen and progestin with the dose of progestin being high enough to suppress endogenous estrogen production. OC are administered daily over a 28-day cycle. With estrogen suppressed, the feedback mechanism in the menstrual cycle, to release FSH and LH is hindered and the follicle will not grow. This inhibits further estrogen and progesterone production and secretion from a growing follicle. The constant level of progesterone throughout the cycle inhibits the egg from implanting. FSH and LH stay at the same low level throughout the 28 days. For 7 out of the 28-day cycle, a placebo or no pill is administered and progestin levels decline and menses occurs. After 7 days of placebo, the next cycle of OC are initiated.

Lopez et al., (2011) provided a meta-analysis of the literature examining the impact of OC use on bone. The assessment of 16 trials indicated a gap in longitudinal studies, fracture risk assessments and consistency in study designs. Injectable (DEPO), combination OC (estrogen and progesterone), progesterone only OC, and implant contraceptives were assessed. Eight of these trials compared between OC formulations on bone parameters (Endrikat, Mih, Dusterberg, Land, Gerlinger et al., 2004; Hartard, Kleinmond, Luppa, Zelger, Egger et al., 2006; Rad, Kluft, de Kam, Meijer, Cohen et al., 2011; Paoletti, Orru, Floris, Mannias, Vacca et al., 2000; Nappi, Di Spiezo Sard, Acunzo, Bifulco, Tommaselli et al., 2003; Berenson, Radecki, Grady, Rickert, & Thomas, 2001; Nappi, Di Spiezo Sardo, Greco, Tommaselli, Giordona et al., 2005; Gargano, Massaro, Morra, Formisano, Di Carlo et al., 2008). Two of the studies compared OC formulations against a placebo and one study compared hormone versus no hormone

contraceptive methods. DEPO was found to negatively affect bone health if used without an estrogen supplement in two trials (Lopez et al., 2011). Combination OC trials indicated there may be a benefit to BMD; however, these trials were not conducted in comparison to a placebo (Lopez et al., 2011). Lopez et al., (2011) assessed the risk of bias in these studies; however they were not reviewed for heterogeneity with meta-analysis due to the varying types of interventions. From this review it was noted that fracture risk was not assessed in any of the randomized control trials (RCT) studies, yet BMD was assessed as an indicator of bone health. Considering fracture-risk was not assessed as an outcome, the applicability of the evidence highlighted the need for longer studies for assessment in this area. It was also found that most of the studies that were reviewed had a greater than 20% loss of participants, a factor which would increase bias risk.

Bitzer and Simon's, (2011) review of hormonal contraceptives indicated that some studies had found combination OC negatively affected BMD but several studies indicated that OC with 30µg ethynilestradiol did not see a subsequent loss in BMD in the maturing skeleton (Llyod, Taylor & Lin, 2000; Beksinska, Kleinschmidt & Smit, 2009; Cromer, Stager & Bonny, 2004). However, the lower dose of 20µg ethynilestradiol does not support optimal bone mineral accrual during adolescence (Cromer, Stager & Bonny, 2004; Bitzer & Simon, 2011).

In the 1996/1997 Statistics Canada Health reported a total of 18% of the population at all ages, was using OC (Wilkins, Johansen, Beaudet & Neutel, 2000). Of females between 15-19 years of age 27% were users. It was also shown that 41 % of 20 -24 year olds used OC, and 33% of 25-29 year olds using OC. Even though less used after the age of 30 there was still a 19% user rate from 30-34 years of age (Wilkins et al., 2000). These statistics indicate that users potentially could be using OC for up to and over 20 years and therefore, may be exposed to extended periods of low estrogen levels, which could negatively impact bone mass. This highlights the importance of longitudinal studies to address the long-term effects of OC usage on bone health, especially since more females are utilizing OC throughout adolescence and young adulthood.

A 2-year cross-sectional analysis by Scholes et al., (2010) assessed over 600 adolescent and adult female OC users, aged 14 to 18 and 19 to 30 respectively. In this study mean duration of use was 9 months for adolescent users and 1 year for young adult users. While there was no impact on aBMD noted during adolescence between users and non-users, the adult females showed a reduced aBMD. Further analysis demonstrated that, as duration of use increased, aBMD decreased. This study indicates that duration of use may impact aBMD in females, and highlights the need to assess this impact longitudinally.

A 5-year study by Polatti, Peroti, Filippa, Gallina and Nappi, (1995) showed young females between the ages of 19-22, who did not use OC had an approximate 8% increase in BMD from pre to post test dates, in comparison to those who used OC. BMD data was collected and examined every year, and non-users showed a significant increase in BMD over the test period, while there were no significant changes observed in the users group. It is important to consider the long-term effects of taking OC and their association with duration of use as a preventative measure for osteoporosis. Studies that choose to look at BMD and BMC over a short period of time may be missing the impact or changes in bone parameters that may occur over the longer period of time, for instance the use of a pharmalogical agent such as OC. It should be cautioned that a one year study, may show an increase, and a positive association with OC use, while in the next few years a decrease in bone mass might actually be occurring.

2.7 Concerns with Oral Contraceptive Use

Age at onset of HC use is gradually decreasing. While the primary medical use of HC is to prevent unplanned pregnancies, other medical conditions often rely on similar hormone therapy as part of the ongoing treatment. For example, OC is the first prescribed method of controlling irregular menstrual function (Hartard, Kleinmond, Kirchbichler, Jeschke, Wiseman et al., 2004), including persons diagnosed with oligomenorrhea and polycystic ovarian syndrome. OC is also often prescribed as an acne medication. It is not unusual therefore to see OC use

beginning as young as 12 years old (Rome, Ziegler, Secic, Bonny, Stager et al., 2004), the age coinciding with average PHV in young females. During this time of peak growth, the influences of estrogen depression may have a significant impact on bone accrual.

In the last 30 years, estrogen levels in OC have been reduced due to health risks. With the reduction of endogenous estrogen in the cycle due to the onset of OC use, and low-estrogen pills, the overall circulation levels of estrogen are compromised. Estrogen is at its highest at the 14th day of the cycle in order to induce an LH surge and release an egg; however, OC suppress estrogen at this time resulting in very low circulation levels. Therefore the question arises: does this lower dose of estrogen and lower circulation levels due to estrogen suppression provide enough estrogen for optimal bone turnover (Cromer et al., 2008).

Liu and Lebrun (2006) conducted a systematic review of 75 articles. Quality of evidence was based on the Oxford Centre for Evidence-based Medicine levels. Under the healthy population category, 10 studies indicated a positive effect of OC use on bone (7 cross-sectional, 3 cohort studies). Twenty-nine studies found no effect of OC use on bone (4 RCT, 9 cohort, 15 cross-sectional), while 7 studies (4 cohort and 3 cross-sectional) reported a negative effect of OC use on bone health. This review concluded that there were positive effects of OC on populations of peri-menopausal and oligio-amenorhhea women. Both these populations suffer from a decreased amount of estrogen, and its believed that the supplemental estrogen improved their circulating levels and in turn optimized their bone mass. However, in healthy pre-menopausal women the research findings are less consistent. The conclusions of this review may be influenced by the differences across the study designs and varying quality of evidence found, from individual cohort study to case series (Levels 2b to 4) (Polatti et al., 1995; Burr, Yoshikawa, Teegarden, Lyle, McCabe et al., 2000; Cromer et al., 2004, Hartard et al., 2004; Rome et al., 2004; Hartard, Bottermann, Bartenstein, Jeschke & Schwaiger, 1997) which reflect a lack of higher levels of evidence. Each study assessed indicated a difference in time-line of study, age groups assessed and type of study (RCT, cohort, cross sectional). The need for

consistency in testing to indicate the impact of OC on bone needs to be considered. Longitudinal design is needed to assess the impact of: lifetime use, duration of use and initiation of use.

Table 2.7 summarizes the quality of evidence assessment for the reviewed literature pertaining to the effects of OC use on bone health. Assessment of evidence was based upon the U.S Preventative Services Task Force (2009), and is defined as follows, from strongest to weakest: 1) meta-analysis/systematic review, 2) RCT, 3) quasi-experimental, 4) controlled observational, 5) observational studies with no controls and 6) expert opinion. Further information regarding quality of evidence can be found from Lopez et al., 2011; Bitzer and Simon, 2011; and Liu and Lebrun, 2006.

Table 2.7 Literature Review Quality of Evidence

Article	**Quality of Evidence Level**	**OC Effect on Bone**	**Study Design**	**Age Group (yrs)**	**Length of Study**	**N**
Lopez 2011	1	-	Meta-analysis	-	-	-
Bitzer 2011	1	-	Review	-	-	-
Scholes 2010	3	Negative	Cross-sectional	14-30	-	606
Polatti 1995	3	Negative	Cohort	19-22	5 years	200
Hartard 2004	3	Negative	Cross-sectional	18-35	-	69
Rome 2004	3	No effect	Cohort	12-18	1 year	370
Liu 2006	1	10 Positive 29 No effect 7 Negative	Systematic-Review	-	-	-
Nappi 2005	2	No effect	RCT	22-34	1 year	71
Ruffing 2007	3	Negative	Cross-sectional			135
Burr 2000	3	Negative	Cohort	18-31	2 years	123
Berenson 2008	3	No effect	Cohort	16-33	3 years	703
Cromer 2008	3	Negative	Cohort	12-18	2 years	433

The study by Nappi et al., (2005) examined two different low-dose OC combinations over a 12-month period on women ages 22-34 years. The 21-day OC formulations consisted of 30-μg ethinyl/estradiol with varied amounts of synthetic progesterone. Combination 1 added 3 mg drospirenone and combination 2 added 30 μg of gestodene. Both combinations showed beneficial effects on bone turnover in the female users groups versus the control group. Also, there were no significant differences in spinal BMD between the two users groups and control group from pre-test to post test values (Nappi et al., 2005). In contrast, a 4-year study by Ruffing et al., (2007), showed a reduction in BMD and BMC in college-aged female cadets who were OC users, with users defined as having used OC for 3 months or more in their lifetime. A 2-year study by Burr et al., (2000), similarly showed that OC use in females aged 18-31, may be associated with a reduced increase of bone mass at the femoral neck (FN). The retrospective study of Hartard et al., (1997) on 128 women from 20-35 years of age also showed that OC use masked the beneficial effect of long-term exercise on bone mass.

The differences in the literature not only highlights the need to further assess OC impact on bone, but also highlight the lack of a longitudinal approach since these studies so far have looked at relatively short-term gains and losses of bone. The inconsistent study designs have included a broad range of chronological and maturational ages (i.e. 12 to 35 years) and have divided the adolescent and adult subgroups differently. Grouping the adolescents by terms of maturation as well as adults by the mature skeleton when addressing bone health is prudent in order to assess the differences OC use has on bone health and maintains consistency among studies. Not only does OC use still need to be addressed, but also the duration of use and timing of initiation in order to evaluate the implications on bone health during the growing and mature phases of bone formation and resorption. Long-term studies are needed to address the gaps in the literature and provide clarity as to the influence of OC on bone development, in order to implement the right preventative strategies for osteoporosis in adolescence and adult life.

2.8 Longitudinal Versus Cross-Sectional Studies

Longitudinal studies are the process of following a selection of same-aged individuals and collecting data serially in order to see developmental patterns (Tanner, 1978). The advantage of this process is that there is no variability between children or ages, as the same child is re-analyzed each year. However, this type of study is costly and demands a larger time commitment. Cross-sectional studies take one measurement at one period and make a prediction based on the outcomes of the sample population. It generalizes the growth of a child over 5 years based on 5 different children at different ages. The positive of this, is data can be collected and analyzed quickly (Tanner, 1978). The limitation of a cross-sectional design is the assumption that children grow at the same time and magnitude (Cromer, 2003). Mixed longitudinal studies have an intake of several age groups over a few years, this allows for a range of data from several individuals over a shorter period of time with the age range of a longer period. Example: Over a span of 3 years, take in cohorts of children aged 8-12, this allows for a data set of ages 8 to 15 years growth and development within a 3-year time span (Tanner, 1978). With a mixed longitudinal study, individual growth patterns can be acquired and used in order to assess differences in skeletal growth.

Considering the 6-year timeline of 50% skeletal growth from 12-18 years (Cromer et al., 2008) and the lifestyle influences, such as pharmacological agents, the timing of initiation of OC use with respect to maturation needs to be examined. Berenson et al., (2008) performed a 3-year study using 16 to 33 year old participants using OC and showed an increase in the spine BMD in the first 12 months, then a gradual decrease. In contrast, a study by Cromer et al., (2008) looked at participants 12-18 years of age and indicated a majority decreased their spine BMD in the first year of OC use. Discrepancy between the two studies may be due to the lack of consideration ascribed to the individual growth patterns. As stated previously, adolescents do not mature at the same rate; therefore, age of maturation (indexed by example PHV) should be used when comparing adolescent OC users. Those participants showing an increase in bone, may in fact be

having a detriment in bone accrual related to OC use but are being recorded as attaining bone, due to the accrual that is occurring as a consequence of their natural growth spurt. OC use in relation to PHV may provide further insight into the impact of OC use on bone accrual.

2.9 Summary of Literature Review

Osteoporosis is a problem that still needs investigation in order to find the best preventative strategies from allowing this disease to occur. Antecedents such as PA and diet in childhood are responsible for up to 45% of skeletal growth. PA, and diet are important factors for bone accrual and need to be accounted for when studying bone growth. Recently, research focusing on the impact of OC use on bone has become more prevalent, thus far however the findings have been equivocal. This leads to the current research questions: Does oral contraceptive use have an effect on bone mineral content with respect to duration of use and initial timing of use? The use of longitudinal assessment is imperative as it allows the impact of OC use on bone from adolescence through adulthood to be examined. This type of assessment also extends the look at the effects of duration of use in conjunction with the normative duration of OC use by women.

Hence it was the **purpose** of this study was to assess the long-term effects of OC use during adolescence and young adulthood on subsequent bone mineral accrual. It is **hypothesized** that non–users of OC will have greater TB, LS, and FN BMC in comparison to OC users. It is also hypothesized that those that have initiated OC during adolescence will have a significant decrease in bone mineral accrual in comparison to the to those that initiate 6 years post peak height velocity (PPHV) and non-users. It is also hypothesized that those who use OC for a greater length of time will have significantly lower BMC at the TB, LS and FN than those that have used for lesser time and non-users.

3.0 METHODS

3.1 Study Design

The University of Saskatchewan's (U of S) Pediatric Bone Mineral Accrual Study (BMAS) is a mixed-longitudinal cohort design that was initiated in 1991. The purpose of the study was to assess bone growth from childhood into adulthood and investigate environmental influences such as physical activity and diet on bone accrual (Bailey, 1997). The study began with eight chronological age clusters (entry age 8 to 15 years) in 1991, with additional recruitments in 1992, and 1993. Data was collected until 1997/98, and was resumed again from 2002 to 2010. This design allowed for a 22-year developmental pattern from 8 to 30 years of age (Table 3.1) to be measured in 11 years.

Table 3.1-Number of subjects by age group and year of test.

Age	1991	1992	1993	1994	1995	1996	1997	2002/03	2003/04	2004/05	2006/07	*Total*
8	3 (7)	5 (11)	0 (2)									8 (20)
9	10 (15)	3 (9)	5 (10)	0 (2)								18 (36)
10	19 (16)	10 (18)	3 (10)	5 (10)	0 (2)							37 (56)
11	17 (12)	18 (15)	12 (18)	3 (9)	5 (10)	0 (2)						55 (66)
12	21 (18)	17 (12)	16 (15)	12 (14)	3 (10)	5 (10)	0 (2)					74 (81)
13	17 (21)	21 (16)	17 (11)	14 (16)	11 (14)	3 (10)	5 (7)					88 (95)
14	17 (16)	15 (20)	20 (15)	14 (11)	14 (16)	10 (12)	3 (9)					93 (99)
15	3 (8)	17 (15)	14 (20)	14 (15)	12 (11)	10 (16)	8 (8)					78 (93)
16		3 (7)	17 (14)	13 (12)	13 (14)	11 (7)	8 (11)					65 (65)
17			3 (7)	13 (11)	13 (12)	12 (12)	9 (6)	0 (2)				50 (50)
18				3 (6)	13 (8)	12 (11)	8 (9)	3 (3)	0 (2)			39 (39)
19					3 (6)	8 (6)	7 (8)	4 (13)	3 (2)	0 (2)		25 (37)
20						4 (3)	5 (3)	6 (13)	2 (12)	5 (4)		22 (35)
21							2 (2)	6 (9)	5 (7)	2 (9)	0 (2)	15 (29)
22								12 (9)	6 (11)	6 (10)	4 (7)	28 (37)
23								8 (10)	10 (8)	7 (9)	3 (7)	28 (34)
24								14 (14)	9 (12)	10 (7)	7 (10)	40 (43)
25								8 (6)	11 (13)	8 (14)	5 (12)	32 (45)
26								3 (5)	9 (6)	10 (11)	7 (8)	29 (30)
27								1 (0)	4 (5)	9 (5)	12 (11)	26 (21)
28										3 (3)	11 (14)	14 (17)
29											9 (5)	9 (5)
30											2 (2)	2 (2)
Total	107 (113)	109 (123)	107 (122)	91 (106)	87 (103)	75 (89)	55 (65)	65 (84)	59 (78)	60 (74)	60 (78)	875 (1035)

Table Description: The total number of participants from 1991- 2007. Male participants (*n*) are indicated per year and age group, followed by the number of female participants (*n*) in brackets indicated for each year and age group for each cohort of the study. 1991, 1992 and 1993 were intake years for the study.

3.2 Participants

BMAS participants were recruited from two elementary schools located in Saskatoon, Saskatchewan (population 200,000). In 1991 a total of 228 students (113 boys and 115 girls) provided written consent to participate and 220 children were DXA scanned. From 2002 – 2007 data was collected on 169 returning participants (85 males and 84 females) (Table 3.1). From 2009 to 2010, 100 participants returned for measurement (35 males and 65 females not shown in table).

For the present analysis, female participants (n=121) were drawn from BMAS. To be included in the study, subjects were required to have had three DXA measures, with at least 2 occurring in childhood. Ethics was obtained through the U of S Biomedical Research Ethics Board (Appendix A). Testing took place at the College of Kinesiology bone growth and anthropometry laboratories at the U of S. Prior to testing the subjects were informed of the testing protocols and informed consent was obtained (Appendix B). During childhood and adolescence, assent was collected from the subjects and consent from their parents. At any point subjects were allowed to withdraw from the study, and could choose to not participate in testing components.

Subjects that were absent due to pregnancy at time of annual data collection were still included at other time points. Only subjects using the oral contraceptive pills (n=48), and contraceptive patches (n=1) were included in this study.

3.3 Anthropometric Measurements

Height, sitting height, and weight were assessed bi-annually from 1991 to 1997, and annually for the remainder of the study. Participants wore light clothing for the anthropometric measurements. Height and sitting height measurements were taken to the nearest 0.01 mm using a stadiometer (Holtain Ltd, Crymych, Dyfed, UK). Body mass was measured to the nearest 0.1

kg using a standard physician scale (Toleda Scale Company, Windsor, Ontario, Model # 2830). A certified exercise physiologist performed all measures.

3.4 Chronological Age, Biological Age and Age of Menstruation

Chronological age was calculated from the decimal age at test date subtracting the decimal age at birth date, for each participant. Age categories were scaled into yearly groups from 8 to 33 years of age. For example, those with the decimal age of 10.50-11.49 were categorized as age 11.

Biological Age (BA), a measure of maturation, was defined by alignment with age of PHV. PHV was determined by measuring the acceleration of the velocity curve of statural growth (cm/year), obtained through serial measures of standing height (Malina et al., 2004) during the child and adolescence periods. PHV is calculated using longitudinal data of annual stature measurements. The process includes fitting a cubic spline to the velocity values of each child per year (Bailey et al., 1999). Therefore, peak linear growth was derived from the result of chronological age at test minus chronological age at PHV (Baxter Jones et al., 2008). Biological age at test was calculated by the decimal age at the test date, minus the decimal age at PHV.

First date of menstruation was collected retrospectively as the subjects participated every six months, and researchers assessed time of menstruation between the two testing dates in which menses occurred. Actual age of first menstruation was determined by subtracting cycles of 28 days from the participant's most current menses. For those who attained menarche between test dates, the date was recorded retrospectively.

3.5 Body Composition

DXA (Hologic 2000 QDR, Hologic, Waltham, MA), was utilized to assess BMC grams (g), aBMD grams per centimeter squared (g/cm^2) and body fat percentage. This method involves the passage of high and low photon beams which attenuate through the body, fat tissues and bone

in order to give an estimate of the composition of soft tissue and bone mineral in the body. The DXA uses two x-ray beams, which exposes the participant to radiation; however, DXA is used due to its low levels of radiation and exposure risk. DXA has equivalent radiation to regular daily life of five days (Malina et al., 2004). A licensed X-Ray technician performed all DXA measurements on the participants. BMC and aBMD were taken at the TB, anterior-posterior LS (L1-L4) and FN sites. All participants were instructed to wear light clothing, free of any metal for the DXA scan. The coefficient of variation (CV) for Hologic 2000 for TB, LS and FN and LS BMC are 0.60, 0.61 and 0.91 respectively and TB, LS, FN aBMD are 0.51, 0.88 and 0.90 respectively (Faulkner, Forwood, Beck, Mafukidze, Russell et al., 2003). The new DXA Hologic 2000 implemented in 2008. BMC and aBMD CV are 0.5% TB, 0.5% LS and 0.7% FN (Cornish & Chilibeck, 2009).

For the TB scan, participants were asked to lie on their back in a horizontal position on the mat in the DXA machine. In order to prevent the superior aspect of the scapula and the mandible from overlapping, the subject's head was positioned with shoulders depressed, chin raised and arms in anatomical position (Erlandson, 2007). The feet were aligned so they rotated internally and any participants unable to hold their feet in this position had their feet taped for immobilization. This position eliminates the tibia and fibula overlap in the scan (Erlandson, 2007). For the LS scan, participants were aligned in order to raise the ribs away from the lumbar spine, by holding their hands behind their head. To reduce the lumbar curve and place the spine in contact with the mat, a box was placed under the participant's lower legs. The FN scans, were taken with the participant laid supine with the foot on the side of the hip being scanned inverted approximately 30 degrees. This position was held with a nylon belt and a positioning wedge (Erlandson, 2007). The arm of the subject was positioned outside the area of the scan.

3.6 Hormonal Contraceptive Therapies

Participants filled out a lifestyle questionnaire (Appendix C) that assessed HC use. HC use was assessed prospectively and retrospectively, as these questions were used in the study from 2002-2010 only. HC specific questions were: Are you currently using oral contraceptives? What type of oral contraceptives do you use? How long have you been using oral contraceptives?

Age of first HC use and duration of use of HC use was calculated retrospectively by dates of last menstruation. The type(s) and brand(s) of HC used, and duration of use were each determined from information provided by the participants.

3.7 Diet and Physical Activity

Participants filled out a 24-hour Food Recall (24-HFR) questionnaire (Appendix D**)**, to assess Ca and vitamin D intake. The 24-HFR questionnaires ask participants to record all the food, beverages, vitamins and supplements they consumed the previous day. A visual aid was available to determine portion sizes for each item the participant listed on the 24-HFR. The correlation coefficient 0.80 for 24-HFR is based on 7 recalls per year (Whiting & Shrestha, 1993). Physical Activity was assessed using the Physical Activity Questionnaires for children (PAQ-C), for adolescents (PAQ-A) and adults (PAQ-AD) (PAQ-AD see Appendix E). This questionnaire determines the level of physical activity a subject participates in, in a typical week. Example: This includes activities that make you sweat, make your legs feel tired or make you breathe hard. In the last 7 days during the morning, how often were you active (none, 1 time, 2 to 3 times, 4 to 5 times, 6 to 7 times last week)? The re-test reliability of PAQ-C in females is $r = 0.82$. The peer-comparison activity rating is $r = 0.73$ for the PAQ-A and the PAQ-AD correlation is $r = 0.53$ to 0.64 (Copeland, Kowalski, Donen, & Tremblay, 2005).

3.8 Data Analysis

Data between non-users and OC users were compared using analysis of variance (ANOVA) to check for differences between descriptive characteristics of height, lean mass, PA, vitamin D and Ca. Data were analyzed to assess the effect of: 1) OC use, 2) Duration of OC use, and 3) Time Frame of initiation of OC use on aBMD and BMC at the TB, LS and FN. Subjects were grouped into OC users (n = 49) and non-users (n= 72). Users were defined as those that used OC for one or more years. The OC users group was sub-grouped by years of OC use; Group a: 1 to 5 years, Group b: 5 to 10 years, and Group c: 10 plus years. They were also grouped in relation to initiation of use. Group i: non-users, Group ii: 1 to 6 years post PHV, and Group iii: 6 plus years post PHV. The users of the OC initiation groups were divided into Group i to simulate the 6 years adolescent growth period after PHV, typically from 12 to 18 years of age, based on timing of maturation. Group ii simulates the mature adult skeleton and adolescent growth has stopped after the age of 18. These two groups are divided by adolescent and adult OC initiation.

Analysis of covariance (ANCOVA) was used to analyze group differences in demographic characteristics (covariates: physical activity and diet) prior to analysis of bone parameters. ANCOVA was then used to analyze group difference amongst the groups. Post hoc tests were used to determine where the differences occurred between the groups. BMC comparisons were adjusted for covariates (height, lean mass, PA, vitamin D and Ca) for each site (TB, LS, FN). Each OC use model was analyzed separately for aBMD and BMC at each site (TB, LS, FN) at each time point for both chronological age and BA. Statistical difference was assessed between groups using Statistical Analysis Software (SPSS version 18, SPSS Inc., Chicago, IL). Level of significance was $p < 0.05$.

Limitations of using ANCOVA in this study are the increase in occurrence of a Type 1 error, meaning that a difference may be shown when there is not one. MANCOVA cannot be used to reduce this error in this study, as a complete data set from 8 to 30 years of age would be needed. The design of this study allows for participants to miss some age points and therefore

group numbers change at each time point. In order to have as many participants at each group for a population mean, ANCOVA is used to assess the differences at each age, with different group numbers. Another assumption of ANCOVA is the normality of distribution of the population. The sample number at the 30-year mark is considerably smaller than at 8 to 10 years of age. The assumption is these 5 individuals at 30 represent the population. Another limitation of this statistical analysis is the homogeneity of variance, meaning the variance is equal across the sample. Once again the low numbers at the age of 30 may contribute to an indiscretion of this assumption.

4.0 RESULTS

4.1 Descriptive Data

Table 4.1 lists the demographic characteristics of the subjects. When aligned by BA, there was a difference in vitamin D, PA score and calcium intake at -1.00, 11.00, 15.00 and 20 between users and non-users groups. Therefore, during analysis of users versus non-users, physical activity, calcium, vitamin D, height and lean mass were used as covariates.

Table 4.1- Characteristics of Non-users and Oral Contraceptive Users

	Non-Users						**Oral Contraceptive Users**					
Biol. Age	n	Height (cm)	Lean Mass (g)	PA Score	Vitamin D (iu)	Calcium (mg)	n	Height (cm)	Lean Mass (g)	PA Score	Vitamin D (iu)	Calcium (mg)
0	33	153.9	3.0	3.1	260.2	1028.1	32	154.6	3.0	3.0	318.6	1081.4
1	36	161.7	3.5	2.8	223.7	968.3	40	160.0	3.4	2.9	258.8	1012.8
2	38	163.5	3.7	2.7	194.1	861.8	13	163.7	3.6	2.8	251.2	1013.1
3	44	165.4	3.7	2.6	227.8	875.6	39	164.8	3.7	2.7	220.0	943.1
4	30	164.7	3.8	2.4	185.9	802.4	31	166.3	3.9	2.6	175.3	175.3
5	21	164.9	3.7	2.3	221.0	836.4	30	166.4	3.9	2.2	239.2	923.1
6	14	164.7	3.9	2.1	215.7	868.2	24	166.4	3.9	2.2	213.9	1042.8
7	11	169.9	4.0	2.0	160.7	779.8	22	167.0	4.0	2.0	160.7	979.3
8	10	170.4	4.2	2.3	190.2	847.1	13	166.5	4.0	2.3	216.3	948.3
9	8	163.7	3.9	2.2	165.5	896.5	11	166.8	3.9	2.3	165.6	872.4
10	8	163.3	3.9	2.2	174.7	687.7	14	167.6	3.9	2.2	155.3	687.7
11	9	163.1	3.7	**2.8***	157,1	851.4	19	166.9	4.0	**2.0***	269.7	975.2
12	8	166.6	4.1	2.3	239.9	1020.3	24	165.5	4.0	2.2	216.6	870.9
13	13	166.6	4.1	2.5	156.1	756.2	30	167.5	4.2	2.24	237.0	982.3
14	7	166.8	4.0	2.1	213.7	902.4	23	165.7	4.1	2.2	204.4	898.8
15	12	167.0	4.3	2.4	**146.8***	800.0	16	165.8	4.2	2.1	**278.5***	1040.2
16	10	167.6	4.4	2.6	228.4	957.8	12	164.3	4.1	2.1	230.5	1126.2
17	8	164.6	4.1	2.5	157.7	644.6	5	163.7	4.0	1.8	170.7	956.5
18	4	168.6	4.4	2.4	146.4	737.1	8	165.6	4.7	2.0	232.8	1029.1
19	6	168.6	4.4	2.1	135.0	982.3	8	166.7	4.6	2.4	454.7	1277.5
20	5	166.9	5.0	1.9	107.2	**473.4***	6	163.2	4.5	2.1	264.3	**1416.1***

Figure Description: Preliminary comparison between non-users and oral contraceptive users of height, lean mass, physical activity (PA), vitamin D and calcium. Biological age from 0 to 20 represents approximately 12-32 years of age. PA score is rated from 1 to 5 (1=inactive, 5=active). Data are expressed as a mean. *Significant values presented in bold between non-users and oral contraceptive users, p. <0.05.

4.2 Analysis of Group Differences in Bone Mineral Density

4.2.1 Non-users vs. OC Users

There was a significant difference between groups in adjusted TB BMC at the BA of 20 years. The non-users had greater mean TB BMC than the users ($p < 0.05$, non-users: 2576.571 +/- SEM 50.193; users: 2226.491 +/- SEM 44.216). There were no significant differences at any other time points between users and non-users. As well, there were no significant differences found at the total LS BMC. There were no other significant differences at the FN BMC between users groups. See Figure 4.2.

Figure 4.2- Total Body, Lumbar Spine and Femoral Neck Bone Mineral Content Between Non-users and Oral Contraceptive Users

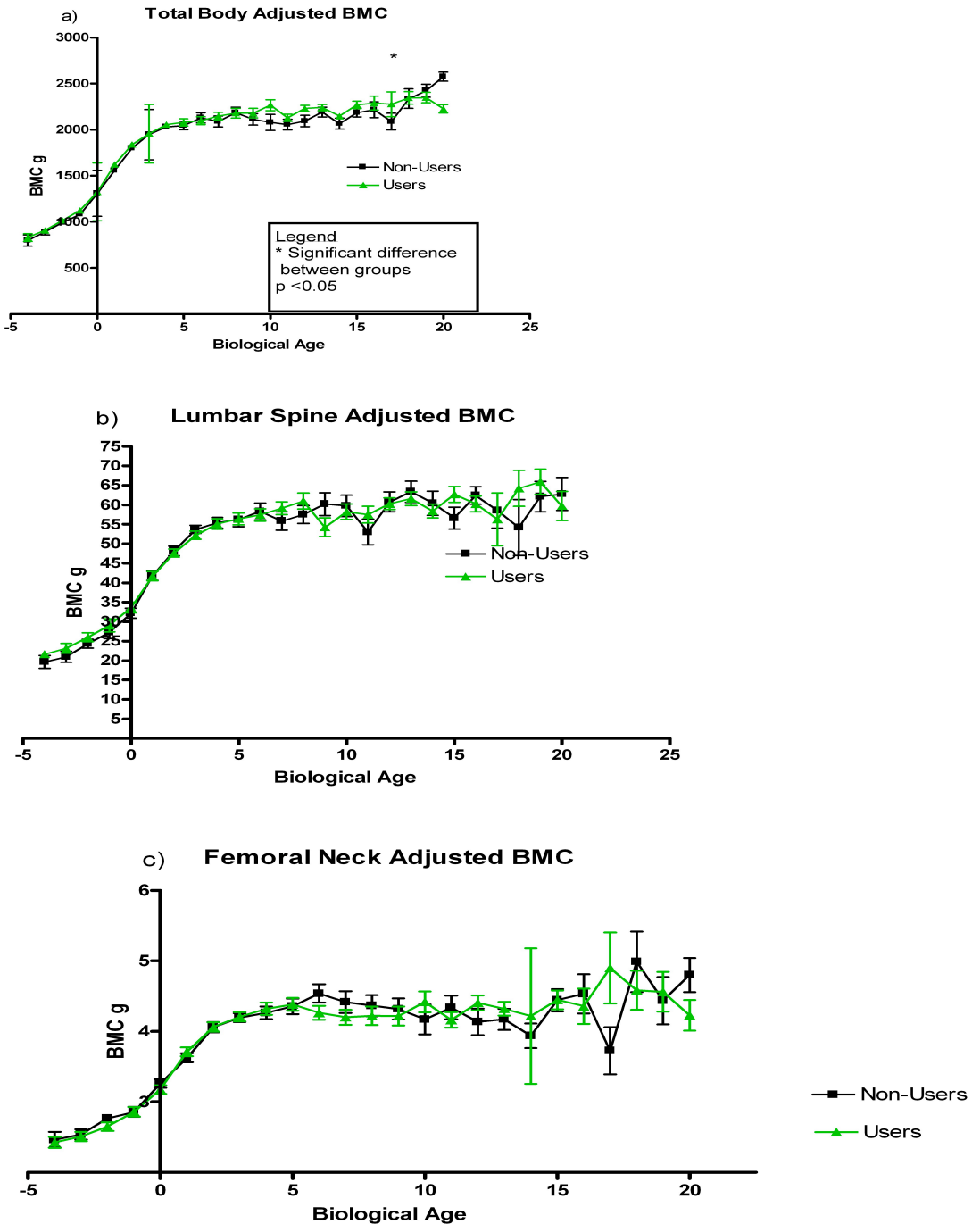


Figure Description: Comparison of a) Total body, b) Lumbar Spine, c) Femoral Neck bone mineral content (BMC) adjusted for covariates (height, lean mass, physical activity, vitamin D and calcium) between non-users and oral contraceptive users. X-axis is biological age aligned by peak height velocity. Data are expressed as mean +/- SEM. * Significant differences between groups $p < 0.05$

4.2.2 Duration of Use

There was no significant difference between user groups at any time point at TB, LS or FN for duration of use analysis.

Table 4.2- Comparison Between Non-users and Users Across Duration of Oral Contraceptive Use in Young Adulthood

	Non User	**User 1-5 yrs**	**User 6-10 yrs**	**User 10^{+} yrs**
n	25	8	18	13
Total Body BMC	2258.46	2343.42	2302.52	2200.91
Lumbar Spine BMC	61.65	63.81	60.75	60.10
Femoral Neck BMC	4.36	7.77	4.40	4.23
Total Body aBMD	1.10	1.14	1.11	1.11
Lumbar Spine aBMD	1.04	1.05	1.023	1.04
Femoral Neck aBMD	0.85	0.88	0.85	0.86

Table Description: Comparison between non-users and oral contraceptive users (OC) duration of use (1-5 years, 6-10 years, 10+ years) adjusted for covariates (height, lean mass, physical activity, vitamin D and calcium) bone mineral content (BMC) and areal bone mineral density (aBMD). BMC expressed as g/cm^2 and aBMD as g/cm^2. Number of participants in each group (*n*). Data are expressed as mean. No significant differences were found between groups $p < 0.05$.

4.2.3 Initiation of Use

Results from the ANCOVA indicated at BA 20, non-users had significantly greater TB BMC than those who initiated OC use 7+ years PPHV. At the BA 7 years there was a significant difference between users groups, where those who initiated OC use 1-6 years PPHV had greater LS BMC than those who initiated 7+ years. There was no significant difference between groups at the LS or FN for adjusted BMC. There was no significant difference between groups at the FN for mean adjusted BMC at any time point.

Table 4.3 – Longitudinal Comparison of Non-users and Age of Initiation of Oral Contraceptive Use

	Non User				User 1-6 yr PPHV				User 7+ yr PPHV			
Time from PHV	PHV 0.00	PPHV 7.00	PPHV 18.00	PPHV 20.00	PHV 0.00	PPHV 7.00	PPHV 18.00	PPHV 20.00	PHV 0.00	PPHV 7.00	PPHV 18.00	PPHV 20.00
Approximate Chronological Age	12	19	30	32	12	19	30	32	12	19	30	32
N	33	11	4	5	14	12	1	3	18	10	7	3
Total Body BMC	1296.9	2095.8	2351.5	2599.6 *	1319.2	2206.1	2219.6	2178.8	1354.1	2073.5	2355.9	2235.8 *
Lumbar Spine BMC	32.2	56.4	51.7	59.6	33.8	63.3 *	86.7	67.3	33.5	53.7 *	62.5	57.5
Femoral Neck BMC	3.3	4.4	5.0	5.1	3.2	4.4	4.8	3.5	3.2	4.0	4.6	4.5

Table Description: Comparison between non-users and initiation of oral contraceptive (OC) users (post peak height velocity (PPHV) 1-6 years, PPHV 7+ years) bone mineral content BMC adjusted for covariates (height, lean mass, physical activity, vitamin D and calcium). Biological age aligned by peak height velocity (PHV). BMC expressed as g/cm. Data are expressed as mean. * Significant differences between groups $p < 0.05$

5.0 DISCUSSION

There were three main findings of this study. There was a significant difference between non-users and OC users at the BA 20, approximately 32 years of age. Those that initiated OC use in adulthood (7+ years PPHV) had significantly less TB BMC than those who had never used OC. The group that initiated OC use during adolescence (1-6 years PPHV) had greater LS BMC at BA 7, approximately 19 years of age in comparison to the group that initiated OC use in adulthood. Up until this point, longitudinal studies exploring the impact of OC use have been limited to 3 to 5 years (Berenson et al., 2008; Pollatti et al., 1995). In addition, none of these studies have incorporated maturational status into the skeletal assessments that were made (Berenson et al., 2008), which does not account for individual timing and growth patterns when comparing across participants and intervention groups. Without a careful look at the growth and development of persons prior to and after PHV (Tanner, 1978), it is impossible to know whether

the change in BMC is due to the natural growth pattern or whether there is causation from OC use. By incorporating the individual growth patterns of each participant in the data analysis this study has overcome this limitation and has provided a stronger analysis of the impact of OC use on skeletal attributes.

5.1 Non-users versus OC users

It was hypothesized that users of OC would have less TB, LS and FN BMC than non-users. The non-users showed a significant increase of TB BMC at the BA of 20, which corresponds to approximately 32 years of age. There were no significant differences at any other time point. The OC users significantly lower TB BMC at 32 years of age may indicate a decline in accrued bone, due to the hormone alterations that only become a detriment to bone later in life. The significantly lower TB BMC may be due to the natural decline in bone mass that starts at the end of the third decade of life (Frost, 1997), which would need to be further assessed with the age groups over 30 to 40. Typically the LS is the final skeletal site to complete peak bone mass accrual, while the FN peaks earlier. A study by Baxter-Jones et al., (2011) indicated that TB peak bone mass was achieved at approximately 19 years of age, while peak LS was at age 17 years and FN at age 15**,** so it would be assumed that a natural decline in BMC would show at the LS or FN earlier; however, there were no significant differences at any other site tested. This lower TB BMC could be because of the natural decline of BMC after the age of 30 and while one might expect specific site differences based on the work of Baxter-Jones et al., (2011) this was not evident in this study. Lack of site-specific difference may be due to the age of the participants, where these specific declinations in BMC may be seen later in life when bone mineral begins to rapidly decline.

The amounts of TB BMC in both groups are within the normal range. Moolgard, Thomsen, Prentice, Cole and Michaelsen (1997) indicates the average TB BMC at 20 years of age is approximately 2250 g. Comparatively, the non-users of this study at 20 years of age had

2211 g of TB BMC and the users had 2108 g. By the age of 32, the non-users had 2576 g and the users had 2226 g of TB BMC, where the non-users had significantly more TB BMC, however the users were still within the normative range for TB BMC. Even though both groups had normative values for TB BMC, future TB BMC may be at risk if the rate of loss is higher due to the hormonal influences from OC use, than the natural loss that occurs after the age of 30. Since the largest drop off of BMC is during the time of menopause, due to a reduction in estrogen levels (Khosla, 2010), it is important to continue addressing the OC use impact after 30 years of age up until the time of menopause in order to further assess whether OC use has an detrimental impact on rate of bone loss. Further assessment through longitudinal studies may be able to address the question of whether the estrogen levels under the influence of OC are enough after the age of 30 when resorption is higher than formation during the years of bone maintenance. Considering estrogen is an osteoblast protective agent (ESHRE Capri Workshop, 2010), the question arises whether the circulating estrogen levels are high enough when bone resorption is higher (ages 30+) in comparison to the time of bone acquisition ages (ages 12-18), where formation is higher than resorption.

Typically persons with oligiomenorrhea and anorexia demonstrate positive effects on bone mineral accrual from OC use (Liu & Lebrun, 2006). Considering the lowest amount of circulating estrogen in a regular menstrual cycle is 30 pol/L **(**Teirmaa et al., 1998**)**, these erratic menstrual cycles may not generate a consistent enough low dose of circulating estrogen lead to higher bone resorption. However, even though persons taking OC have a reduced circulating estrogen level, they may still have a consistent dose of estrogen. This in itself may maintain enough circulating estrogen to regulate bone remodeling.

Another aspect is to look at further is the physical activity groups within non-user and user groups. This may indicate whether PA has had any protective or masking effect on this analysis. A study by Burr et al., (2000), indicated that those who exercise and took OC had the greatest increase in bone mineral accrual at the FN. Furthermore, if those who are active have a

greater total BMC by approximately 10 % (Baxter-Jones et al., 2011), than those who are less active, this may also mask any effects that OC have on bone parameters of active individuals, whether it beneficial or detrimental. Even though there were no preliminary differences between groups for PA level, considering PA has influence on bone accrual (Bailey et al., 1999), further analysis using more sophisticated analytical techniques may give further insight to whether or not OC have any benefits or detriment on adolescent, young adult or adult bone health. The type of PA should also be considered as those individuals that are more exposed to the benefits high impact loading activities (McKay et al., 2000), may still benefit from continuous accrual of bone regardless of detriments hormonal alterations may have. It is important to further research these mechanisms on bone accrual and bone maintenance. Singling out each factor that contributes to bone mineral acquisition is important in order to get the most benefit from environmental and lifestyle choices.

The finding of decreased TB BMC is consistent with that of Scholes et al., (2010), where it was found that adult users of OC, with a mean use of over 3 and half years, had a significant decrease in whole body BMD, and the higher decrease in BMD was associated with increase in duration of use. It has been suggested that long duration of OC use may be detrimental to TB BMC later in life; however, this finding needs to be further assessed into ages 30 through 50, by looking at a further duration of use on BMC.

There was no significant difference at the LS or FN where fracture risk is most prominent, an indication that OC use may not enhance osteoporotic bone loss. OC users did not indicate a detriment to LS or FN BMC by the alterations to circulating estrogen and progestin levels. However, analyzing initiation and duration of use may indicate whether timing and prolonged use impact healthy BMC levels.

5.2 Duration of Use

It was hypothesized that longer duration of use would have a negative effect on TB, LS, and FN BMC at all sites. However, a positive finding from this study is that duration of use did not show any significant detriments at any of the sites tested, at any age. This analysis suggests that the TB, LS and FN BMC were not affected by prolonged use of OC. This would suggest that the amount of estrogen needed to aid in the balance of cell apoptosis of osteoblasts (Bradford, Gerace, Roland, & Chrzan, 2010) is still enough despite the reduced circulating levels regulated by OC use. Typically regular menstruation leads to circulation estradiol levels of 143 +/- 78 pmol/l and 35pg/ml (converts to 128pmol/l) (Teirmaa et al., 1998; Ginther, MaGastal, Gastal, Baerwald & Pierson, 2005). Teirmaa et al., (1998) indicated approximately 30-pmol/l level of estradiol throughout the 21 days of OC use. This low circulating level may be effective in maintaining bone homeostasis and the reason why those with irregular menstruation benefit from OC use (Liu & Lebrun, 2006).

Studies so far have looked at users versus non-users over a short duration of time, Cromer et al., (2008) for 2 years and Berenson et al., (2008), for 3 years. This is of interest as our study looked at over 20 years of data, and had a mean usage of 7.94 years. This is the first study to investigate OC use over 5 years and since the analysis of users versus non-users does not account for duration of use the grouping of users into short term and long-term was able to further analyze OC use on bone. This study has taken it two steps further in order to analyze the specific details of timing of use and years of use.

Finally, users for over 10 years had no detriment to their BMC, which is interesting considering the prolonged levels of reduced peak estrogen levels in the cycle. This is an opposing outcome to Scholes et al., (2010) who demonstrated a decrease in BMD with increase in duration of use. This discrepancy in findings may be due to the nature of the data analysis in the current study, specifically the aligning of participants by skeletal maturation and accounting for differences in growth patterns and development.

5.3 Initiation of Use

It was hypothesized that those who initiated OC in the adolescent period would have lower BMC at the TB, FN and LS than those who initiated in adulthood and non-users. Interesting findings of the study are outcomes that are slightly opposing in respects to initiation of use. Participants that had never used OC had significantly greater TB BMC at (BA 20yrs) 32 years of age, than those who had initiated OC use at BA 7, approximately age 19 or later. There was no difference for those that initiated use 1-6 years PPHV at this site at any time point. This would suggest a detriment to late start of OC, which is what was hypothesized; however, it was also hypothesized that an early start of OC use would be a detriment, but there was no association. Further the data indicates that initiation of OC within adolescence (1-6 PPHV) had a significant increase in LS BMC at BA 7, approximately age 19, in comparison to the adult initiation (7+ PPHV) LS BMC. This further refutes the premise that OC use during the delicate growing years may deplete optimal bone levels, and may in fact suggest that initiation in adolescent years is more beneficial than initiation in young adulthood. At this point the initiation of use at an adult age is more controversial than those starting in adolescence, affecting the TB BMC as well as the LS BMC. From these findings we can conclude that initiation of OC use may be most beneficial during the adolescent years. It should be noted however, that the lack of difference between groups at 30 years of age would suggest that this benefit is not prolonged. This may have to do with the duration of use for those that initiated in adolescence and leaves the question of whether the increase was due to OC use for the entire adolescent period or portion thereof.

Scholes et al., (2010) indicated that users who used for a longer period of time had lower BMC, however, timing of maturation was not accounted for within the study and thus bone may have shown a detriment that could be confirmed using a longitudinal model. Using a longitudinal design to assess OC use allowed for alignment of maturation using PHV to address changes in bone parameters (Tanner, 1962). Since 20-40% of bone accrual is affected by

lifestyle and environmental factors (Ruffing et al., 2007) it was crucial to look at the impact of hormonal changes by pharmalogical agents on bone during adolescence, when the majority of bone is accrued. Participants that have already reached adult BMC should still be studied to analyze the effects of OC use on BMC; however, it is imperative to account for differences in growth and development to compare across individuals, which is why accounting for growth prior to and after PHV is essential (Tanner, 1978).

There were no significant differences at the FN between any of the groups at any age. This is of interest since the FN is the first of these sites to obtain peak bone mass, and is one of the three most common fragility fracture risk areas. This finding indicates that use of OC on bone may not be a detriment and therefore not a risk factor for osteoporosis related fractures. Furthermore, with the decrease in age of initiation of use of OC, it is important to include users prior to adulthood, as a growing number of users are under the age of 19 and utilizing OC for numerous medical conditions. These findings suggest that usage of OC during adolescence can be used without a detrimental effect on bone. With the benefit shown in LS BMC, OC did not appear to affect the crucial magnitude of bone accrual when initiated in adolescence. Even though a majority of users are 20-30 years old, over 25 % of Canadian users (Wilkins et al., 2000) use OC within 6 years of PPHV. Since a detriment was not found, but a benefit, this indicates the possibility to address menstrual irregularities and acne with OC without the penalty to BMC and risk of osteoporosis.

Also, these findings may indicate the impact of OC use on bone in adolescence may not be as detrimental to BMC levels as a teenage pregnancy. Pregnant women, provide calcium to the fetus for development of the skeleton (NIAMS, 2011). Ca is provided through the diet, but if inadequate will be derived from the stores in the maternal bone; therefore, decreasing BMC (NIAMS, 2011). Teenage pregnancy is a concern, as during the times of optimal bone accrual, the calcium is needed for the baby and can lead to low levels of BMC and increase the risk of osteoporosis (NIAMS, 2011). The use of OC in adolescence in this study has not shown a

detriment, but yet an improvement at the LS BMC; therefore, the continuation of OC prescription to prevent teenage pregnancy should still be considered.

5.4 Limitations of the Study

One of the limitations to this study is that it is an observational study and does not regulate uncontrollable factors such as type of OC use and selection of groups. All types of OC formulations were included in the study, and most of the users had switched OC brands and formulation types; therefore, not allowing for one type of OC to be individually assessed. A RCT would be needed to further solidify the potential benefits of OC use in adolescence found in the study. Another limitation of this observational study design is that participants were included throughout the study even with only 3 time points. This varied the amount of individuals included in the group mean at each age point. At the approximate age of 12, there were over 30 participants per group, but by age 32 there were only 5 non-users and 6 OC users. This low participant number may have contributed to the significant difference in TB BMC at the age of 32. The mean of 5 non-users and 6 OC users is calculated as a representative of the population and the variation in TB BMC between these two groups, due to small numbers, may be contributing to this significant difference. Thus this significant finding may be due to a Type 1 error and therefore caution should be used when considering the real effect of this outcome.

In this study some retrospective data for OC use was necessary since by the date the questionnaire was implemented, some participants were already users. Medical records could be used to specifically date time of OC use in combination with questionnaires in order to increase reliability between prescription start dates and length of use. Even though most questionnaires were given within the timing of initiation of use, some participants did not remember the exact times of start of OC use. Additionally, some participants were indicating different start times from year to year.

Another limitation was the variety of OC types that the users were utilizing. While the majority used the OC brand Tri-Cyclen (n=18), other OC types used were Allesse (n=13), Marvelon (n=5), Lo-Estren (3), Diane 21 (n=3), Yasmin (n=1), Triquilar (n=1), Min-Ovral (n=4), Demulin (n=1), and the Evra Patch (n=1). It was not one brand or formulation of OC but rather a multitude of OC formulations. Even though the groups were divided into users and non-users, OC brands vary in combinations of progesterone and estrogen. These variations may separately have an impact on bone health. This study also allowed for a long duration of use to be observed. Considering females may change birth control types throughout their duration of use, there is no detailed information to which formulation, if any, is causing bone alterations. Specifically, a certain brand may be causing a benefit or detriment, and being masked by another.

Another limitation of the study was the low sample size at the later time points of data collection. Seven participants per group are needed for 80% statistical power. As the study continues the number of data for time points over 30 years of age will be increased and another analysis of the data should be performed to see if any further changes in BMC at the TB, LS and FN are evident.

There are three limitations to the use of DXA to assess bone parameters. One is that DXA is a 2 dimensional image of a 3 dimensional object. This does not allow for data to be derived of the bone structure and leads to assumptions of bone health based on BMC or aBMD. Second, since DXA has only 2 rays to attenuate the 3 different tissues of the body (lean mass, fat mass and bone) (Bolotin, Sievanen, & Grashuis, 2003) lean and fat mass data are derived as soft tissue and then calculated in order to derive compositions of each. An assumption with DXA is that the tissues scanned are consistent (Malina et al., 2004); however, the varying fat mass at the bone site between patients may lead to unreliable measures as with increase in fat mass the more soft tissue for the ray to attenuate in order to measure the bone (Bolotin et al., 2003). Further, OC use can have an effect on lipid profiles as the 17β-estradiol has shown to increase high-density

lipoprotein cholesterol and low-density lipoprotein cholesterol (Bitzer & Simon, 2011). These changes in lipid profiles may lead to unreliable measures from patient to patient, and years on and off use of OC. The third limitation of DXA in this study is in 2008 a second machine was utilized. Changes in bone mass may be due to the change in equipment and variability between machines; however, a conversion equation was applied to the new data to make it comparable with the first machine, so this should not be a significant factor.

5.5 Strengths of Study

The longitudinal aspect of this study as well as the skeletal measurements prior to and after maturation are important strengths of this study. With these markers we were able to tease out individual growth patterns and align participants by maturational status instead of chronological age. This accounts for individual timing and magnitudes of growth and allows for a more valuable and reliable comparison. This is the only study to analyze OC use in accordance with maturation. Also this is the only study with an OC use data span of over 20 years in relation to bone health.

5.6 Future Directions

Considering there was a significant decline in TB BMC after the age of 30 in the OC user group, it would be imperative to follow this group throughout the next decade of life.
Also, it is important to look at physical activity groups in comparison with duration of use, to assess for any PA benefits to OC users. And finally, a future study needs to look at initiation of use and duration of use of those who initiated OC within 1-6 years of PPHV and used OC for 1-5 years, 5-10 years and 10+ years, in order to determine further answers to why LS BMC was greater at 19 years of age in OC users, but did not show a difference later on. Does the combined effect of timing of OC initiation and duration of use have an effect on BMC? Considering that

LS BMC went up at 19 years of age for those who initiated 1-6 years PPHV it would be interesting to see the interaction between lengths of use within this initiation period.

5.7 Conclusion

This is the first study to examine the impact of OC use on bone parameters using a longitudinal perspective with maturation alignment. Up until now, there have been equivocal results in OC use on bone. The short-term RCT so far in the literature does not account for the changes of bone parameters over a long term, or the absence of markers prior to and after maturation. Short term RCT or intervention studies neglect the ability to align individuals by PHV for maturational reliable comparisons.

This study indicated users that initiated OC in adulthood (7 + years PPHV) had less TB BMC at 32 years of age than adolescent OC users. It also indicates benefits to LS BMC at 19 years of age from adolescent initiation of OC use. Interestingly enough there were no detriments to the FN site, irrespective of timing of initiation or duration of use at any age point. This is a positive finding as the FN is a fracture risk site (Osteoporosis, 2011), which is linked with morbidity (Ioannidis et al., 2009). The other benefit of these findings is the LS showed an increase at 19 years of age with adolescent initiation of OC use, and otherwise did not show any significant differences amongst the analysis. The LS being another significant area of fragility fracture risk and increased morbidity, it is important to further assess the question of the detriments of OC use on this site.

In conclusion, the majority of the study hypotheses were not supported, with two exceptions: at 32 years of age OC users had significantly more TB BMC than non-users and those that initiated OC use in adulthood had significantly more TB BMC at the same age point. Given these results it seems promising that OC use in adolescence may not be as detrimental as previously hypothesized.

6.0 REFERENCES

Bachrach, L.K., Hastie, T., Wang, M.C., Narasimhan, B., & Marcus, R., (1999). Bone mineral acquisition in healthy Asian, Hispanic, Black and Caucasian youth: a longitudinal study. *Journal of clinical endocrinology and metabolism.* (84) pp. 4702-4701.

Baerwald, A., Adams, G.P., & Pierson, R.A., (2011). Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Human Reproduction Update Advanced Access.* (0) 0 pp. 1-19.

Baerwald, A.R., & Pierson, R.A., (2003). Ovarian follicular development during the use of oral contraception: a review. *Journal of Obstetrics and Gynaecology Canada.* (26) 1 pp. 1-6.

Bailey, D.A., (1997). The Saskatchewan pediatric bone mineral accrual study: bone mineral acquisition during the growing years. *International Journal of Sports Medicine.* (18) pp. *191-194.*

Bailey, D.A., McKay, H.A., Mirwald, R.L., Crocker, P.R.E., & Faulkner, R.A., (1999). A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. *Journal of Bone and Mineral Research*. (14) pp. 1672-1679.

Baxter-Jones, A.D.G., Mirwald, R.L., McKay, H.A., & Bailey, D.A., (2003). A longitudinal analysis of sex differences in bone mineral accrual in healthy 8-19-year-old boys and girls. *Annals of human biology*. (30) 2 pp. 160-175.

Baxter-Jones, A.D.G., Kontulainen, S.A., Faulkner, R.A., & Bailey, D.A., (2008). A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. *Bone*. (43) pp. 1101-1007.

Baxter-Jones, A.D.G., Faulkner, R.A, Forwood, M., Mirwald, R.L., & Bailey, D.A., (2011). Bone mineral accrual from 8 to 30 years of age: An estimation of peak bone mass. *Journal of Bone Mineral Research.* Doi: 10.102/jbmr.4.12.

Beksinska, M.E., Kleinschmidt, I., & Smit, J.A., (2009). Bone mineral density in a cohort of adolescents during use of norethisterone enanthate, depot-medroxy progesterone acetate or combined oral contraceptives and after discontinuation of norethisterone enanthate. *Contraception* (79) pp. 345-349.

Berenson, A.B., Radecki, C.M., Grady, J.J., Rickert, V.I., Thomas, A., (2001). A prospective, controlled study of the effects of hormonal contraception on bone mineral density. *Obstetrics and Gynecology.* (98) pp. 576-582.

Berenson, A.B., Breitkopf, C.R., Grady, J.J., Rickert, V.I., & Thomas, A., (2004). Effects of hormonal contraception on bone mineral density after 24 months of use. *The American College of Obstetricians and Gynecologists.* (103) 5 pp. 899-905.

Berenson, A.B., Rahman, M., Breitkopf, C.R., & Bi, L.X., (2008). Effects of depot medroxyprogesterone acetate and 20-microgram oral contraceptives on bone mineral density. *American College of Obstetricians and Gynecologists*. (112) 4 pp. 788-799.

Bitzer, J., and Simon, J.A., (2011). Current issues and available options in combine hormonal contraception. *Contraception.* (84) pp. 342-256.

Bolotin, H.H., Sievanen, H., and Grashuis, J.L., (2003). Patient-specific DXA bone mineral density inaccuracies: Quantitative effects of non uniform extraosseous fat distributions. *Journal of Bone and Mineral Research*. (18) 6 pp. 1020-1027.

Bradford, P.G., Gerace, K.V. Roland, R.L., & Chrzan, R.B., (2010). Estrogen regulation of apoptosis in osteoblasts. *Physiology & Behavior*. (99) pp. 181-185.

Burr, D.B., Yoshikawa, T., Teegarden, D., Lyle, R. McCabe, G., McCabe, L.D., & Weaver, C.M., (2000). Exercise and oral contraceptive use suppress the normal age-related increase in bone mass and strength of the femoral neck in women 18-31 years of age. *Bone*. (27) 6 pp. 855-863.

Busen, N.H., (2004). Bone mineral density in adolescent women using depot medroxyprogesterone acetate. *Journal of the American Academy of Nurse Practitioners*, (16) 2 pp. 57-62.

Consensus Development Conference Statement, (2000). Osteoporosis Prevention, Diagnosis and Therapy. *National Institution of Health*.

Cromer, B.A., (2003). Bone Mineral density in adolescent and young adult women on injectable or oral contraception. *Obstetrics and Gynecology*. (15) pp. 353-357. Doi: 10.1097.01.gco.0000094695.87578.57.

Cromer, B.A., Stager, M., & Bonny, A., (2004). Depot medroxyprogesterone acetate, oral contraceptives and bone mineral density in a cohort of adolescent girls. *Journal of Adolescent Health.* (35) pp. 434-441.

Cromer, B.A., Bonny, A.E., Stager, M., Lazebnik, R., & Secic, M. (2008). Bone mineral density in adolescent females using injectable or oral contraceptives: a 24-month prospective study. *Fertility and Sterility*. *(90)* 6.

Cornish, S.M., Chilibeck, P.D., (2009). Alpha-linolenic acid supplementation and resistance training in older adults. *Applied Physiology, Nutrition and Metabolism.* (34) 1 pp. 49-59.

Copeland, J.L., Kowalski, K.C., Donen, R.M., & Tremblay, M.S., (2005). Convergent validity of the physical activity questionnaire for adults: the new member of the PAQ family. *Journal of Physical Activity.* (2) pp. 216-229.

Corwin, E. (2008). Handbook of Pathophysiology. *Williams & Wilkins, Philadelphia*. (3).

Cutler, G.B., (1997). The role of estrogen in bone growth and maturation during childhood and adolescence. *Journal of Steroid Biochemistry and Molecular Biology.* (61) 3-6, pp. 141-144.

Dayton Children's: The Children's Medical Centre of Dayton. (Artist) (1995-2011). *Growth plate injuries.* [Web]. Retrieved from <http://www.childresdayton.org/cms/kidshealth /ae22683b01214bf2/index.html>

Dennison, D., Mohamed & M.A., Cooper, C., 2006. Epidemiology of osteoporosis. *Rheumatic Disease Clinics of North America*. Doi:10.1016/j.rdc.2006.08.003

Drake, R.L., Vogl, W., & Mitchell, A.W.M., (2005). Gray's anatomy for students. *Elsevier, Philadelphia.* pp. 14-17.

Ellis, K.J., Shypailo, R.J., Hergenroeder, A., Perez, M., & Abrams, S., (1996). Total body calcium and bone mineral content: comparison of dual-energy x-ray absorptiometry with neutron activation analysis. USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA.

Endrikat, J., Mih, E., Dusterberg, B., Land, K., Gerlinger, C., and Schmidt, W., (2004). A 3-year double-blind, randomized, controlled study on the influence on two oral contraceptives containing either 20µg or 30µg ethinylestradiol in combination with levonorgestrel on bone mineral density. *Contraception*, (78), pp. 10-15.

Erlandson, M.C., (2007). The effects of a gymnastics program on early childhood (4-6 yrs) body composition development. Unpublished masters thesis, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

ESHRE Capri Workshop Group, (2010). Bone fractures after menopause. *Human Reproduction Update*. pp. 1-13. Doi: 10.1093/humupd/dmq008

Faulkner, R.A., Forwood, M.R., Beck, T.J., Mafukidze, J.C., Russell, K., & Wallace, W., (2003). Strength indices of the proximal femur and shaft in prepubertal female gymnasts. *Medicine & Science in Sports & Exercise.* Doi: 10.1249/01.Mss.0000053724.33480.8B

Ferretti, J. L., Capozza, R. F., Cointry, G. R., Garcia, S. L., Plotkin, H., Alvarez, Filgueira, M.L., & Zanchetta, J.R., (1998). Gender-related differences in the relationship between densitometric values of whole-body bone mineral content and lean body mass in humans between 2 and 87 years of age. *Bone* (22) pp. 683-690.

Frost, H.M., (1997). On Our Age-Related Bone Loss: Insights from a New Paradigm. *Journal of Bone and Mineral Research.* (12) 10 pp.1539-1546.

Fuchs, R.K., Bauer, J.J., & Snow, C.M., (2001). Jumping improves hip and lumbar spine bone mass in prepubescent children: A randomized controlled trial. *Journal of bone and mineral research*. (16) pp. 148-156.

Gargano, V., Massaro, M., Morra, I., Formisano, C., Di Carlo, C., & Nappi, C., (2008). Effects of two low-dose combined oral contraceptives containing drospirenone on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. *Contraception.* (78) pp. 10-15

Ginther, O.J., Beg, M.A., Gastal, E.L., Gastal, M.O., Baerwald, A.R., & Pierson, R.A., (2005). Systematic concentrations of hormones during the development of follicular waves in mares and women: a comparative study. *Eutheria Foundation, Wisconsin USA*. (130) 2 pp. 379-388.

Gunter, K., Baxter-Jones, A.D.G., Mirwald, R.L., Almstedt, H., Fuschs, R.K., Durski, S., & Snow, C., (2008). Impact exercise increases BMC during growth: an 8-year longitudinal study. *Journal of Bone and Mineral Research*. (23) 7 pp. 986-993. Doi: 10.31359/JBMR.071201.

Hartard, M., Bottermann, P., Bartenstein, P., Jeschke, D., & Schwaiger, M., (1997). Effects on bone mineral density of low-dosed oral contraceptives compared to and combined with physical activity. *Contraception*. (55) pp. 87-90.

Hartard, M., Kleinmond, C., Kirchbichler, A., Jeschke, D., Wiseman, M., Wissenbacher, E.R., Felsenberg, D., & Erben, R.G., (2004). Age at first oral contraceptive use as a major determinant of vertebral none mass in female endurance athletes. *Bone*. (*35*) pp. 836-841.

Hartard, M., Kleinmond, C., Luppa, P., Zelger, O., Egger, K., Wiseman, M., (2006). Comparison of skeletal effects of the progestogens desogesterel and levonorgestrel in oral contraceptive preparations in young women: controlled open, party randomized investigation over 13 cycles. *Contraception,* (74) pp. 367-375.

Ioannidis, G., Papaioannou, A., Hopman, W.M., Noori, A., Anastassiades, T., Pickard, L., Kennedy, C.C., Prior, J.C., Olszynski, W.P., Davison, K.S., Goltzman, D., Thabane, L., Gafni, A., Papadimitropoulos, E.A., Brown, J.P., Josse, R.G., Hanley, D.A., & Adachi, J.D., (2009). Relation between fractures and mortality: results from the Canadian multicentre osteoporosis study. *CMAJ*. (5) pp.181. Doi: 10.1503/cmaj.081720.

Institute of Medicine, (2010). DRI Dietary reference intakes: Calcium vitamin D: committee to review dietary reference intakes for vitamin D and calcium. National Academy Press, Washington, DC. Pp. 1104. Web accessed < http://www.nap.edu/openbook.php?record_id=13050&page=R1>.

Kanis, J.A., Melton III, J., Christiansen, C., Johnston, C., & Khaltaev, N., (2009). The diagnosis of osteoporosis. *Journal of Bone and Mineral Research*. (9) 8 pp. 1137-1141. Doi: 10.1002/jbmr. 5650090802.

Kannus, P., (1999). Preventing osteoporosis, falls, and fractures among elderly people: promotion of lifelong physical activity is essential. *BMJ*.

Khan, K., McKay, H.A. Kannus, P., Bailey, D.A., Wark, J., & Bennell, K. (2001). *Physical Activity and Bone Health*. Champlaign, Human Kinetics, Champaign, IL.

Kholsa, S. (2010). Update on estrogen and the skeleton. *Journal of Clinical Endocrinology & Metabolism*. (95) 8 pp. 3569-3577.

Lanyon, L.E. (1996). Using functional loading to influence bone mass and architecture: objectives, mechanisms, and relationship with estrogen of the mechanically adaptive process in bone. *Bone.* (18) pp. 37-43.

Liu, S.L., & Lebrun, C.M. (2006). Effect of oral contraceptives and hormone replacement therapy on bone mineral density in premenopausal and perimenopausal women: a systematic review. *British Journal of Sports Medicine*. (40) 11.

Llyod, T., Taylor, D.S., & Lin H.M., (2000). Oral contraceptive use by teenage women does not affect peak bone mass: a longitudinal study. *Fertility and Sterility.* (74) pp. 734-738.

Lopez, L.M., Grimes, D.A., Schulz, K.F., & Curtis, K.M., (2011). Steroidal contraceptives: effect on bone fractures in women (review). *The Cochrane Collaboration*. (7) pp. 1-77.

Mackelvie, K.J., Khan, K.M., Petit, M.A., Janssen, P.A., & McKay, H.A., (2003). 2-year randomized controlled trial in girls: a school-based exercise intervention elicits substantial bone health benefits. *Pediatrics.* (112).

McKay, H.A., Bailey, D.A., Mirwald, R.L., Davison, R.S, & Faulkner, R.A., (1998). Peak bone mineral accrual and age at menarche in adolescent girls: a 6-year longitudinal study. *Journal of Pediatrics*. (133) pp. 682-687.

McKay, H.A., Petit, M.A., Schutz, R.W., Prior, J.C., Barr, S.I., & Khan, K.M., (2000). Augmented trochanteric bone mineral density after modified physical education classes: A randomized school-based exercise intervention study in prepubescent and early pubescent children. *The Journal of Pediatrics.* (136) 2 pp. 156-162.

Malina, R.M., (1978). Adolescent growth and maturation: Selected aspects of current research. *Yearbook of Physical Anthropology*. University of Texas, Austin. (21) pp. 63-94.

Malina, R.M., Bouchard, C., & Bar-Or, O., (2004). Growth, maturation, and physical activity. *Human Kinetics, Champaign, IL.* (2).

Maynard, L.M., Guo, S.S., Chumlea, W.C., Roche, A.F., Wisemandle, W.A., Zeller, C.M., Towne, B., & Siervogel, R.M., (1998). Total-body and regional bone mineral content and areal bone mineral density in children aged 8-18 y: the Fels Longitudinal Study. *American Journal of Clinical Nutrition*. (68) pp. 1111-1117.

Molina, P.E., (2009) "Female Reproductive System. *Endocrine Physiology*, (2) <www.accessmedicine.com> Com/content.aspx?aID=2365235>.

Moolgard, C., Thomsen, B.L., Prentice, A., Cole, T.J., & Michaelsen, K.M., (1997). Whole body bone mineral content in healthy children and adolescents. *Archives of Disease in Childhood.* (76) 9, pp. 9-15

Molgaard, C., Thomsen, B.L., & Michaelsen, K.F., (1999). Whole body bone mineral accretion in healthy children and adolescents. *Archives of Disease in Childhood*. (81) pp. 10-15.

National Institute of Arthritis and Musculoskeletal and Skin Diseases (2011). NIH Osteoporosis and related bone diseases national resource center. <http://www.niams.nih.gov/Health_Info/Bone/Bone_Health/Pregnancy/default.asp>.

Nappi, C., Di Spiezo Sardo, A., Acunzo, G., Bifulco, G., Tommaselli, G.A., & Guida, M., (2003).
Effects of a low-dose ultra-low-dose combined oral contraceptive use on bone turnover and bone mineral density in young fertile women: a prospective controlled randomized study. *Contraception.* (67) pp. 355-369.

Nappi, C., Sardo, A.D.S., Greco, E., Tommaselli, G.A., Giordano, E., & Guida, M., (2005). Effects of an oral contraceptive containing drospirenone on bone turnover and bone mineral density. *The American College of Obstetricians and Gynecologists*. (105) 1.

Osteoporosis Canada, (2011). Osteoporosis: Towards a fracture free future [White Paper]. <www.osteoporosis.ca/index.php/ci_id/6385/la_id/1.htm>.

Osteoporosis Canada, (2011). What is osteoporosis? <www.osteoporosis.ca/index.php/ci_id/5526/la_id/1.htm>

Pack, A.M., Morrell, M.J., McMahon, D.J., & Shane, E., (2011). Normal vitamin D and low free estradiol levels in women on enzyme-inducing antiepileptic drugs. *Epilepsy & Behavior.*

Paoletti, A.M., Orru, M., Floris, S., Mannias, M., Vacca, A.M.B., & Ajossa, S., (2000). Evidence that treatment with monophasic oral contraceptive formulations containing ethynilestradiol plus gestodene reduces bone resorption in young women. *Contraception.* (61) pp. 259-263.

Polatti, F., Peroti, F., Filippa, N., Gallina, D., & Nappi, R.E., (1995). Bone Mass and Long-Term monophasic oral contraceptive treatment in young women. *Contraception*. (51) pp. 221-224.

Rad, M., Kluft, C., de Kam, M.L., Meijer, P., Cohen, A.F., & Grubb, G.S., (2011). Metabolic profile of a continuous versus a cyclic low-dose combined oral contraceptive after one year of use. *European Journal of Contraception and Reproductive Health Care*. (16) 2 pp. 85-94.

Rauch, F. (2005). Bone growth in length and width: the yin and yang of bone stability. *Journal of Musculoskeletal Neuronal Interact* 2005; (5) 3 pp.194-201.

Reed, S.D., Scholes, D. LaCroix A.Z., Ichikawa, L.E., Barlow, W.E., & Ott, S.M., (2003). Longitudinal changes in bone density in relation to oral contraceptive use. *Contraception*. (68) pp. 177-182.

Rickenlund, A., Carlstrom, K., Ekblom, B., Brismar, T., Schoutlz, B., & Hirschberg, A. (2004). Effects of oral contraceptives on body composition and physical performance in female athletes. *Journal of Clinical Endocrinology and Metabolism*. (9) 89.

Rome, E., Ziegler, J., Secic, M., Bonny, A., Stager, M., Lazebnik, R., & Cromer, B.A. (2004). Bone biochemical markers in adolescent girls using either depot medroxyprogesterone acetate or an oral contraceptive. *North American Society for Pediatric and Adolescent Gynecology*. (9) 13.

Ruffing, J.A., Nieves, J.W., Zion, M., Tendy, S., Garrett, P., Lindsay, R., & Cosman, F., (2007). The influence of lifestyle, menstrual function and oral contraceptive use on bone mass and size in female military cadets. *Nutrition and Metabolism*. (4) 17. Doi: 10.1186./1743-7075-4-17.

Seeman, E., (2003). Physiology of aging, invited review: genesis of osteoporosis. *Journal of applied Physiology*. (95) pp. 2142-2151. doi10.1152/japplphysiol.00564.2003.

Scholes, D., Ichikawa, L., LaCroix, A.Z., Spangler, L., Beasley, J.M., Reed, S., Ott, S.M., (2010). Oral Contraceptive use and bone density in adolescent and young adult women. *Contraception*. (81) pp. 35-40.

Tanner, J.M., (1978). Foetus into Man: Physical Growth from Conception to Maturity *Open Books Publishing Ltd*. Goodwin's Court, London.

Tanner, J. M., (1962). Growth at Adolescence. *Blackwell Scientific Publications*, Oxford.

Teirmaa, T., Luukkaa, V., Rouru, J., Koulu, M., & Huupponen, R., (1998). Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. *European Journal of Endocrinology.* (138) pp. 190-194.

Theintz, G., Buchs, B., Rizzoli, R., Slosman, D., Clavien, H., Sizonenko, P.C., and Bonjour, J.P., (1992). Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *Journal of Clinical Endocrinology and Metabolism.* (75) pp. 1060-1065.

Teegarden, D, Legowski, P., Gunther, C.W., McCabe, G.P, Peacock, M., & Lyle, R., (2005). Dietary calcium intake protects women consuming oral contraceptives from spine and hip bone loss. *Journal of Clinical Endocrinology and Metabolism*. (90) 9.

U.S Preventative Services Task Force, (2009). http://www.ahrq.gov/data/.

Vantanparast, H., Bailey, D.A., Baxter-Jones, A.D.G., & Whiting, S. J., (2009). Calcium Requirements for bone growth in Canadian boys and girls during adolescence. *British Journal of Nutrition.* (103) pp. 575-580.

Vantanparast, H., Calvo, M.S., Green, T.J., & Whiting, S.J., (2010). Despite mandatory fortification of staple foods, vitamin D intakes of Canadian and adults are inadequate. *Journal of Steroid Biochemistry and Molecular Biology.* (121) pp. 301-303.

Vieth, R., Cole, G.A., Hawker, H.M., Trang, H.M., & Rubin, L.A., (2001). Wintertime vitamin D insufficiency is common in young Canadian women and their vitamin D intake does not prevent it. *European Journal Clinical Nutrition*. (55) pp. 1091-1097.

Wei, S., Winzenberg, T., Laslett, L.L., Venn, A., & Jones, G., (2010). Oral contraceptive use on bone. *Current Osteoporosis Report*. Doi: 10.1007/s11914-010-0031-9

Whiting, S.J., & Shrestha, R.M., (1993). Dietary assessment of elementary school-age children and adolescents. *Journal of Canadian Dietic Association.* (54) 4 pp.193-195

Wilkins, K., Johansen, H., Beaudet, M.P., & Neutel, I., (2000). Oral Contraceptive Use. *Statistics Canada, Catalogue: Health Reports.* (11) 4 pp. 25-37.

APPENDIX A: Ethics

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB)

Certificate of Approval
Study Amendment

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Adam Baxter-Jones	Kinesiology	88-102

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
College of Kinesiology
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SUB-INVESTIGATOR(S)
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STUDENT RESEARCHER(S)
Marta Erlandson, Andrew Frank, Stefan Jackowski

FUNDER(S)
CANADIAN INSTITUTES OF HEALTH RESEARCH (CIHR)
ROYAL UNIVERSITY HOSPITAL FOUNDATION

TITLE
Protocol NHRDP #6608-126-OS: Relationship of Growth and Lifestyle to Peak Bone Mass

APPROVAL OF	APPROVED ON	CURRENT EXPIRY DATE
Acknowledgement of: Addition of Royal University Hospital Foundation as Funder Addition of Research Personnel to the Certificate of Approval: Sub-Investigators: Dr. Bob Faulkner, College of Kinesiology Dr. Don Bailey, College of Kinesiology Dr. Saija Kontulainen, College of Kinesiology Dr. David Cooper, College of Medicine Dr. David Leswick, Medical Imaging, College of Medicine id RUH Dr. Hassanali Vatanparast, College of Pharmacy and Nutrition Dr. James Johnston, College of Engineering Dr. Lauren Sherar, College of Kinesiology Student Researcher's: Marta Erlandson, PhD student, College of Kinesiology Stefan Jackowski, PhD student, College of Kinesiology Andrew Frank, MSc student, College of Kinesiology	13-Apr-2011	20-Oct-2011

Full Board Meeting ☐ Delegated Review ☒

CERTIFICATION
The study is acceptable on scientific and ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

FIRST TIME REVIEW AND CONTINUING APPROVAL
The University of Saskatchewan Biomedical Research Ethics Board reviews above minimal studies at a full-board (face-to-face meeting. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project. For more information visit http://www.usask.ca/research/ethics_review/.

ase send all correspondence to

Research Ethics Office
University of Saskatchewan
Box 5000 RPO University
1607-110 Gymnasium Place
Saskatoon SK S7N 4J8

APPENDIX B: Consent Form

Bone Mineral Accrual Study
College of Kinesiology, University of Saskatchewan

Research Participant Information and Consent Form

Principal Investigator
- Dr. Adam Baxter-Jones, College of Kinesiology, phone 966-1078

Sub-Investigator(s)
- Dr. Saija Kontulainen, College of Kinesiology
- Dr. David Leswick, Medical Imaging, RUH

Sponsor
- Canadian Institute of Health Research, grants no. MOP 83391, ROP 85582 and MOP 98002.

Introduction:
You are invited to continue participating in the *Bone Mineral Accrual Study.* Your participation in this study is entirely voluntary and you may withdraw at any time without penalty. Before you decide to participate it is important that you understand what the research involves. This consent form will tell you about the study, why the research is being done, and your role as a volunteer along with the possible benefits, risks and discomforts involved. If you decide to participate you will be required to sign this consent form. Please read this form carefully and feel free to ask any questions you might have.

Purpose:
High peak bone mass and optimal bone structures are important in determining lifetime's risk of osteoporosis. Thus, determination of factors that enhance bone development during growth is important in developing intervention strategies for osteoporosis prevention. The Bone Mineral Accrual Study is the only study internationally to date to have monitored yearly bone mineral accrual through childhood, adolescence and early adulthood. The continuation of testing into the adult years will answer the question of when peak bone mass occurs, and identify factors during the growing years that affect peak bone mass, structure and strength.

Procedures:
Procedures will be the same as previous testing. However, this year you have the option of taking part in another part of the study, which will involve additional measures of bone structure and estimated strength. Assessment of bone, nutrition, physical activity and lifestyle questionnaires, anthropometry and the assessment of blood and urine will take place at the College of Kinesiology. MRI measures of bone will take place at the Royal University Hospital. The following procedures will be performed:

a) Assessment of Bone Mineral Density and Structure: Your total body, lumbar spine and proximal femur bone mineral density will be evaluated using dual-energy x-ray absorptiometry (DXA). This procedure is routinely used in clinical medicine. Your bone structure will be measured with two peripheral quantitative computer tomography (pQCT) scanners. Your wrist, forearm, ankle and calf will be scanned. Each scan will take approximately 2 minutes. The effective radiation dose from the DXA and pQCT measurements is small, about 23 μSv(micro Sievert). The average

annual background radiation in North America due to natural sources is approximately 2500 μSv per year. Your hip bone structure will also be measured with magnetic resonance imaging (MRI). This will be done in the Royal University Hospital. MRI does not produce radiation.

b) Nutrition, physical activity and lifestyle questionnaires. These are standard questionnaires that will ask you to identify your physical activity patterns and typical nutritional intake. You will also be asked about other lifestyle behaviors such as tobacco and alcohol use.
c) Anthropometric and muscle strength assessment: Height, weight, waist circumference and skinfold thickness will be measured. Your grip strength will be measured by a hand held dynamometry and your leg strength by a vertical jump.
d) Blood assessment and urine: Approximately 5 mL of blood will be drawn from a catheter that is inserted into a vein in your arm and you will be asked to provide a urine sample. The purpose of the blood and urine collection is to measure indicators of health (i.e. glucose, triglycerides and cholesterol). There may be some discomfort during the drawing of blood. Although unlikely, there is a risk of bruising and infection with the drawing of blood but care will be taken to minimize these risks.
e) Resting blood pressure will be assessed.

The total time commitment for this study will be approximately three hours.

Potential Benefits:
The benefits of participating in this experiment include an increased knowledge of your overall health status, including bone mass, structure and strength, body composition and activity and dietary patterns. There is no guarantee that the participant will receive any direct benefit from the study.

Potential Risks:
The risks of this experiment involve exposure to small amounts of radiation during DXA and pQCT scanning.

Research-Related Injury:
There will be no cost to you for participation in this study. You will not be charged for any research procedures. In the event that you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

Alternative Procedures or Course of Treatment:
You do not have to participate in this study to have your body composition assessed. These tests can be arranged through appointment in the College of Kinesiology.

New Findings:
If, during the course of this study, new information becomes available that may relate to your willingness to continue to participate, this information will be provided to you by the investigators.

Confidentiality:
While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this

research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

Future consent
With your permission we may also contact you in the future for reassessment and to provide you with your results.

Right to Withdraw:
It is understood that you will be free to withdraw from any or all parts of the study at any time without penalty.

Questions:
Please be assured that you may ask any question at any time. We will be glad to discuss your results with you when they become available and we welcome your comments and suggestions. Should you have any concerns about this study or wish further information please contact:

Dr. Adam Baxter-Jones (phone: 966-1078, email baxter.jones@usask.ca) or
Dr. Saija Kontulainen (phone: 966-1077, email saija.k@usaks.ca)

If you have any questions regarding your rights as a research subject or concerns about your experiences while participating in this study, you should **contact the Chair of the Biomedical Research Board Ethics Board (Bio-REB), c/o, University of Saskatchewan at 306-966-4053**.

APPENDIX C: Lifestyle Questionnaire

Bone Mineral Accrual Study
Females Only Questionnaire

1. How old were you when you started to have menstrual cycles?_____ years old

   Did it occur in:
   - ❑ Spring
   - ❑ Summer
   - ❑ Fall
   - ❑ Winter

2. Are you currently using oral contraceptives?
   - ❑ No
   - ❑ Yes

   If yes, for how long have you used them? _____ Years _____ Months

   What is the brand name of the oral contraceptives that you use? _____

   If no, have you used them in the past?

   - ❑ No
   - ❑ Yes

   If yes, for how long had you used them? _____ Years _____ Months

   What brand name of oral contraceptives did you use? _____

3. How many periods do you have in a year?
   - ❑ Over 13 periods
   - ❑ 9 to 13 periods
   - ❑ 3 to 8 periods
   - ❑ less than 3 periods

4. Have you had a period in the past three months?
   - ❑ No
   - ❑ Yes

5. What is the date of the first day of your last period? _____

6. Have you ever had an absence or loss of periods (pregnancy and lactation not included)?
   - ❑ No
   - ❑ Yes

If yes, at what age(s) did you miss a period(s)?
_____ years old
_____ years old

For how long did your periods stop?
_____mos. _____ yrs
_____mos. _____ yrs

Legally, you cannot be scanned if you are pregnant.

7. Are you pregnant?
   - ❑ No
   - ❑ Yes
   - ❑ I don't know

8. How many children have you given birth to? _____ If none, go to next page

   List their birthdates:

   Child 1: _____ Did you breastfeed? ❑ No ❑ Yes
   If yes, how many months? _____

   Child 2: _____ Did you breastfeed? ❑ No ❑ Yes
   If yes, how many months? _____

   Child 3: _____ Did you breastfeed? ❑ No ❑ Yes
   If yes, how many months? _____

   Child 4: _____ Did you breastfeed? ❑ No ❑ Yes
   If yes, how many months? _____

APPENDIX D: 24-Hour Food Recall

University of Saskatchewan
Bone Mineral Accrual Study
24 Hour Recall Questionnaire

Name: _____ Subject # _____ Date: _____

Please list every FOOD and DRINK that you ate yesterday

Time	Food Items	Type & Preparation	Amount	Brand Name or Where Bought	Code
Morning					
Mid-morning					
Noon Meal					
Midday					

Evening Meal					
Before Bed					
EXAMPLE	***CEREAL***	***CORN FLAKES***	***1 cup***	***Kellogg***	

Was this intake usual? Circle one: Yes No (*if No, explain why not* _____)

Did you take any vitamins/minerals during this time? Circle one: Yes No (if Yes, list names: _____

APPENDIX E: Physical Activity Questionnaire

Bone Mineral Accrual Study
Physical Activity Questionnaire (Adults)

We are trying to find out about your level of physical activity from ***the last 7 days*** (in the last week). This includes activities that make you sweat, make your legs feel tired, or make you breathe hard, such as team sports, running, strenuous occupational activities, and others.

Remember:
1. There are no right and wrong answers — this is not a test.
2. Please answer all the questions as honestly and accurately as you can — this is very important.

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1. Physical activity in your spare time: Have you done any of the following activities **in the past 7 days** (last week)? If yes, how many times? (Mark only one circle per row.)

	No	1-2	3-4	5-6	7 times or more
Rock climbing.....	❍	❍	❍	❍	❍
Rowing/canoeing.....	❍	❍	❍	❍	❍
Tennis/squash	❍	❍	❍	❍	❍
Stair climber (or other similar equipment).....	❍	❍	❍	❍	❍
Walking for exercise.....	❍	❍	❍	❍	❍
Heavy yard work	❍	❍	❍	❍	❍
Jogging or running.....	❍	❍	❍	❍	❍
Bicycling	❍	❍	❍	❍	❍
Aerobics (or other exercise class)...	❍	❍	❍	❍	❍
Swimming	❍	❍	❍	❍	❍
Baseball, softball	❍	❍	❍	❍	❍
Dance	❍	❍	❍	❍	❍
Football	❍	❍	❍	❍	❍
Badminton	❍	❍	❍	❍	❍
Soccer.....	❍	❍	❍	❍	❍
Street/floor hockey.....	❍	❍	❍	❍	❍
Volleyball	❍	❍	❍	❍	❍
Basketball	❍	❍	❍	❍	❍
Skating (in-line/ice).....	❍	❍	❍	❍	❍
Cross-country skiing	❍	❍	❍	❍	❍
Ice hockey/ringette	❍	❍	❍	❍	❍
Martial arts.....	❍	❍	❍	❍	❍
Weight training.....	❍	❍	❍	❍	❍
Other:					
_____.....	❍	❍	❍	❍	❍
_____.....	❍	❍	❍	❍	❍

2. **In the last 7 days**, *during the morning*, how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity, strenuous household or child rearing tasks)? (Check one only.)

None..……..	❍
1 time last week.....	❍
2 or 3 times last week.....	❍
4 or 5 times last week.....	❍
6 or 7 times last week.....	❍

3. **In the last 7 days**, *after lunch and before supper*, how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity, strenuous household or child rearing tasks)? (Check one only.)

None.....	❍
1 time last week	❍
2 or 3 times last week	❍
4 or 5 times last week.....	❍
6 or 7 times last week.....	❍

4. **In the last 7 days**, *during the evening,* how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity, strenuous household or child rearing tasks)? (Check one only.)

None	❍
1 time last week	❍
2 or 3 times last week	❍
4 or 5 last week.....	❍
6 or 7 times last week	❍

5. **On the last weekend**, how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity, strenuous household or child rearing tasks)? (Check one only.)

None .....……	❍
1 time.....	❍
2 — 3 times.....	❍
4 — 5 times.....	❍
6 or more times.....	❍

6. Which *one* of the following describes you best for the **last 7 days**? Read *all five* statements
before deciding on the *one* answer that describes you.

A. All or most of my free time was spent doing things that involve little physical effort ..... ❍

B. I sometimes (1 — 2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics) .....❍

C. I often (3 — 4 times last week) did physical things in my free time .....…….❍

D. I quite often (5 — 6 times last week) did physical things in my free time ……..❍

E. I very often (7 or more times last week) did physical things in my free time. …❍

7. Mark how often you did physical activity (for example: playing sports, exercise classes, strenuous occupational activity).

	None	Little bit	Medium	Often	Very often
Monday	❍	❍	❍	❍	❍
Tuesday	❍	❍	❍	❍	❍
Wednesday.....	❍	❍	❍	❍	❍
Thursday.....	❍	❍	❍	❍	❍
Friday	❍	❍	❍	❍	❍
Saturday	❍	❍	❍	❍	❍
Sunday	❍	❍	❍	❍	❍

8. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

Yes ..... ❍
No ..... ❍

If Yes, what prevented you? _____