Bone Health In Adolescent Female Athletes: The Influence of Menstrual and Oral Contraceptive Status on Bone Mineral Density and Content of the Lumbar Spine and Proximal Femur

by

Shana L DeNeef Ooms
B.Sc., University of Victoria, 1996

A Thesis submitted in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

in the School of Physical Education

We accept this thesis as conforming
to the required standard

[redacted]
Dr. Catherine A. Gaul, Supervisor (School of Physical Education)

[redacted]
Dr. Howard A. Wenger, Departmental Member (School of Physical Education)

[redacted]
Dr. Richard Backus, Outside Member

[redacted]
Dr. Marie Hoskins, Outside Member (School of Child and Youth Care)

[redacted]
Dr. Nancy Sherwood, External Examiner (Department of Biology)

Supervisor: Dr. Catherine A. Gaul

Abstract

The purpose of this research was to investigate the influence of menstrual status and oral contraceptive status on areal bone mineral density (aBMD) and bone mineral content (BMC) of the lumbar spine and proximal femur in 31 adolescent, athletic Caucasian, non-smoking females, aged 14 to 21 years (mean 18.8 ± 1.3 years). Participants were grouped by menstrual and oral contraceptive status: eumenorrheic, non-oral contraceptive users (ENOC, n=15), eumenorrheic, oral contraceptive users (EOC, n=11), and irregularly menstruating (amenorrheic and oligomenorrheic), non-oral contraceptive users (INOC, n=5). A secondary purpose was to determine if the ENOC, EOC and INOC groups were distinguishable by other factors which have been associated with aBMD and BMC, such as anthropometrics, age at menarche, physical activity, calorie and calcium intake, and eating attitudes. Subjects participated in a regular program of vigorous weight bearing activity that involved a minimum of 3 times per week for at least 45 minutes each time. Measures of aBMD and BMC of the lumbar spine (LS, L_{1-4}), proximal femur (PF) and its sub regions, the femoral neck (FN) and trochanter (FT), were taken using a Hologic QDR 2000 dual energy x-ray absorptiometer (DXA) in array mode. Descriptive information regarding menstrual and oral contraceptive history, familial history of osteoporosis, medical history and current physical activity levels was gathered with a general health questionnaire. A three-day dietary record was used to assess calorie and calcium intake, and the Eating Attitudes Test (EAT-26) was used to evaluate restrictive eating tendencies. Anthropometrics including stature, weight, body mass index (BMI), and sum of five skin folds (So5S) were also assessed.

ENOC, EOC and INOC groups were similar in terms of chronological age, age at menarche, anthropometrics, current physical activity and resistance training (hours per week), and all dietary measures. Height and weight correlated significantly with measures of BMC and bone mineral area (BMA) ($p<0.05$), but not with measures of aBMD. Weight bearing activity and resistance training correlated significantly with aBMD of the FT and FN, respectively ($p<0.05$). Age at menarche, calorie and calcium

intake, EAT-26 scores, and So5S did not correlate significantly with any of the bone mineral measures.

A one-way ANOVA revealed that in non-oral contraceptive users (ENOC and INOC), menstrual status did not influence aBMD and BMC at the LS and PF. Conversely, in the eumenorrheic groups, oral contraceptive users (EOC) had significantly higher BMC at the PF ($p=0.028$) and FT ($p=0.020$) compared to non-oral contraceptive users (ENOC). Oral contraceptive use did not influence BMC at the LS or aBMD at any site. BMC at the PF may be influenced by initiation of oral contraceptive use prior to 20 years of age and before adult peak bone mass is achieved. Skeletal growth during puberty reflects increases in BMC more so than in aBMD because of increases in bone size. A longer period of bone mineral accrual after longitudinal growth has ceased, therefore, might be necessary before any influence of oral contraceptive use on aBMD is observed.

Examiners:

[redacted]

Dr. Catherine A. Gaul, Supervisor (School of Physical Education)

[redacted]

Dr. Howard A. Wenger, Departmental Member (School of Physical Education)

[redacted]

Dr. Richard Backus, Outside Member

[redacted]

Dr. Marie Hoskins, Outside Member (School of Child and Youth Care)

[redacted]

Dr. Nancy Sherwood, External Examiner (Department of Biology)

Table of Contents

Abstract ii
Table of Contents iv
List of Tables vi
List of Figures vii
Acknowledgements viii
Introduction 1
Purpose 6
Research Questions 7
  Experimental Hypotheses 7
Delimitations 8
Limitations 8
Assumptions 8
Operational Definitions 9
Methods 10
  Subjects 10
  Research Design and Procedures 11
    Information Session 11
    Bone Mineral Density and Content 12
    General Health Questionnaire, EAT-26 and Anthropometry 14
  Statistical Analyses 14
Results 16
  Total Sample Descriptive Characteristics 16
  Total Sample Correlations 16
    Age 17
    Anthropometry 17
    Exercise 17
    Diet 17
  ENOC, EOC, and INOC Group Characteristics 19
    Chronological Age and Age at Menarche 19

Anthropometric Measurements 19
Eating Attitudes Test (EAT-26) 19
Exercise 21
3-Day Dietary Record 21
Bone Mineral Density, Content and Area Measurements 23
Discussion 25
Physical Characteristics of ENOC, INOC and EOC 26
Measures of Bone Mineralization 26
Anthropometrics 26
Age at Menarche 27
Menstrual Status (ENOC and INOC) and Bone Mineral Measures 28
Oral Contraceptive Status (ENOC and EOC) and Bone Mineral Measures 33
Bone Mineral Density 33
Bone Mineral Content 34
Conclusions and Implications for Future Research 37
References 40
Appendices 49
Appendix A: Review of Literature 49
Appendix B: Recruitment Poster 100
Appendix C: Informed Consent 102
Appendix D: Three Day Dietary Information and Record 105
Appendix E: General Health Questionnaire 109
Appendix F: EAT-26 114

List of Tables

Table 1	Significant Bivariate Pearson Product Moment Correlations (r) Between aBMD, BMC and BMA Measurements and Height, Weight, Exercise and Dietary Fat	18
Table 2	Mean (SD) Height, Weight, Body Mass Index, So5S According to Menstrual and Oral Contraceptive Status Groups	20
Table 3	Mean (SD) Total Eating Attitudes Test and Subscale Item Scores According to Menstrual and Oral Contraceptive Status Groups	20
Table 4	Mean (SD) Hours per Week Vigorous Weight-Bearing Activity and Resistance Training According to Menstrual and Oral Contraceptive Status Groups	21
Table 5	Mean (SD) Caloric and Nutrient Intakes Averaged From 3-Day Dietary Records and Reported According To Menstrual and Oral Contraceptive Status Groups	22
Table 6	Mean (SD) aBMD, BMC and BMA Measurements for ENOC, EOC and INOC Groups	24
Table 7	Retrospective Studies Examining the Association Between Past Oral Contraceptive Use and Current Bone Mineral Measures	73

List of Figures

Figure 1	Lumbar spine (L1-4) DXA scan position (Hologic QDR 2000, array mode)	13
Figure 2	Proximal femur DXA scan position (Hologic QDR 2000, array mode)	13

Acknowledgements

While I have not discovered a cure for osteoporosis with my study, I have learned a tremendous amount about the significant challenges and rewards involved in "the process" of research. For this, I am sincerely grateful to those whose contributions made this experience possible. I would like to express gratitude to my supervisor, Kathy, for her unwavering patience and dedication. I know that when she took me on as a grad student she was not expecting me to finish so far in the future, and nor was that my plan. Her flexibility and understanding, however, have allowed me to reach not only my academic goals but also to gain valuable work experiences. She enthusiastically devoted her time, both at work and at home, expertise and energy to each stage of this study. Beyond this study, Kathy has provided me with numerous opportunities over the years, which have helped me grow not only as a student, but also as a person.

I would like to express genuine gratitude to my committee: Richard for his funding and subject recruitment ideas, and for meeting me at Health Services despite being injured in a cycling accident the day before; Howie, for his open door, his genuine care and concern and his amazing ability to provide guidance in such a way that you feel as though you arrived at the solution to a problem yourself, when in reality his critically thinking mind did all the work. I feel fortunate and thankful to have had Howie as an influence in my life; Marie, for her flexibility, graciousness, and the unique perspective that she brought to the study. Special thanks also to Walter Muir, who was not on my committee but still gave up retirement time to go over my statistics.

The cooperation and commitment of many individuals at the Royal Jubilee Hospital's Nuclear Medicine Department were integral to the success of this project. Thank you to Larry Shorter, Bob Brandt, Marion Faust, Chris Jang, 'Bev' the receptionist, and "Quincey" Carefoot for your time and enthusiasm. My deepest appreciation to Andy Jeffs who continuously made himself available for DXA testing even though he was extremely busy with his own classes, and he always did so with a smile on his face and in his voice. I would also like to acknowledge the contributions of Susan Boegman and Sally Willis who each generously volunteered their time and

allowed me to come into their homes and draw on their expertise in the areas of nutrition and research.

I would like to acknowledge the participants of this research for energetically volunteering their time and, in particular, I would like to thank them for putting so much care and thought into completing the dietary records. It was a pleasure to meet each of the young women in this study and I believe I learned more from them than they did from me. Access to many of the subjects would not have been possible without the eager cooperation of coaches Keith Butler and Brent Fougner, and dance instructor Peggy Bowman.

I highly regard and appreciate the grad students I have come to know over the years. Some assisted me directly on this project while others helped indirectly by providing support, encouragement, advice and several great moments of fun. Thank you to Jill, Sean, Jen, Bob, Adrian, Meghan, Dona, Lindsay, Sam, Kirstin, Guy, and Ben. I would also like to recognize and thank the P.E. office staff, Norma, Gladys, Nancy and Jill, who graciously found the time to help me out repeatedly over the years.

Finally, I would like to express wholehearted appreciation to my friends and family for being my strongest advocates throughout this process. Your limitless words of encouragement and supportive actions will be cherished and reflected on as I face future challenges. Deepest thanks to my Mom, whose love has fostered within me the strength and dedication necessary to complete this degree, and to my Dad, whose love and refreshingly grounded approach to life have always taught me that it doesn't matter if I fail at something, but that it is more important to put my best effort forth-for in your best effort there is no failure, only success. To my husband Peter who has truly given so much of himself in order to help me achieve this goal: While I am profoundly thankful for his tireless efforts, I am most grateful to him for showing me that academia is only one piece of this amazing life puzzle.

Introduction

Osteoporosis is a chronic disease that is defined pathologically as an absolute decrease in the amount of bone concomitant with microarchitectural degeneration leading to skeletal fragility and fractures with minimal trauma (Eriksen, 1992). Demographically it is estimated that in Canada hip fractures resulting from osteoporosis will increase by 72.8% from 1987 to 2006 (Martin et al., 1991). Undoubtedly, health care costs associated with fractures are already placing a tremendous burden on the health care system and decreasing the quality of life for many older people (Eriksen, 1992). Adult bone mass, the strongest predictor of osteoporotic fracture risk, can be described as the peak bone mass of early adulthood less subsequent bone loss (Bailey, Martin, McKay, Whiting, & Mirwald, 2000). Much of prior research surrounding osteoporosis has focused on strategies for the reversal of bone loss or for prevention of bone loss only after bone loss has already begun (Eriksen, 1992). An alternative strategy in the last decade, brought about by advancements in techniques for measuring bone mineral content and density, is to concentrate on enhancing peak bone mass for future fracture prevention. A greater understanding of the timing of bone mineral acquisition relative to other maturational events, as well as knowledge of those factors that can enhance or detract from this process, is valuable to this strategy of maximizing peak bone mass (Bailey et al., 2000). Hormonal status has been identified as a modifiable determinant of bone mass in hypoestrogenic women of varying ages (Snow-Harter, 1994; Warren & Stiehl, 1999; Compston, 1992). The efficacy of hormonal modulation with oral contraceptives or hormone replacement therapy (HRT) in hypoestrogenic irregularly menstruating adolescents is unclear, although HRT is prescribed in approximately 77.6% of amenorrheic anorexic cases (Robinson, Bachrach & Katzman, 2000). Even less is known about the potential for oral contraceptives to affect the skeleton of eumenorrheic adolescents (Lloyd et al., 2000).

The prevalence of menstrual irregularities attributed to vigorous exercise, such as complete cessation of menstruation (secondary amenorrhea) and irregular menstruation (oligomenorrhea), ranges from 2-45% depending on exercise intensity and type of exercise and the specific definitions used to describe each menstrual irregularity (Sanborn

& Wagner, 1988; Keizer & Rogol, 1990). Aesthetic sports, such as ballet and gymnastics, as well as sports which combine low body weight with high intensity exercise (cross country running, rowing) appear to have the highest incidences of menstrual irregularities (Sanborn & Wagner, 1988). Sedentary females experience similar menstrual irregularities at a rate of only 2-5% of the population (Snow-Harter, 1994) (See Appendix A: Review of Literature).

In contrast to the athletic adult population, adolescent females commonly experience irregular and anovulatory menstrual cycles during the first postmenarcheal years independent of exercise involvement. It has been reported that amenorrhea, oligomenorrhea, irregular cycles, abnormal uterine bleeding and dysmenorrhea represent 50% of their gynecologic complaints, regardless of exercise involvement (Caufriez, 1991). It has been observed that adolescent girls who begin intense exercise before menarche may experience delayed onset of menarche when compared to sedentary controls (Malina, 1983). Introduction of intense exercise at a fragile time in the development of the immature hypothalamic-pituitary-ovarian axis, therefore, may increase the incidence and duration of menstrual irregularities (Warren & Stiehl, 1999).

The pathophysiological relationship between intense exercise and the induction of menstrual irregularities remains unclear. The unequivocal involvement of the endocrine system, whether or not it plays a causative or consequential role, is characterized by measurable changes in hormone profiles of athletes experiencing menstrual irregularities. Females experiencing menstrual irregularities associated with chronic intense exercise, such as amenorrhea and oligomenorrhea, typically exhibit depressed resting levels of circulating luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol and progesterone when compared to chronically exercising eumenorrheic controls. In addition, chronically exercising, eumenorrheic athletes exhibit lower resting levels of these hormones than eumenorrheic, sedentary controls (Cumming & Rebar, 1985; Loucks et al., 1989; Creatsas et al., 1992; Ding, et al., 1988).

A depressed level of sex steroids in irregularly menstruating young athletes mimics the postmenopausal endocrine profile, suggesting a similar potential for negative impact on bone mineral. In the postmenopausal female, for example, low levels of sex steroids result in increased bone mineral turnover and increased resorption of bone

relative to formation in each basic multicellular unit (Manolagas, 2000). Low levels of estrogen have been shown to affect remodeling by altering the genesis and lifespan of osteoclasts and osteoblasts, extending the lifespan of osteoclasts and shortening the lifespan of osteoblasts. This creates a negative remodeling balance and manifests as bone loss and degeneration of trabecular architecture (Eriksen, 1992; Manolagas, 2000). Depressed sex steroid levels in irregularly menstruating female athletes may also have a similar negative impact on bone mineral density (Keen & Drinkwater, 1997; Cann, Martin, Genant & Jaffe, 1984; Lloyd et al., 1988) (See Appendix A: Review of Literature).

Spinal bone density has been shown to be decreased by as much as 10-29% in amenorrheic and oligomenorrheic young adult athletes and non-athletes when compared to eumenorrheic controls ($p<0.001$). Peripheral bone mineral values may be decreased by 5-10% (Cann et al., 1984;Wolman et al., 1990; Lloyd et al., 1988). Bone mineral loss appears to occur at its greatest rate in the first 1-5 years of amenorrhea with 3-5% bone mineral loss occurring per year in the first 2 years alone (Biller et al., 1991). Although the rate of bone density loss appears to decrease or stabilize after 5 years of skeletal exposure to the amenorrheic condition, it has also been shown that increased years of skeletal exposure to amenorrhea and oligomenorrhea (>5 years) results in a significantly lowered bone mineral density and lowered adult peak bone mass of the lumbar spine when compared to females whose menses have been regular throughout their lives (Micklesfield et al., 1995; Biller et al., 1991). Resumption of normal menstruation has not demonstrated a return to bone mineral density levels comparable to age-matched controls. Keen & Drinkwater, (1997), investigated even longer term effects of resumption of menses by measuring bone mineral density in athletic subjects 6-10 years after the return of normal menstruation. Formerly oligomenorrheic or amenorrheic athletes had mean vertebral bone mineral densities that were 84.4% of the eumenorrheic athletic controls despite several years of normal menses or use of oral contraceptives ($p<0.05$). These data suggest that exercise and diet-related hypothalamic amenorrhea or oligomenorrhea in young adulthood may have permanent negative effects on adult bone mass and that there is a critical period following the development of menstrual

irregularities wherein bone loss occurs. Hormone interventions, such as with oral contraceptives, may prove to be beneficial in limiting these negative effects.

Adolescents are at particular risk for experiencing long-term effects of menstrual irregularities considering the timing of bone mineral accrual. Longitudinal research in the area of bone mineral acquisition has shown that there is a specific period of time during skeletal development when the greatest amount of bone accretion occurs in females. This peak level of bone accretion (peak bone mineral content velocity) occurs primarily during adolescence, specifically, within the first year subsequent to the cessation of longitudinal growth (peak height velocity) and the closing of the epiphyseal growth plates (McKay et al., 1998). This two-year period of peak skeletal growth adds 26% of adult calcium to the skeleton (Bailey et al., 2000). During this time, the average female gains 40-50% of her skeletal mass, which translates to approximately 1,000 g of bone mineral during adolescence (Lloyd et al., 2000). While rapid acquisition of bone mineral density in females occurs between 11 and 14 years of age, a plateau in bone mineral acquisition follows this period of growth from age 17-20 years (Katzman et al., 1991). The timing of peak bone mass is site specific with peak hip bone mineral density reached well before peak lumbar vertebral bone mineral density. While the timing of total body peak bone mass remains uncertain, there appears to be only small increments in bone mineral density beyond the second decade in females (Gilsanz et al., 1988; Theintz et al., 1992). Thus, adolescence is a critical time for bone mineral accrual. Adolescent athletes who experience exercise-induced menstrual irregularities during a period when most of their skeletal mass is acquired are not only at risk for decreased sport performance due to associated health problems such as injury to bone, but are susceptible to lowered peak bone mass affecting future risk of osteoporosis (Hergenroeder et al., 1997; Myburgh, Hutchins, Fataar, Hough, & Noakes, 1990). In addition, calcium intake, which facilitates the development of peak bone mass, appears to decline in girls as they reach adolescence. This could compound the deleterious effects that exercise-related menstrual irregularities have on bone mineral density during adolescence (Matkovic & Ilich, 1993).

Hormonal modulation of bone mineral accrual with oral contraceptives is an area that has only been explored retrospectively in older females, or in hypoestrogenic

anorexic females. Women who report a history of oral contraceptive use are significantly less likely to have low bone mineral density (Kritz-Silverstein & Barrett-Connor, 1993). Measurements indicate that the bone density of premenopausal oral contraceptive users may be as much as 12% higher than non users ($p<0.01$) and that vertebral bone mass may increase by about 1% for each year of exposure to oral contraceptives (Lindsay, Tohme, & Kanders, 1986). The degree of protection from bone mineral loss appears to be related to increased duration of exposure to oral contraceptives with oral contraceptive use for 6-10 years or more affording the greatest protection against low bone mineral density (Kritz-Silverstein & Barrett-Connor, 1993; Kleerekoper et al., 1991; Tuppurainen et al., 1994). Timing of this exposure may also be important. Grainge et al. (2001) demonstrated that oral contraceptive use before the age of 23 years was significantly associated with increased bone density. There are many confounding variables in these retrospective studies however, and formulations of oral contraceptives are lower today than those considered in the research. These results must be interpreted with caution and more prospective research is needed to clearly identify the influence of oral contraceptives on bone mineral, particularly in the adolescent population.

Prospective studies utilizing oral contraceptive interventions have involved mostly young adults (20-40 years) or adolescent anorexic females. Few studies have been conducted on oral contraceptive use in adolescent athletes (irregularly menstruating and eumenorrheic). Of those that have been conducted, results are equivocal at all ages with no effects (Mazess & Barden, 1991; Gremion et al., 2001), positive effects (Recker et al. 1992; Hergenroeder, 1995), and negative effects being demonstrated (Burr et al., 2000; Polatti, Perotti, Fillippa, Gallina & Nappi, 1995). Hergenroeder et al. (1997) assessed the effectiveness of oral contraceptive and medroxyprogesterone treatment in a randomized, controlled clinical trial using young women 14-28 years of age with hypothalamic amenorrhea and oligomenorrhea. This research indicates that the spine and total body bone mineral density of irregularly menstruating athletes may be significantly improved in those who ingest oral contraceptives for only 12 months when compared to females taking a placebo for the same amount of time. This provides support for oral contraceptive intervention in young amenorrheic females and indicates that a net increase in bone mineral density may occur with as little as one year of intervention. In contrast,

Lloyd et al. (2000) reported that oral contraceptive ingestion for the purpose of enhancing skeletal integrity in eumenorrheic adolescent females was ineffective. Contradictions in research findings involving oral contraceptive use and bone health in young women and adolescents (amenorrheic, oligomenorrheic or eumenorrheic) illustrate the need for further research in this area.

Purpose

Athletes experiencing menstrual irregularities exhibit depressed levels of sex steroids, a condition that has the potential to negatively affect bone mass. Adolescent athletes experiencing menstrual irregularities may be at a greater risk for associated bone health disturbances as most of peak bone mass is accrued during adolescence with limited increases beyond the second decade. Oral contraceptive use has been observed to have a positive effect on bone density in pre and postmenopausal women, which has led to the suggestion that oral contraceptives may have the potential to protect young female athletes from bone loss associated menstrual irregularities during a critical period of bone growth.

The purpose of this research was to compare measures of areal bone mineral density and content between eumenorrheic and irregularly menstruating adolescent athletes (menstrual status) and between adolescent athletes who were using oral contraceptives and those who were not using oral contraceptives (oral contraceptive status). A secondary aspect of this study was to determine if these groups were distinguishable on other factors that have been associated with bone mineral density and content, such as height and weight, age at menarche, physical activity, caloric and calcium intake, and attitudes toward food. Insight into maximizing peak bone mass might assist in the protection against progressive age and menopause-related bone loss, and associated lifelong health consequences such as increased fracture risk and osteoporosis.

Research Questions

Using a cross sectional, non intervention design:

1. Do adolescent athletes experiencing menstrual irregularities have lower bone mineral density and content than their regularly menstruating counterparts?

2. Does physician prescribed oral contraceptive (OC) use positively influence bone mineral density and content in regularly menstruating (eumenorrheic) adolescent athletes?

3. Does physician prescribed OC use positively influence bone mineral density in adolescent athletes experiencing exercise-associated menstrual irregularities (amenorrhea and oligomenorrhea)?

Experimental Hypotheses

1. Non-OC using adolescent athletes experiencing menstrual irregularities (INOC) will exhibit lower bone mineral density and content of the lumbar spine and proximal femur when compared to non-OC using eumenorrheic adolescent athletic controls (ENOC).

2. OC-using eumenorrheic adolescent athletes (EOC) will exhibit higher bone mineral density and content at the lumbar spine and proximal femur when compared to non-OC using eumenorrheic adolescent athletes (ENOC).

Delimitations

1. Results of this study are reflective of a western Canadian athletic population of adolescent females between 15 and 20 years of age.
2. QDR Hologic 2000 model of dual energy x-ray absorptiometer (array mode) was used. Bone mineral density and content values are comparable only with those obtained from the same type of densitometer.

Limitations

1. Sample size for each of the groups was small, and varied across groups. This limits the external validity of findings and decreases the degrees of freedom for statistical analyses.
2. Subjects were not randomly selected but were recruited based on menstrual status, oral contraceptive status and amount of weight bearing activity per week.
3. Subjects participated in various types of weight bearing exercise that are known to produce different mechanical strains on bone.
4. This study did not include a non-exercising control group.
5. This study did not measure hormone levels associated with menstrual status and oral contraceptive status.
6. Maturation was not measured in this investigation and it was assumed that subjects were at similar stages of maturation.

Assumptions

1. The General Health Questionnaire, EAT-26, and Dietary Records were answered accurately and honestly.

Operational Definitions

Menstrual Irregularities

Amenorrhea: 1-3 menstrual cycles in the year preceding the study

Oligomenorrhea: 4-9 menstrual cycles in the year preceding the study

Eumenorrhea: 10-12 menstrual cycles in the year preceding the study

Athlete: Involved in regular program of vigorous, weight bearing activity, minimum three times per week for forty-five minutes each time.

Methods

Subjects

Thirty-three healthy female adolescent athletes between the ages of 14.9-21.1 years (mean=18.8 years, SD=1.3) volunteered for this research. Subjects were recruited through local sports and recreation facilities, local high school teams and from local medical offices via poster and pamphlet advertising. This poster was also provided to athletes, coaches and parents/guardians (Appendix B). Criteria for participant selection included: involvement in a program of regular exercise that included vigorous physical activity at least three times a week for a minimum of 45 minutes each time and involving weight bearing activities (i.e. running, gymnastics, dance, field hockey, etc.); a recent physical exam by a physician to ensure good health and to rule out potential causes of menstrual irregularities other than exercise.

Participants were grouped by menstrual and oral contraceptive status: eumenorrheic, oral contraceptive users (EOC, n=11), eumenorrheic, non oral contraceptive users (ENOC, n=15), and irregularly menstruating, non oral contraceptive users (INOC). Irregularly menstruating subjects consisted of both amenorrheic (2) and oligomenorrheic (3) athletes. Criteria for duration of oral contraceptive use was at least 8 months of continuous use immediately prior to the study. There were no criteria for type/dosage of oral contraceptive. Formulations of oral contraceptives used were as follows: 0.020-0.035mg ethinyl estradiol combined with either norgestimate, levonorgestrel or desogestrel progestins. Seven of 11 participants were taking the same oral contraceptive (Tri-Cyclen, ethinyl estradiol and norgestimate combination). Participants were not prescribed oral contraceptives for inclusion in this cross-sectional study, but continued with previous (to the investigation) oral contraceptive prescriptions.

Data from one of the original 33 volunteers was not included in the analyses because she was on depot medroxyprogesterone acetate (Depo-Provera) injectable contraceptive instead of oral contraceptives. This type of contraceptive has been shown to alter bone remodeling by causing bone resorption to exceed bone formation (Ott et al., 2001). This negative remodeling balance has been observed to result in significantly

decreased bone mineral density of the lumbar spine over two years of intervention in post-menarcheal females, 12-21 years of age, when compared with other oral contraceptive users and controls (Cromer, Blair, Mahan, Zi & Naumovski, 1996). Data from a second subject (EOC) was excluded from analyses because she had an immediate (maternal) history of clinically diagnosed osteoporosis. A third subject (EOC) completed the DXA testing but did not complete the anthropometric or nutritional analysis portions of the study. Her data were included only in the bone mineral statistical analyses and excluded from any other analyses.

Research Design and Procedures

Using a cross sectional research design, subjects attended one information session and were then tested on two occasions separated by approximately a week: The information session was held at the University of Victoria, the first testing session at the Royal Jubilee Hospital Nuclear Medicine Department and the second testing session at the University of Victoria Sport and Fitness Centre. This study was conducted in accordance with the Human Research Ethics Committee at the University of Victoria and was also approved by the Research Review and Ethical Approval Committee-Capital Health Region, School District #61 (Greater Victoria) and administrators of Oak Bay Secondary School.

Information Session

Prior to any data collection, a preliminary Information Session was conducted during which participants (and parent/guardian) were familiarized with the purpose, methods and procedures of the study, and given a package containing three dietary record forms and information on how to complete them as accurately as possible. In addition, participants filled out Informed Consent Forms at this time or brought them home for parent/guardian signatures (Appendix C). Three-Day Dietary Records were used to assess mean caloric and calcium intake as well as other macronutrients, minerals and vitamins (Schoeller, 1990) (Appendix D). Subjects were shown how to fill out a three-day dietary record using food models and examples from the participants themselves. Participants took the records with them and returned the completed records at one of the testing sessions. These records were analyzed using The Food Processor Nutritional

Analysis Software-Version 7.7 (Esha Research, 2001) and a registered dietician reviewed results for any inconsistencies.

Bone Mineral Density and Content

Bone mineral measures were conducted at the Royal Jubilee Hospital Nuclear Medicine Department. All scans were completed by one of two registered Nuclear Medicine Technologists. Combined cortical and cancellous areal bone mineral density (aBMD, g/cm^2) and content (BMC, in grams) within a projected area (BMA, in cm^2) were measured at the lumbar spine (L1-L4) and the proximal femur (sub regions: femoral neck and trochanter) using a Hologic QDR 2000 (array mode) dual energy x-ray absorptiometer (DXA). Procedures followed are outlined in the Supine Lateral Bone Densitometer Operator's Manual and User Guide (Hologic, 1994). Participants were required to wear clothing with no metal near selected scan sites (torso/hip area). Participants were weighed prior to DXA measurement and then positioned on the scan table in a supine position according to the selected scan site. Lumbar spine measures required participants to be positioned with the hips and knees flexed over a support so as to eliminate lumbar lordosis (Figure 1). Scanning the left proximal femur required the same leg to be slightly abducted and internally rotated by the use of a positioning device so as to bring the proximal femur parallel to the top of the scan table (Figure 2). These procedures took approximately 15-20 minutes.

DXA involves the radiation transmission of two discrete energies (70 and 140 kVp alternating at 60/s) from an x-ray source. The energies that are used are optimal for separating soft tissue (i.e. muscle and fat) from the mineralized components (i.e. bone) of the area analyzed. Advantages of this technique over previous bone density measurement techniques (i.e. single photon absorptiometry, computed tomography) include higher resolution, increased speed of the procedure and better precision (1% at the lumbar spine, 1-2% at the proximal femur) while using minimal radiation (Adams, 1995). The effective dose equivalent for the DXA technique is 1-6μSv per site examined in women. This is considerably less than that of a common chest x-ray, which is 60 μSv, and background environmental radiation per annum, which is 2400 μSv (Kalender, 1992). Thus, there is negligible radiation exposure with this method and it has been found to be a safe, accurate and precise technology for assessing bone mineral density (Kellie, 1992).

The extremely low doses involved in this procedure make it a suitable tool for the investigation of skeletal development in children (Adams, 1995).

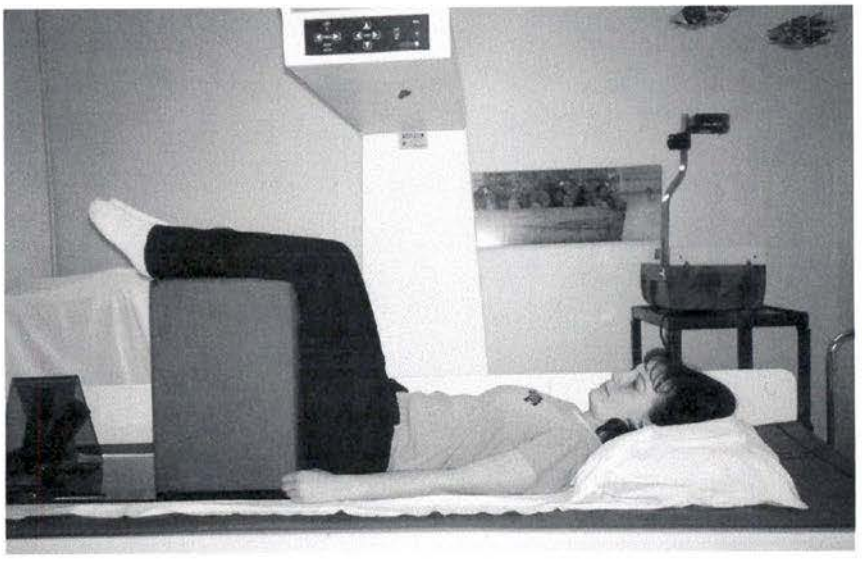


Figure 1. Lumbar spine (L1-4) DXA scan position (Hologic QDR 2000)

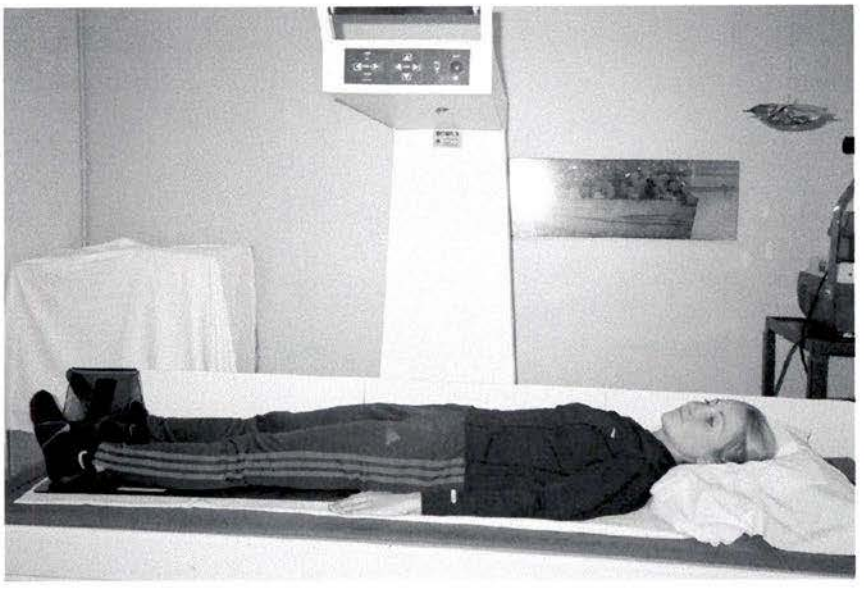


Figure 2. Proximal femur DXA scan position (Hologic QDR 2000)

General Health Questionnaire, EAT-26 and Anthropometry

The second testing session took place at the University of Victoria's Sport and Fitness Centre. At this session participants completed a General Health Questionnaire (Appendix E) and an Eating Attitudes Test (EAT-26, Appendix F). The general questionnaire provided information regarding oral contraceptive and menstrual histories, physical activity levels, caffeine and alcohol consumption, smoking history, individual medical history and familial history of osteoporosis, medication and vitamin intake and participant age. The purpose of the questionnaire was to provide details about the independent variables as well as to indicate factors other than the independent variables that may have influenced the participant's bone mineral results. The Eating Attitudes Test provided information regarding tendencies toward eating disorder behavior and allowed further description of the sample. This test has an established validity coefficient of $r = 0.85$, $p<0.001$ (Garner & Garfinkel, 1997).

A wall stadiometer was used to measure height to the nearest 0.1 cm, during maximal inspiration and with shoes off. Weight was measured to the nearest 0.1 kg using a balanced scale with subjects wearing light clothing and no shoes. Body mass index (BMI) was determined as weight (kg)/height $(m)^2$. Five standard skin fold measures (biceps, triceps, subscapular, iliac crest, medial calf) were taken using Harpenden calipers (British Indicator Ltd.) and according to the methods of the Canadian Society for Exercise Physiology's Professional Fitness and Lifestyle Appraiser Manual (Canadian Society for Exercise Physiology, 1995) in a confidential manner in a private area of the Sport and Fitness Centre. The sum of five (So5S) skinfolds was calculated for each subject to reflect adiposity and for comparison to normative values derived from the 1981 Canada Fitness Survey (Canadian Society for Exercise Physiology, 1996).

Statistical Analyses

Statistical analysis was performed using SPSS version 10.0 statistical package (SPSS Inc., 2001). Descriptive statistics were used to characterize the whole sample together (n=31) and each group (ENOC, EOC, INOC) separately in terms of age, age at menarche, anthropometrics, nutrition, exercise, and bone mineral variables. Pearson

product moment correlations were used to analyze bivariate associations between independent and dependent variables. One-way analysis of variance was used to determine if the ENOC, EOC, and INOC subject groups were similar with respect to chronological age, age at menarche, anthropometric measures, nutrient intake and physical activity. One-way ANOVA was then used to compare differences between the means of ENOC, EOC, and INOC with respect to the dependent variables aBMD, BMC and BMA of the lumbar spine, proximal femur and subregions. Following this, analyses were repeated to control for any effect height and weight might have had on these bone measures. Statistical significance was set at $p \leq 0.05$.

Results

This section summarizes the results of the descriptive statistics and Pearson product moment correlations for all subjects considered together regardless of group status (total n = 31) and separately by oral contraceptive and menstrual status group (ENOC, n=15; EOC, n=11; INOC, n=5). Comparative results (one way ANOVA) of the three subject groups on each independent variable to determine the level of homogeneity between the groups with respect to these variables are summarized. Finally, comparative results of the means for the ENOC, EOC, and INOC subject groups with respect to the dependent bone mineral variables (aBMD, BMC and BMA) of the lumbar spine and proximal femur (including femoral neck and femoral trochanter subregions) are summarized.

Total Sample Descriptive Characteristics

The mean chronological age, height and weight of the volunteer subjects (n=31) was 18.8 ± 1.3(SD) years (range=14.9-21.1 years), 167.2 ± 5.0(SD) cm (range=159.0–176.0 cm) and 58.7 ± 6.3(SD) kg (range=50.1-77.0 kg), respectively. Mean body mass index was 21.0 ± 6.3(SD) kg/m^2 (range=18.8-26.0 kg/m^2). These data exhibited a "normal" bell shaped distribution about the mean but there tended to be slight negative skewness in weight measures with more subjects weighing less than the group mean than those subjects whose weight was greater than the mean (skewness=0.832). Most subjects were between 18 and 20 years of age (skewness = -0.618).

Total Sample Correlations

Table 1 provides the significant bivariate correlations found between the dependent variables (aBMD, BMC and BMA) at the lumbar spine (LS) and proximal femur (PF), sub regions femoral neck (FN) and trochanter (FT), and factors other than menstrual and oral contraceptive status that may relate to aBMD, BMC or BMA. These factors include age, anthropometric variables, exercise and diet and are described below.

Age

No significant correlations were observed between age at menarche, or chronological age and any of the aBMD, BMC or BMA measures.

Anthropometry

Height correlated significantly with all of the BMA measures for the proximal femur, but not lumbar spine. Height was also significantly correlated with measures of BMC at the proximal femur and lumbar spine. Height did not correlate significantly with aBMD measures. Weight correlated significantly with BMA of the proximal femur but not with the femoral subregions or with BMA of the lumbar spine. Weight correlated significantly with all BMC measures. Similar to height, weight did not correlate significantly with any of the aBMD measures. Body mass index and sum of 5 skinfolds did not correlate significantly with any of the bone measures.

Exercise

The only bone measure demonstrating any statistically significant correlation to exercise was aBMD. Vigorous weight bearing activity correlated significantly with aBMD_FT. A trend in correlations between vigorous weight bearing activity and the femoral subregions, aBMD_PF ($r=0.313$, $p=0.092$) and aBMD_FN ($r=0.333$, $p=0.072$) was observed, however, these did not reach statistical significance. Resistance training (hrs/wk) correlated significantly with aBMD_FN. A trend in correlations between resistance training and aBMD_PF ($r=0.337$, $p=0.068$) and aBMD_FT ($r=0.353$, $p=0.056$) was also observed, however these did not achieve statistical significance. Vigorous weight bearing exercise and resistance training did not correlate with aBMD of the lumbar spine nor with any measure of BMC and BMA.

Diet

Dietary fat ingestion correlated significantly with all aBMD proximal femur measures as well as BMC_PF and BMC_FN. No significant relationship was observed between dietary fat and any lumbar spine bone measure. Caloric, calcium, carbohydrate, protein, potassium, phosphorus, vitamin C and D intakes did not correlate significantly with any of the bone measures nor did the Eating Attitudes Test (26) (total score and subscale items scores).

Table 1

Significant Bivariate Pearson Product Moment Correlations (r) Between aBMD, BMC and BMA Measurements and Height, Weight, Exercise and Dietary Fat.

		ENOC, EOC, and INOC Combined (n=30)		
Dependent Variables		Independent Variable(s)	R	Significance (p) *
Areal Density	aBMD_LS	*No significant correlations*		
	aBMD_PF	Dietary Fat (g)	0.483	0.007
	aBMD_FN	Resistance Training (hrs/wk)	0.333	0.017
		Dietary Fat (g)	0.364	0.048
	aBMD_FT	Vig Weight-bearing Activity (hrs/wk)	0.373	0.042
		Dietary Fat (g)	0.382	0.037
Content	BMC_LS	Height (cm)	0.478	0.009
		Weight (kg)	0.533	0.003
	BMC_PF	Height (cm)	0.432	0.017
		Weight (kg)	0.483	0.007
		Dietary Fat (g)	0.385	0.035
	BMC_FN	Weight (kg)	0.401	0.028
		Dietary Fat (g)	0.444	0.014
	BMC_FT	Weight (kg)	0.377	0.040
Area	BMA_PF	Height (cm)	0.605	0.000
		Weight (kg)	0.432	0.017
	BMA_FN	Height (cm)	0.394	0.031
	BMA_FT	Height (cm)	0.373	0.042

* 2-tailed

ENOC, EOC, and INOC Group Characteristics

Levine's statistic for homogeneity of variance between ENOC, EOC and INOC groups across all variables revealed that their variances were homogeneous on every variable except aBMD_LS ($p = 0.047$).

Chronological Age and Age at Menarche

Examination of chronological age and age at menarche according to menstrual and oral contraceptive groups by one-way ANOVA revealed that groups were not significantly different (Table 2). Groups approached being significantly different with respect to their age at menarche, ($F(2,28)=2.63$, $p=0.09$), with the INOC group being slightly older at menarche than the other groups, although this did not attain statistical significance.

Anthropometric Measurements

Descriptive statistics for the anthropometric results according to menstrual and oral contraceptive groups are also presented in Table 2. One-way ANOVA revealed no significant differences between the ENOC, EOC and INOC groups on any of the anthropometric variables.

Eating Attitudes Test (EAT-26)

Table 3 presents descriptive statistics for each group with respect to their EAT-26 results. One-way ANOVA revealed no significant differences between the groups for any of the EAT-26 scores and subscale items. Though it was not significant, the INOC group scored almost twice as high on the EAT-26 and subscale items than the ENOC and EOC groups.

Table 2

Mean (SD) Height, Weight, Body Mass Index, So5S According to Menstrual and Oral Contraceptive Status Groups

Variable	Subject Groups		
	ENOC *(n=15)*	EOC *(n=10)*	INOC *(n=5)*
Chronological Age	18.4(1.4)	19.3(1.3)	18.9(1.0)
Age at Menarche	13.2(1.6)	13.4(1.8)	15.1(1.8)
Height (cm)	167.4(5.8)	168.1(4.9)	164.7(1.6)
Weight (kg)	59.2(7.6)	58.9(4.7)	56.8(5.7)
BMI ($kg \cdot m^{2\,(-1)}$)	21.1(2.2)	20.8(1.3)	20.9(1.7)
So5S (mm)	59.4(16.7)	54.8(9.6)	60.5(10.2)

Table 3

Mean (SD) Total Eating Attitudes Test and Subscale Item Scores According to Menstrual and Oral Contraceptive Status Groups

Variable	Subject Groups		
	ENOC *(n=15)*	EOC *(n=10)*	INOC *(n=5)*
Total EAT-26 Score	6.73(8.2)	5.70(6.9)	12.80(8.2)
Subscale Items:			
Dieting	4.80(6.6)	3.70(4.9)	8.60(7.1)
Bulimia and Food Preoccupation	0.93(1.6)	1.10(1.9)	2.00(2.9)
Oral Control	1.00(1.4)	0.90(1.0)	2.20(1.9)

Note. Scores are proportional to disordered eating behaviours. A score of 20 or greater indicates possible presence of an eating disorder and supports the inclusion of secondary interview (Garner & Garfinkel, 1997).

Exercise

Descriptive results for ENOC, EOC and INOC groups with respect to time spent performing vigorous weight bearing activity and resistance training (hrs/wk) are presented in Table 4. One-way ANOVA revealed that the amount of vigorous weight-bearing physical activity and resistance training performed per week was similar for each of the three groups.

Table 4

Mean (SD) Hours per Week of Vigorous Weight-Bearing Activity and Resistance Training According to Menstrual and Oral Contraceptive Status Groups

Variable	Subject Groups		
	ENOC *(n=15)*	EOC *(n=10)*	INOC *(n=5)*
Vigorous Weight-bearing Activity (hrs/wk)	8.1(3.9)	6.4(4.5)	6.3(2.3)
Resistance Training (hrs/wk)	2.0(2.3)	2.2(2.5)	1.7(1.7)

3-Day Dietary Record

Table 5 presents the results from the 3-Day Dietary Record analyses according to menstrual and oral contraceptive status groups. One-way ANOVA revealed that there were no significant differences between the groups on any of the dietary variables. The INOC group ingested about 400-500 calories a day less than the EOC and ENOC groups but this was not significant ($p=0.072$). Calcium intake was highest in the ENOC group and lowest in the INOC group but all groups achieved the Canadian Recommended Nutrient Intake for calcium of 700mg per day based on the mean age of each group. Groups did not differ significantly with respect to alcohol or caffeine consumption, none of the subjects smoked, and all subjects experienced at least twenty minutes of sunshine on the face and hands for each day that food was recorded (corresponds to Vitamin D production).

Table 5

Mean (SD) Caloric and Nutrient Intakes Averaged from 3-Day Dietary Records and Reported According To Menstrual and Oral Contraceptive Status Groups

Dietary Variable	Subject Groups		
	ENOC *(n=15)*	EOC *(n=10)*	INOC *(n=5)*
Calories (cal/d)	2065(302)	2006(467)	1596(418)
Protein (g/d)	81(18)	76(24)	59(22)
Carbohydrates (g/d)	327(60)	322(78)	280(75)
Fat (g/d)	52(14)	50(21)	34(16)
Potassium (mg/d)	3247(664)	2977(803)	2816(958)
Phosphorus (mg/d)	1278(326)	1183(498)	961(314)
Calcium (mg/d)	1065(365)	956(404)	761(222)
Vitamin C (mg/d)	180(75)	173(99)	144(60)

Bone Mineral Density, Content and Area Measurements

Mean (SD) aBMD, BMC and BMA measurements of the lumbar spine (LS) and proximal femur (PF) with its femoral neck (FN) and trochanter (FT) sub regions are presented for each group in Table 6. One-way ANOVA revealed that the ENOC, EOC and INOC groups differed significantly with respect to most BMC measures including: BMC_LS [$F(2,27)=3.53$; $p=0.044$; $\eta^2=0.21$], BMC_PF, [$F(2,28)=4.22$, $p=0.025$; $\eta^2=0.23$], and BMC_FT [$F(2,28)=3.93$, $p=0.031$; $\eta^2=0.22$]. BMA_PF approached but did not achieve significance [$F(2,28)=3.29$, $p=0.052$, $\eta^2=0.19$]. There were no other significant differences between ENOC, EOC and INOC for any of the other bone mineral measures.

Post-hoc testing (Fisher LSD) further revealed that BMC_LS was significantly greater in the EOC group compared to the INOC group ($p=0.017$; mean difference=11.21; 95% CI=2.21-20.20). The EOC group also had a significantly greater BMC_PF than the ENOC group ($p=0.028$; mean difference=3.15; CI=0.36 to 5.93) and the INOC group ($p=0.016$; mean difference= 4.75; CI=0.97 to 8.53). BMC_FT was also significantly greater in the EOC group compared to both the ENOC ($p=0.020$; mean difference=1.18; CI=0.20-2.16) and INOC ($p=0.033$; mean difference=1.46; CI=0.13-2.79) groups. These differences remained after controlling for height and weight.

Table 6

Mean (SD) aBMD, BMC and BMA Measurements for ENOC, EOC and INOC Groups

Variable		Subject Groups ENOC *(n=15)*	EOC *(n=11)*	INOC *(n=5)*
Areal Density	aBMD_LS (g/cm^2)	1.017(0.104)	1.039(0.065)	0.964(0.139)
	aBMD_PF (g/cm^2)	0.986(0.087)	1.026(0.091)	0.968(0.121)
	aBMD_FN (g/cm^2)	0.892(0.114)	0.923(0.102)	0.886(0.155)
	aBMD_FT (g/cm^2)	0.761(0.089)	0.828(0.095)	0.729(0.122)
Content	BMC_LS (g)	59.57(7.89)	65.40(5.76)*	54.20(11.81)
	BMC_PF (g)	31.23(2.69)	34.37(4.13)†*	29.63(3.74)
	BMC_FN (g)	4.38(0.42)	4.47(0.60)	4.20(0.28)
	BMC_FT (g)	7.87(0.91)	9.06(1.55)†*	7.60(1.13)
Area	BMA_LS (cm^2)	58.51(3.75)	61.56(7.25)	55.76(4.77)
	BMA_PF (cm^2)	31.73(2.11)	33.46(1.86)	30.73(3.04)
	BMA_FN (cm^2)	4.94(0.38)	4.84(0.28)	4.81(0.50)
	BMA_FT (cm^2)	10.37(0.89)	10.90(1.14)	10.47(0.75)

* EOC significantly different than INOC (LSD; $p \leq 0.05$)

†EOC significantly different than ENOC (LSD; $p \leq 0.05$)

Discussion

The major objective of this investigation was to determine if areal bone density and content at the lumbar spine and proximal femur in female adolescent athletes were affected by menstrual status and/or oral contraceptive use. The original cross sectional design of the study called for the inclusion of four groups to reflect two levels of menstrual status (eumenorrheic and amenorrheic/oligomenorrheic) and two levels of oral contraceptive use (oral contraceptive use and non use). An inability to recruit subjects who were both irregularly menstruating and taking oral contraceptive resulted in the removal of this group from the research design. As a consequence, this research could not address whether or not oral contraceptive use by irregularly menstruating adolescent athletes ameliorates the detrimental effect of irregular menstruation on measures of areal bone density and content.

This study is limited by the small sample sizes and variability in sample size between the groups. Considering this limitation and the possibility that the observed trends reflect a true physiological difference that may not have attained statistical significance, observed trends and factors associated with them will be discussed cautiously in conjunction with the major findings of this study.

A major finding of this study was that menstrual status had no significant effects on aBMD and BMC of the lumbar spine and proximal femur in adolescent athletes. The eumenorrheic group (ENOC) tended to have higher values on all measures of bone mineral content and density than the irregularly menstruating group (INOC), however these differences did not achieve significance. The second major finding was that oral contraceptive use by eumenorrheic adolescent athletes (EOC) had a significant positive effect on BMC of the proximal femur, but not of the lumbar spine or on any aBMD measure when compared to non-oral contraceptive using eumenorrheic adolescent athletes (ENOC). The EOC group did demonstrate a trend toward higher BMC and aBMD measures at all sites when compared to the ENOC group, however these did not attain statistical significance. Mean chronological age, age at menarche, height, weight, BMI, So5S, amount of physical activity, and dietary factors were similar between the groups and, therefore, can be discounted as contributing to these bone mineral results.

Physical Characteristics of ENOC, INOC and EOC

Measures of Bone Mineralization

The study of bone mineral in the pediatric population has been significantly enhanced in the last decade due to the advent of new measurement technologies, such as the dual energy x-ray absorptiometry (DXA), that allow for rapid, highly reproducible and valid assessment of bone mineral with a low radiation dose (Bonjour et al., 1991; Katzman et al., 1991; Faulkner et al., 1996). Consequently, there are few longitudinal studies involving the assessment of bone mineral accrual, content and density, as well as determinants of these measures, during growth and adolescence in the literature. In addition, due to slight variations in values produced by different DXA models and measurement sites observed, there are few "normative" values for comparison to bone mineral measures obtained in this present study. The Saskatchewan Pediatric Longitudinal Growth study of Canadian Caucasian children aged 8-17 years, however, utilized the same densitometer (QDR Hologic 2000 in array mode) and measured similar sites as this present study (Faulkner et al., 1996). This group found no significant differences in BMC or BMD at any sites for females between the ages of 17 and 21. Faulkner's data are representative of the slightly older girls in this present study. Comparison of the ENOC, EOC, and INOC mean values for bone mineral content and density of the lumbar spine and proximal femur with these Canadian data provides evidence that all group means are within the normal range. Upon further inspection of the data, the INOC group mean was at the low end of normal on all content and density measurements, while the EOC group mean was at the upper end of the normal range for all content and density measurements.

Anthropometrics

Mean weight did not differ significantly between ENOC, INOC and EOC groups. Each of the groups was similar in mean weight to the established median normative ratings for Canadian girls of similar age (Crawford, 1996). Mean stature was not significantly different between each of the groups and was slightly above the median normative rating for Canadian girls of similar age (Crawford, 1996). Further examination of these data found that the INOC group was slightly shorter and the ENOC group was

slightly taller than Canadian normative data, though not significantly. Mean sum of five skinfolds for ENOC, INOC, and EOC groups were slightly below the established median normative rating for Canadian girls of similar age (Martin & Ward, 1996). This finding could reflect a higher level of physical activity in these groups than that of the participants of the 1981 Canada Fitness Survey.

The significant correlations between body weight and BMC and BMA measures suggest that body weight influences aBMD even though body weight was not significantly different between the ENOC, INOC and EOC groups. This relationship is supported by research that suggests almost half of the population variance in bone mineral density is due to variations in weight (Heaney, 1996). Considering that body weight is supported by the skeleton, thus imposing a load on the skeleton through muscle contraction, it follows that if a greater load is imposed this would result in increased bone mineral accrued (Parfitt, 1994). This relationship is also supported by research with children that has found body weight to be strong predictor of bone mineral density (Katzman et al., 1991; Lloyd et al., 1992). Considering that areal bone density is partially dependent on size, the necessity for controlling for height and weight in different sized populations becomes evident. The findings of this research, therefore, are strengthened by the fact that weight and height were not significantly different between the ENOC, INOC and EOC groups, and controlling for height and weight did not alter the bone mineral findings of this present study.

Age at Menarche

It has been suggested that early menarche reflects a positive association with total estrogen exposure and may confer skeletal benefits into adulthood (Dhuper, Warren, Brooks-Gunn & Fox, 1990). McKay and colleagues (1998) demonstrated that girls who reach menarche earlier exhibit greater peak bone mineral content velocity and peak height velocity than late maturing girls. The contribution of these factors to adult peak bone mass could not be examined, however, as participants had not reached adult bone mass. Age at menarche provides information about the hormonal milieu of an individual, the timing of puberty relative to skeletal growth and is reflected in current measures of bone mineral (Constantini, 1994). Mean age at menarche was found to be average in the ENOC and EOC groups and late in the INOC group when compared to other Canadian

adolescent females (McKay et al., 1998), although these group differences did not achieve statistical significance. The slight delay in menarche observed in the INOC group is supported by findings that irregular menstrual cycles are prevalent in females who experience delayed menarche associated with vigorous exercise prior to menarche (Hergenroeder, 1995; Frisch et al., 1981). Although it was not quantified, the INOC group in this present study indicated a history of vigorous physical activity prior to menarche. The average age at menarche in gymnasts has been observed to be 15.6 years (Warren & Stiehl, 1999) and two of the five INOC subjects had a long history of competitive gymnastics participation. The later menarche seen in the INOC group, therefore, may support these earlier reports.

Menstrual Status (ENOC and INOC) and Bone Mineral Measures

The occurance of menstrual irregularities (amenorrhea and oligomenorrhea) in young adult athletes (20-40 years of age) has commonly been associated with decreased bone mineral density (Keen & Drinkwater, 1997; Snead et al., 1992; Myburgh et al., 1993; Biller et al., 1991; Cann et al., 1984). Decreases have been observed mainly in the lumbar spine (Cann et al., 1984; Biller et al., 1991; Snead et al., 1992) but have also been found in appendicular sites such as the proximal femur and femoral shaft (Myburgh et al., 1993; Keen & Drinkwater, 1997). Menstrual status did not have a significant effect on aBMD density or BMC of the lumbar spine or proximal femur in the adolescent athletes in this present investigation. Compared with the ENOC and INOC groups, subjects in the adult investigations described above were at a different stage of bone mineral acquisition, remodeling balance and near or at their peak bone mass (Szulc, Seeman & Delmas, 2000; Parfitt, 1994; McKay et al., 1998; Teegarden et al., 1995). This could alter their susceptibility and response to menstrual irregularities and associated determinants of bone density as compared to the adolescents in this investigation.

Few data are available on menstrual irregularities and bone mineralization in adolescent athletes. The menstrual status results of this study are supported by those of Baer et al. (1992) and Moen et al. (1998). Baer et al. (1992) found no significant differences in lumbar bone mineral density between amenorrheic and eumenorrheic adolescent runners 15 to16 years of age. Similarly, Moen et al. (1998) found lumbar

spine bone mineral density to be similar in amenorrheic (< 5 menstrual periods in the last year) and eumenorrheic runners (15.1-18.8 years of age). Their subjects had trained for 1-5 years and averaged 59 km per week in the 9 months prior to the bone mineral testing. Moen et al. (1998) also observed expected trends in bone mineral density based on adult findings such that lumbar spine bone mineral density was lower in the amenorrheic runners compared to eumenorrheic runners. The INOC group in the present study exhibited lower aBMD and BMC content measures at the lumbar spine and proximal femur compared to the ENOC group, although this difference did not achieve significance.

In contrast to the menstrual status results of this study, Dhuper et al. (1990), found that lumbar bone mineral density was significantly lower in adolescent amenorrheic compared to eumenorrheic dancers (13-20 years). Inclusion of younger adolescents in the Dhuper study makes it difficult to compare their findings with those of this present study due to potential differences in maturity. Pubertal stage has been found to be a major determinant of change in bone mineral density during the growing years (Bailey et al., 1999). The influence of menstrual status on bone mineral in younger females, therefore, may reflect variations in skeletal growth and bone mineral accretion at this age and affect bone mineral measures differently than in older adolescents. Also in contrast to the results of this present study, Pearce, Bass, Formica, Young & Seeman (1996) found that oligomenorrhea in ballet dancers 15-20 years of age resulted in significantly decreased bone mineral density at non-weight bearing sites (arms, $p<0.05$; ribs, $p<0.0001$) when compared to eumenorrheic controls. Lloyd, Myers, Buchanan & Demers (1988) found that oligomenorrhea in athletes prior to 20 years of age resulted in trabecular lumbar bone mineral density that was 69% of eumenorrheic controls ($p<0.05$).

There are a number of possible reasons why significant differences in bone mineral density and content were not observed between eumenorrheic and amenorrheic/oligomenorrheic adolescent athletes as hypothesized in this investigation. It may be that a greater length of time post menarche, or a longer period of irregular menstruation is required before differences in bone mineral density appear between these types of individuals. The ENOC group was 5 years post menarche on average, while the INOC group was only 3.5 years post menarche. Duration of amenorrhea and

oligomenorrhea have been negatively correlated to measures of bone mineral density (Seeman et al., 1992; Pearce et al., 1996) and have been associated with lower circulating levels of estrogen when irregularly menstruating young adult athletes are compared to normally menstruating athletic controls (Snow-Harter, 1994; Snead et al., 1992; Jonnavithula et al., 1993). The role of estrogen in the adult female with respect to bone is to exert a tonic suppression of trabecular bone remodeling and to maintain a balance between osteoclastic resorption and osteoblastic formation that would maintain, not increase, current bone mass (Turner, Riggs & Spelsburg, 1994). The role of estrogen in late puberty is to effect closing of the epiphyseal growth plates and cessation of longitudinal growth. In later adolescence, estrogen influences a positive balance in bone remodeling such that total body bone mass continues to accumulate until peak bone mass is achieved sometime between 20-30 years of age. This increased bone remodeling in adolescence has been observed through higher levels of biochemical markers of bone turnover, such as osteocalcin and pyridinoline, when compared to young adult values (Szulc, Seeman & Delmas, 2000). Thus, because the rate of bone remodeling is higher indicating more of a positive balance in adolescents, any effect of menstrual irregularities mediated by lower levels of sex steroids may not be observed until the early adult years. Longitudinal studies are needed to explore this prospect further. In addition, it may be that sex steroid levels were not low enough in the INOC group to have a negative impact on bone mineral accrual at this age. A negative correlation between bone mineral density and estradiol levels has been observed (Biller, 1991) and it has been suggested that there is a threshold below which estradiol levels are too low to maintain bone mass (Chen & Brzyski, 1999). Sex steroids were not measured in this investigation but it may be that estradiol levels in the INOC group were not at that threshold, even though menstruation was irregular.

It is also possible that the type of exercise in which the INOC group was participating had a protective effect on their bone mineral density and content. The mechanical strain on bone produced by exercise loads is thought to increase bone mass (or maintain bone mass in postmenopausal or hypoestrogenic women) by reducing activation frequency of bone remodeling units and the force of generalized muscle contraction invokes an osteogenic stimulus in bone as a result of the pull of the muscle

tendons at the insertion site on a specific skeletal site. The muscle tendon pull is thought to stimulate the rate of osteoblast recruitment (bone formation) although the exact mechanisms are still unclear (Lanyon, 1992; Parfitt, 1994).

Two of the five INOC subjects had a history of competitive gymnastics prior to and following menarche. The other three subjects were competitive cross-country runners. A recent investigation by Helge & Kanstrup (2002) on bone mineral density in amenorrheic and oligomenorrheic adolescent (15-20 years) gymnasts found that in spite of menstrual irregularities, artistic and rhythmic gymnasts demonstrated 25-30% higher bone mineral density of the lumbar spine, femoral neck and trochanter when compared to moderately active controls (low impact activity less than 4 hours per week). These data support that gymnastics involvement may have protected bone mineral density in the two gymnasts within the INOC group and resulted in higher mean bone density and content values of the INOC group.

Robinson et al (1995) demonstrated that bone mineral density values of the lumbar spine and femur in gymnasts were 30-35% greater than runners, despite similar prevalence of menstrual irregularities. Within the INOC group, gymnasts exhibited lumbar and proximal femur density values that were 30% and 35% higher, respectively, than those of the runners in the INOC group. Running has been shown to have both a protective effect and a detrimental effect on bone measures in irregularly menstruating athletes, depending on the length of amenorrhea/oligomenorrhea and associated declination in sex steroid levels, as well as on the bone site (Gremion et al., 2001; Pearce et al., 1996).

Body weight has been shown to be lower in irregularly menstruating athletes compared to regularly menstruating controls (Snow-Harter, 1994). Low body weight and body fat have been suggested to mediate the occurrence of menstrual irregularities in athletes (Frisch et al., 1993). Body weight, BMI and sum of five skinfolds in the INOC group, however, were within normal ranges for their age as compared to established ratings for Canadian females of similar age (Canadian Physical Activity and Lifestyle Appraisal, Health Canada, 1996; Martin & Ward, 1996) and were not significantly different than the mean body weight of the ENOC group. This suggests that body weight in the INOC group may not have mediated changes in menstruation with vigorous

exercise and also that the load on the skeleton provided by a similar body weight as the ENOC group may have had a protective effect on bone mineral in this group (Parfitt, 1994). This is partially supported by research that has demonstrated a greater rate of bone mineral loss at the hip and spine in premenopausal (40-55 years of age) women as a result of simple weight loss (Salamone et al., 1999).

The contribution of genetics to bone mineral accrual and density during adolescence may be another reason why the INOC group did not have significantly lower bone measures compared to the ENOC group. Genetics have been suggested to account for as much as 60-80% of the variability in bone density while environmental factors, such as menstrual irregularities associated with vigorous exercise and decreased caloric intake, are suspected to account for only 20-40% (Slemenda et al., 1991a). Considering that 90% of skeletal mass is accumulated before 20 years of age (Faulkner et al., 1996), genetics play a substantial role in bone mineral accrual during puberty and adolescence. Matkovic et al. (1990) demonstrated that by 14 years, bone size, mass and density in adolescent females approached the corresponding values of their mothers. By 16 years of age the girls had accumulated 97% of the bone mass of their premenopausal mothers. More recently, evidence has been found for a major gene of codominant inheritance for spinal bone mineral density in idiopathic osteoporotic families. It is suggested that this gene exerts its effects during bone mineral accrual rather than subsequent to attainment of peak bone mass (Cardon et al., 2000). Thus, the influence of genetic factors on bone mineral density in the INOC athletes may have been more profound than the influence of menstrual status, resulting in sufficient bone mineral accrual when compared to normative values for this age (Faulkner et al, 1996).

Finally, the trends observed when comparing ENOC and INOC groups suggest that significant differences in aBMD and BMC as a result of menstrual status might be observed with a larger sample size in the INOC group. INOC subjects consistently exhibited lower mean aBMD and BMC at all sites. The INOC group experienced a later age of menarche, exhibited decreased caloric and calcium intakes and more of a tendency toward eating disorders as measured by the EAT-26, although these differences did not achieve significance. At the same time, exercise amounts were similar suggesting similar energy output in ENOC and INOC groups. All of these factors have been implicated as

contributing to lower bone mineral density values in both adolescents and young adults and their contributions to bone measures may be masked by a small sample size in this group (Dhuper et al., 1990; Warren & Stiehl, 1999; Keen & Drinkwater; 1997; Snow-Harter, 1994; Barr et al., 2001).

Oral Contraceptive Status (ENOC and EOC) and Bone Mineral Measures

Oral contraceptive status in eumenorrheic adolescent athletes did not have a significant effect on any of the aBMD measures (lumbar spine and proximal femur) or on BMC of the lumbar spine when compared to the ENOC group. Oral contraceptive use in eumenorrheic adolescent athletes, however, did have a significant effect on BMC of the proximal femur and its trochanteric subregion when compared to eumenorrheic non-users. These similarities and differences remained after controlling for height and weight.

Bone Mineral Density

Results of studies evaluating the association between oral contraceptive use and bone mineral density have been equivocal. Oral contraceptive use has been associated with a positive (Hergenroeder et al., 1997; Recker et al 1992; Grainge et al., 2001), neutral (Gremion et al, 2001; Mazess & Barden, 1991) and negative effect on bone mineral density (Prior et al., 2001; Burr et al., 2000; Hartard et al., 1997) at various skeletal sites. Many of these studies used cross sectional and retrospective research designs, involving pre and postmenopausal women. Few studies have included an adolescent population, with those that have being primarily concerned with the effects of oral contraceptive use in young females experiencing a hypoestrogenic state associated with anorexia nervosa and menstrual irregularities (Klibanski, Biller, Schoenfeld, Herzog & Saxe, 1995; Seeman et al., 1992). Few investigations have considered oral contraceptive use and bone mineral density in eumenorrheic adolescents.

A recent longitudinal study by Lloyd et al. (2000) investigated the effects of oral contraceptive use on bone mineral in 62 eumenorrheic adolescents over an eight-year time span during 12 to 20 years of age. These researchers found that oral contraceptive use did not affect the acquisition of peak bone mass as measured by total body bone mineral density and dedicated hip bone mineral density at 20 years of age. Their results

support the bone mineral density findings of the present study. Lloyd et al. (2000) did not measure sex steroid levels or markers of bone turnover and did not consider the different formulations of oral contraceptives that were used, although they were reported as all being low dose monophasics. Inclusion of these analyses may have helped to explain the findings of the present study. Since oral contraceptive use has been shown to suppress endogenous hormone production (Hartard et al., 1997), the ingestion of exogenous sex steroids may not result in higher serum levels of these hormones, and therefore, oral contraceptive use would not be expected to provide a greater impact on bone mineral density. Having not measured sex steroids in this present study, however, makes this argument speculative as applied to these findings.

In addition, oral contraceptive use has been observed to decrease biochemical markers of bone turnover in young women aged 18 to 39 years (Ott et al., 2001). These researchers demonstrated decreased levels of N-telopeptide to helix in urine, a marker of bone resorption, and lower levels of osteocalcin, a marker of bone formation, when compared to non oral contraceptive users. Bone mineral density at all anatomic sites (spine, femur, total body) was not different, however, between users and non-users of oral contraceptives (0.035 mg combined with progestins norethindrone or levonorgestinel). This suggests that although these markers of bone turnover were suppressed, the net balance of bone formation and resorption was similar between users and non-users of oral contraceptives and there was not a unique effect on bone mineral density. These data support the finding in this study that oral contraceptive users did not have significantly different bone mineral density values at the lumbar spine and proximal femur.

Bone Mineral Content

The significantly higher bone mineral content at the proximal femur in the EOC group, accompanied by higher, yet not statistically significant areal bone mineral density at this site, is more difficult to explain. With the exception of those studies that monitored growth in prepubertal and pubertal participants, there have been few investigations that have reported bone mineral content values along with density. A significantly higher bone mineral content at the proximal femur in the EOC group may reflect exogenous sex steroid mediated bone mineral accrual, differences in mechanical

loading patterns between the groups, a difference in bone dimension that is not accounted for with DXA, or it may demonstrate differences in social and lifestyle factors during growth that this study does not consider such as activity based hobbies or a variety of dietary practices (i.e. macrobiotic vegan diet).

Although almost 90% of peak bone mass in females is thought to be accumulated before the age of 20 years (Faulkner et al., 1996), increases in bone mineral density and content have been observed beyond 20 years of age (Teegarden et al., 1995; Recker et al., 1992). Increases in bone mineral content during growth predominantly reflect increases in the dimension of bone (Katzman et al., 1991). From 7-17 years of age femur and lumbar spine bone mineral content increase by 50-150% while volumetric bone mineral density (g/cm^3) increases by only 10-30% (Bass et al., 1999). It may be therefore, that increases in bone density associated with oral contraceptive use will be seen only after longitudinal growth has ceased and bone mineral accrual (positive remodeling) without concomitant increases in bone size has proceeded for some, as yet to be determined, time period. Increases in bone mineral content under such conditions would affect bone mineral density values more so than during a period of longitudinal growth (Katzman et al., 1991; Bass et al., 1999). The significant increase in proximal femur bone mineral content in the EOC group might reflect increased bone mineral accrual associated with oral contraceptive use and the slightly higher mean bone density (non significant) observed in this group might have been measurably different from the ENOC group had there been a longer period of positive remodeling at the consolidation phase of bone growth (Turner et al., 1994). Longitudinal monitoring of aBMD and BMC is essential to test such a prospect. Retrospective research has found oral contraceptive use prior to the age of 23 years to be significantly associated with increased bone mineral density at all sites except the total radius when measured in women 45-61 years of age (Grainge et al., 2001). This supports the concept that a longer period of bone remodeling may be necessary to observe significant increases in density associated with oral contraceptive use in the EOC group.

The concept that oral contraceptives could positively affect bone mineral accrual is supported by research on the effects of lowered sex steroids in postmenopausal women. Decreased sex steroids have been demonstrated to suppress osteoblastogenesis, increase

osteoclastogenesis, decrease the lifespan of osteoblasts and increase the lifespan of osteoclasts (Manolagas, 2000). Introduction of exogenous sex steroids by using oral contraceptives, therefore, may have the opposite effect on bone cells and result in increases in bone mineral accretion, as was observed in the proximal femur of the EOC group. Inclusion of sex steroid measures in the present research design would have provided valuable information regarding the mechanism of increased bone mineral content. The lack of such data limits the interpretation of the present findings.

It has been demonstrated that bone mass at the proximal femur reflects loading patterns, such as weight bearing, vigorous exercise. Conversely, bone mass at the lumbar spine represents the influence of endocrine factors (Parfitt, 1994). This may be due to the higher trabecular component of the lumbar vertebrae, which is more metabolically active (Eriksen, 1992). Consequently, it would be expected that differences between the EOC and ENOC groups would be seen in lumbar spine bone mineral content and not at the proximal femur. Although the ENOC and EOC groups were similar in the frequency and duration of weight bearing physical activity and resistance training, they were involved in many different types exercises that were not fully accounted for in this study. Hiking, step aerobics, highland dance, gymnastics, field hockey, volleyball, track and field, gymnastics are examples of the variety of weight bearing activities in which subjects participated. As was described previously, different types of exercise have been shown to produce varying mechanical loads on bone creating varying levels of osteogenic response (Chillibeck, Sale & Webber, 1995). Unilateral loading studies suggest that the osteogenic response is site specific. Young adult female competitive squash players had higher bone mineral density and content in the proximal humerus and ulnar shaft of their playing arm than their non playing arm (Haapasalo et al., 1998). This provides support that the significantly greater bone mineral content at the proximal femur in the EOC group compared to the ENOC group reflects variation between the groups with respect to exercise type. Differences in mechanical loading patterns and associated strain rate and magnitude at the proximal femur might have initiated an osteogenic adaptive response that altered the recruitment of osteoblasts and lead to a higher bone mineral content (Parfitt, 1994).

The observation that bone mineral content in the EOC group is significantly greater than the ENOC group without a concomitant difference in areal bone mineral density may not reflect a physiological difference but a limitation of the densitometer technique. DXA measures bone mineral area on two dimensions (width and length) and does not take into account antero-posterior depth or the thickness of the bone. Consequently, areal bone mineral density does not present a true volumetric density, which can only be measured with computed tomography (Compston, 1995). This can make comparison of areal bone mineral density of bones of different thickness difficult and may lead to overestimating density in large or, more specifically, thicker bones, and underestimating density in thinner bones (Kroger et al., 1992). The dependency of areal bone mineral density values on bone size is seen most dramatically in growing children where increases in bone mineral content reflect increases in bone size as growth builds a bigger but only moderately denser skeleton (Bass et al., 1999). Thus, if the EOC group had thicker bones than the ENOC group, this would be reflected in a higher bone mineral content if the two-dimensional measure of bone mineral area was similar between the groups, as observed in the present study. Correcting for antero-posterior bone thickness might reveal that the higher content in the EOC group is a result of a thicker bone, and is not necessarily a denser bone (Compston, 1995).

Conclusions and Implications for Future Research

Based on the results of this study, menstrual status and oral contraceptive status between the ages of 15 and 20 years do not affect aBMD measured at the lumbar spine and proximal femur in late adolescence. Oral contraceptive use, however, may confer benefits to BMC at the proximal femur. The trend indicated that oral contraceptive users have a slightly higher bone mineral density and content than non-users, and that regularly menstruating athletic adolescents have a higher bone mineral density and content than irregularly menstruating athletic adolescents. These trends may reflect real physiological differences between menstrual and oral contraceptive status groups that did not reach statistical significance in this study due to small samples sizes, and have implications that should be addressed.

From a health standpoint, it is encouraging that oral contraceptive use affected bone mineral measures positively relative to non users, but subjects would have to be followed longitudinally to confirm this finding and whether or not oral contraceptive use by adolescents affects attainment of higher peak bone mass than non users. It is also encouraging that irregular menstruation in the INOC group did not result in significantly lower bone measures and possible benefits conferred by weight bearing physical activity to the skeleton merit further consideration, specifically in this age group. Though sample sizes were small, the results of this study contribute to the body of knowledge in this area, which at this point is limited, especially in the area of oral contraceptive use and bone mineral in adolescents. From a clinical perspective, the results of this study provide further information about normative values for bone mineral in adolescents under different hormonal influences. From an educational perspective, the participants (and parents of participants) in this study expressed interest in and learned about factors that could influence their bone density. Education may be essential in helping young females to maximize bone mineral accrual during critical growing years. A curriculum package could be developed for health and physical education teachers including a theoretical component to learn about the factors affecting bone mass.

The results of this study suggest the following future research considerations:

1. A limitation of this study was its small sample size which may have accounted for unexpected findings. Future research in the area of bone health in athletic adolescents under different hormonal influences should employ a large number of both regularly menstruating and irregularly menstruating participants and/or a larger number of oral contraceptive and non oral contraceptive users.

2. Few data are available on bone mineral in healthy adolescent oral contraceptive users. A longitudinal design that takes into account different exercise modes, dietary intakes and serum hormone levels and follows participants until adult peak bone mass is achieved is necessary.

3. This study did not answer whether oral contraceptive use confers skeletal benefits to irregularly menstruating athletic adolescents. A prospective intervention study that places irregularly menstruating athletic females on oral contraceptives as a form of low dose hormone therapy is needed. In addition, it could be that irregularly menstruating adolescent athletes who begin taking oral contraceptives may start to menstruate regularly. If this is the case, the time course of this in association with losses/gains in bone mineral needs to be addressed. This could provide an explanation for why it was difficult to find amenorrheic/oligomenorrheic oral contraceptive users for this study.

4. A randomized sample to establish the actual prevalence of secondary amenorrhea in athletic adolescent females and associated bone mineral density and content would be valuable from a clinical, educational and health perspective. Another dimension of this question would be to distinguish cultural differences in the prevalence of menstrual irregularities as a part of the female athlete triad.

References

Adams, J.E. (1995). Quantitative measurements in osteoporosis. In F.I. Tovey & T.C. Stamp (Eds.), ***The measurement of metabolic bone disease*** (pp.107-142). New York: Parthenon.

Baer, J.T., Taper, L.J., Gwazdauskas, F.G., Walberg, J.L., Novascone, M., & Ritchy, S.J. (1992). Diet, hormonal and metabolic factors affecting bone mineral density in adolescent amenorrheic and eumenorrheic female runners. ***Journal of Sports Medicine and Physical Fitness, 32,*** 51-58.

Bailey, D.A., Martin, A.D., McKay, H.A., Whiting, S., & Mirwald, R. (2000). Calcium accretion in girls and boys during puberty: A longitudinal analysis. ***Journal of Bone and Mineral Research, 15***(11), 2245-2250.

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

Barr, S.I., Petit, M.A., Vigna, Y.M., & Prior, J.C. (2001). Eating attitudes and habitual calcium intake in peripubertal girls are associated with initial bone mineral content and its change over two years. ***Journal of Bone and Mineral Research, 16***(5), 940-947.

Bass, S., Delmas, P.D., Pearce, G., Hendrich, E., Tabensky, A., & Seeman, E. (1999). The differing tempo of growth in bone size, mass, and density in girls is region-specific. ***The Journal of Clinical Investigation, 104***(6), 795-804.

Biller, B., Coughlin, J., Saxe, V., Schoenfeld, D., Spratt, D., & Klibanski, A. (1991). Osteopenia in women with hypothalamic amenorrhea: A prospective study. ***Obstetrics and Gynecology, 78***, 996-1001.

Bonjour, J.P., Theintz, G., Buchs, B., Slosman, D., & Rizzoli, R. (1991). Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. ***Journal of Clinical Endocrinology and Metabolism, 73(3),*** 555-563.

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), 855-863.

Canadian Society for Exercise Physiology (1996). ***The Canadian Physical Activity, Fitness and Lifestyle Appraisal – CSEP's Plan for healthy active living.*** Author.

Canadian Society for Exercise Physiology (1995). ***Certified Fitness Appraiser: Resource Manual.*** Author.

Cann, C., Martin, M., Genant, H., & Jaffe, R. (1984). Decreased spinal mineral content in amenorrheic women. ***Journal of the American Medical Association, 251***(5), 626-629.

Cardon, L.R., Garner, C., Bennett, S.T., MacKay, I.J., Edwards, R.M., Cornish, J., Hedge, M., Murray, M.A.F., Reid, I.R., & Cundy, T. (2000). Evidence for a major gene for bone mineral density in idiopathic osteoporotic families. ***Journal of Bone and Mineral Research, 15***(6), 1132-1137.

Caufriez, A. (1991). Menstrual disorders in adolescence: Pathophysiology and treatment. ***Hormone Research, 361,*** 156-159.

Chen, E., & Brzyski, R. (1999). Exercise and reproductive function. ***Fertility and Sterility, 71***(1), 1-6.

Chillibeck, P.D., Sale, D.G., & Webber, C.E. (1995). Exercise and bone mineral density. ***Sports Medicine, 19,*** 103-122.

Compston, J.E. (1995). Bone density: BMC, BMD, or corrected BMD? ***Bone, 16***(1), 5-7.

Compston, J.E. (1992). HRT and osteoporosis. ***British Medical Bulletin, 48***(2), 309-344.

Constantini, N.W. (1994). Clinical responses of athletic amenorrhoea. ***Sports Medicine, 17***(4), 213-223.

Crawford, S.M. (1996). Anthropometry. In D. Docherty (Ed.), ***Measurement in pediatric exercise science*** (pp.17-86). Champaign, IL: Human Kinetics.

Creatsas, G., Salakos, N., Averkiou, M., Miras, K., & Aravantinos, D. (1992). Endocrinological profile of oligomenorrheic strenuously exercising adolescents. ***International Journal of Gynecology and Obstetrics, 38***, 215-221.

Cromer, B.A., Blair, J., Mahan, J.D., Zi, L., & Naumovski, Z. (1996). A prospective comparison of bone density in adolescent girls receiving depot medroxyprogesterone acetate (Depo-Provera), levonorgestrel (Norplant), or oral contraceptives. ***Journal of Pediatrics, 129,*** 671-676.

Cumming, D., & Rebar, R. (1985). Hormonal changes with acute exercise and with training in women. ***Seminars in Reproductive Endocrinology, 3***(1), 55-64.

Dhuper, S., Warren, M.P., Brooks-Gunn, J., & Fox, R. (1990). Effects of hormonal status on bone density in adolescent girls. ***Journal of Clinical Endocrinology and Metabolism, 71***(5), 1083-1088.

Ding, J-H., Sheckter, C., Drinkwater, B., Soules, M., & Bremner, W. (1988). High serum cortisol levels in exercise-associated amenorrhea. ***Annals of Internal Medicine, 108***(4), 530-534.

Eriksen, E. (1992). ***Osteoporosis: Pathogenesis and treatment therapy manual.*** Denmark: Novonordisk.

Esha Research (2001). ***Professional nutrition anlysis software databases: The food processor (version 7.7)***. Salem, OR: Esha Research.

Faulkner, R.A., Bailey, D.A., Drinkwater, D.T., McKay, H.A., Arnold, C. & Wilkinson, A.A. (1996). Bone densitometry in Canadian children 8-17 years of age. ***Calcified Tissue International, 59,*** 344-351.

Frisch, R.E., Gotz-Welbergen, A.V., McArthur, J.W. et al. (1981). Delayed menarche and amenorrhea of college athletes in relation to age of onset of training. ***Journal of the American Medical Association, 246,*** 1559-1563.

Frisch, R.E., Snow, R., Johnson, L., Gerard, B., Barbieri, R., & Rosen, B. (1993). Magnetic resonance imaging of overall and regional body fat, estrogen metabolism, and ovulation of athletes compared to controls. ***Journal of Clinical Endocrinology and Metabolism, 77,*** 471-477.

Garner, D.M., & Garfinkel, P.E. (1979). The eating attitudes test: An index of the symptoms of anorexia nervosa. ***Psychological Medicine, 9,*** 273-279.

Garner, D.M., Olmsted, M.P., Bohr, Y., & Garfinkel, P.E. (1982). The Eating Attitudes Test: Psychometric features and clinical correlates. ***Psychological Medicine, 12,*** 871-878.

Garner, D.M., & Garfinkel, P.E. (Eds.). (1997). ***Handbook of treatment for eating disorders*** (2nd ed.). New York: Guilford Press.

Gilsanz, V., Gibbens, D., Roe, T., Carlson, M., Senac, M., Boechat, M., Huang, H., Schulz, E., Libanati, C., & Cann, C. (1988). Vertebral bone density in children: Effect of puberty. ***Radiology, 166,*** 847-850.

Grainge, M.J., Coupland, C.A.C., Cliffe, S.J., Chilvers, C.E.D., & Hosking, D.J. (2001). Reproductive, menstrual and menopausal factors: Which are associated with bone

mineral density in early postmenopausal women? ***Osteoporosis International, 12,*** 777-787.

Gremion, G., Rizzoli, R., Slosman, D., Theintz, G., & Bonjour, J. (2001). Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. ***Medicine and Science in Sports and Exercise, 33***(1), 15-21.

Haapasalo, H., Kannus, P., Sievanen, H., Pasanen, M., Uusi-Rasi, K., Heinonen, A., Oja, P., & Vuori, I. (1998). Effect of long term unilateral activity on bone mineral density of female junior tennis players. ***Journal of Bone and Mineral Research, 13,*** 310-319.

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,*** 87-90.

Heaney, R.P. (1996). Pathogenesis of post-menopausal osteoporosis. In M.J. Favus (ed.), ***Primer on the metabolic bone diseases and disorders of mineral metabolism*** (3rd ed., pp.252-254). Philadelphia: Lippincott-Raven.

Helge, E.W., & Kanstrup, I.-L. (2002). Bone density in female elite gymnasts: Impact of muscle strength and sex hormones. ***Medicine and Science in Sports and Exercise, 34,*** 174-180.

Hergenroeder, A.C. (1995). Bone mineralization, hypothalamic amenorrhea, and sex steroid therapy in female adolescents and young adults. ***The Journal of Pediatrics, 126(5),*** 683-689.

Hergenroeder, A.C., Smith, E., Shypailo, R., Jones, L., Klish, W., & Ellis, K. (1997). Bone mineral changes in young women with hypothalamic amenorrhea treated with oral contraceptives, medroxyprogesterone, or placebo over 12 months. ***American Journal of Obstetrics and Gynecology, 176***(5), 1017-1025.

Hologic QDR 2000 (1994). ***Supine lateral bone densitometer operator's manual and user's guide.*** Waltham, MA: Hologic Inc.

Jonnavithula, S., Warren, M., Fox, R.P., & Lazaro, M.I. (1993). Bone density is compromised in amenorrheic women despite return of menses: A two year study. ***Obstetrics and Gynecology, 81***(5), 669-674.

Kalender, W. (1992). Effective dose values in bone mineral measurements by photon absorptiometry and computed tomography. ***Osteoporosis International, 2,*** 82-87.

Katzman, D.K., Bachrach, L.K., & Carter, D.R. (1991). Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. ***Journal of Clinical Endocrinology and Metabolism, 72(6),*** 1332-1339.

Keen, A., & Drinkwater, B. (1997). Irreversible bone loss in former amenorrheic athletes. ***Osteoporosis International, 7,*** 311-315.

Keizer, H., & Rogol, A. (1990). Physical exercise and menstrual cycle alterations. ***Sports Medicine, 10***(4), 218-235.

Kellie, S. (1992). Measurement of bone density with dual energy x-ray absorptiometry (DEXA). ***Journal of the American Medical Association, 267***, 286-294.

Kleerekoper, M., Brienza, R., Schultz, L., & Johnson, C. (1991). Oral contraceptive use may protect against low bone mass. ***Archives of Internal Medicine, 151,*** 1971-1976.

Klibanski, A., Biller, B.M.K., Schoenfeld, D.A., Herzog, D.B., & Saxe, V.C. (1995). The effects of estrogen administration on trabecular bone loss in young women with anorexia nervosa. ***Journal of Clinical Endocrinology and Metabolism, 80,*** 898-904.

Kritz-Silverstein, D., & Barrett-Connor, E. (1993). Bone mineral density in postmenopausal women as determined by prior oral contraceptive use. ***American Journal of Public Health, 83,*** 100-102.

Kroger, H., Kotaniemi, A., Vainio, P., & Alhava, E. (1992). Bone densitometry of the spine and femur in children by dual-energy x-ray absorptiometry. ***Bone and Mineral, 17***, 75-85.

Lanyon, L.E. (1992). Control of bone architecture by functional load bearing. ***Journal of Bone and Mineral Research, 7***(Suppl.2), S369-S375.

Lindsay, R., Tohme, J., & Kanders, B. (1986). The effect of oral contraceptive use on vertebral bone mass in pre and post menopausal women. ***Contraception, 34***(4), 333-340.

Lloyd, T., Meyers, C., Buchanan, J.R., & Demers, L.M. (1988). Collegiate women athletes with irregular menses during adolescence have decreased bone density. ***Obstetrics and Gynecology, 72***(4), 639-642.

Lloyd, T., Rollings, N., Andon, M., Demers, L., Eggli, D., Kieselhorst, K., Kulin, H., Landis, J., Martel, J., Orr, G., & Smith, P. (1992). Determinants of bone density in young women. ***Journal of Clinical Endocrinology and Metabolism, 75***(2), 383-387.

Lloyd, T., Taylor, D.S., Lin, H.M., Matthews, A.E., Eggli, D.F., & Legro, R.S. (2000). Oral contraceptive use by teenage women does not affect peak bone mass: A longitudinal study. ***Fertility and Sterility, 74***(4), 734-738.

Loucks, A.B., Mortola, J., Girton, L., & Yen, S. (1989). Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axes in athletic women. ***Journal of Clinical Endocrinology and Metabolism, 68,*** 402-411.

Malina, R. (1983). Menarche in athletes: A synthesis and hypothesis. ***Annals of Human Biology, 10***(1), 1-24.

Manolagas, S.C. (2000). Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. ***Endocrine Reviews, 21***(2), 115-137.

Martin, A.D., Silverthorn, M.D., Houston, C.S., Bernhardson, S., Wajda, A., & Roos, L.L. (1991). The incidence of fracture of the proximal femur in two million Canadians from 1972-1984. ***Clinical Orthopaedics, 266,*** 111-118.

Martin, A.D., & Ward, R. (1996). Body Composition. In D. Docherty (Ed.), ***Measurement in pediatric exercise science*** (pp.87-128). Champaign, IL: Human Kinetics.

Matkovic, V., Fontana, D., Tominac, C., Goel, P., & Chesnut, C.H. (1990). Factors that influence peak bone mass formation: A study of calcium balance and the inheritance of bone mass in adolescent females. ***American Journal of Clinical Nutrition, 52,*** 878-888.

Matkovic V., & Ilich, J. (1993). Calcium requirements for growth: Are current recommendations adequate? ***Nutrition Reviews, 51***(6), 171-180.

Mazess, R., & Barden, H. (1991). Bone density in premenopausal women: Effects of age, dietary intake, physical activity, smoking, and birth control pills. ***American Journal of Clinical Nutrition, 53,*** 132-142.

McKay, H., Bailey, D., Mirwald, R., Davison, K.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***, 682-687.

Micklesfield, L., Lambert, E., Fataar, A., Noakes, T., & Myburgh, K. (1995). Bone mineral density in mature, premenopausal ultramarathon runners. ***Medicine and Science in Sports and Exercise, 27***(5), 688-696.

Moen, S., Sanborn, C., Dimarco, N., Gench, B., Bonnick, S., Keizer, H., & Menheere, P. (1998). Lumbar bone mineral density in adolescent female runners. ***Journal of Sports Medicine and Physical Fitness, 38,*** 234-239.

Myburgh, K., Bachrach, B., Lewis, B., Kent, K., & Marcus, R. (1993). Low bone mineral density at axial and appendicular sites in amenorrheic athletes. ***Medicine and Science in Sports and Exercise, 25,*** 1197-2202.

Myburgh, K., Hutchins, J., Fataar, A., Hough, S., & Noakes, T. (1990). Low bone density is an etiologic factor for stress fractures in athletes. ***Annals of Internal Medicine, 113,*** 754-759.

Ott, S.M., Scholes, D., Lacroix, A.Z., Ichikawa, L.E., Yoshida, C.K., & Barlow, W.E. (2001). Effects of contraceptive use on bone biochemical markers in young women. ***Journal of Clinical Endocrinology and Metabolism, 86***(1), 179-185.

Parfitt, A.M. (1994). The two faces of growth: Benefits and risks to bone integrity. ***Osteoporosis International, 4,*** 382-398.

Pearce, G., Bass, S., Young, N., Formica, C., & Seeman, E. (1996). Does weight-bearing exercise protect against the effects of exercise induced oligomenorrhea on bone density? ***Osteoporosis International, 6,*** 448-452.

Polatti, F., Perotti, F., Filippa, N., Gallina, D., & Nappi, R.E. (1995). Bone mass and long-term monophasic oral contraceptive treatment in young women. ***Contraception, 51***, 221-224.

Prior, J.C., Kirkland, S.A., Joseph, L., Kreiger, N., Murray, T.M., Hanley, D.A., Adachi, J.D., Vigna, Y.M., Berger, C., Blondeau, L., Jackson, S.A., & Tenenhouse, A. (2001). Oral contraceptive use and bone mineral density in premenopausal women: Cross-sectional, population-based data from the Canadian multicentre osteoporosis study. ***Canadian Medical Association Journal, 165***(8), 1023-1029.

Recker, R., Davies, K., Hinders, S., Heaney, R., Stegman, M., & Kimmel, D. (1992). Bone gain in young adult women. ***Journal of the American Medical Association, 268***(17), 2403-2408.

Robinson, E., Bachrach, L.K., & Katzman, D.K. (2000). Use of hormone replacement therapy to reduce the risk of osteopenia in adolescent girls with anorexia nervosa. ***Journal of Adolescent Health, 26***(5), 343-348.

Robinson, T., Snow-Harter, C., Taaffe, D.R., Gillis, D., Shaw, J., & Marcus, R. (1995). Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea and oligomenorrhea. ***Journal of Bone and Mineral Research, 10,*** 26-35.

Salamone, L.M., Cauley, J.A., Black, D.M., Simkin-Silverman, L., Lang, W., Gregg, E., Palermo, L., Epstein, R.S., Kuller, L.H., & Wing, R. (1999). Effect of a lifestyle intervention on bone mineral density in premenopausal women: A randomized trial. ***American Journal of Clinical Nutrition, 70***, 97-103.

Sanborn, C., & Wagner, W. (1988). The female athlete and menstrual irregularity. In J. Puhl, C.H. Brown, & R. Voy (Eds.), ***Sport science perspectives for women*** (pp. 111-130). Champaign, IL: Human Kinetics.

Schoeller, D.A. (1990). How accurate is self–reported dietary energy intake? ***Nutrition Reviews, 48,*** 373-378.

Seeman, E., Szmukler, G., Formica, C., Tsalamandris, C., & Mestrovic, R. (1992). Osteoporosis in anorexia nervosa: The influence of peak bone density, bone loss, oral contraceptive use, and exercise. ***Journal of Bone and Mineral Research,*** *7*(12), 1467-1474.

Slemenda, C., Christian, J., Williams, C., Norton, J., & Johnston, C. (1991a). Genetic determinants of bone mass in adult women: A reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. ***Journal of Bone and Mineral Research, 6***(6), 561-567.

Slemenda, C., Miller, J., Hui, S., Reister, T., & Johnston, C. (1991b). Role of physical activity in the development of skeletal mass in children. ***Journal of Bone and Mineral Research, 6,*** 1227-33.

Snead, D.B., Weltman, A., Weltman, J.Y., Evans, W.S., Veldhuis, J.D., Varma, M.M., Teates, C.D., Dowling, E.A., & Rogol, A.D. (1992). Reproductive hormones and bone mineral density in women runners. ***Journal of Applied Physiology,*** *72*(6), 2149-2156.

Snow-Harter, C. (1994). Bone health and prevention of osteoporosis in active and athletic women. ***Clinics in Sports Medicine, 13***(2), 389-404.

SPSS Inc. (2001). ***SPSS user's guide.*** Chicago: SPSS Inc.

Szulc, P., Seeman, E., & Delmas, P.D. (2000). Biochemical measurements of bone turnover in children and adolescents. ***Osteoporosis International, 11***, 281-294.

Teegarden, D., Proulx, W.R., Martin, B.R., Zhao, J., McCabe, G.P., Lyle, R.M., Peacock, M., Slemenda, C., Johnston, C.C., & Weaver, C.M. (1995). Peak bone mass in women. ***Journal of Bone and Mineral Research, 10***(5), 711-715.

Theintz, G., Buchs, B., Rizzoli, R., Slosman, D., Clavien, H., Sizonenko, P., & Bonjour, J.-P.H. (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*(4),** 1060-1065.

Tuppurainen, M., Kroger, H., Saarikoski, S., Honkanen, R., & Alhava, E. (1994). The effect of previous oral contraceptive use on bone mineral density in perimenopausal women. ***Osteoporosis International, 4,*** 93-98.

Turner, R.T., Riggs, B.L., & Spelsberg, T.C. (1994). Skeletal effects of estrogen. ***Endocrine Reviews, 15***(3), 275-300.

Warren, M. & Stiehl, A. (1999). Exercise and female adolescents: Effects on the reproductive and skeletal systems. ***Journal of the American Medical Women's Association, 54,*** 115-120.

Wolman, R., Clark, P., McNally, E., Harries, M., & Reeve, J., (1990). Menstrual state and exercise as determinants of spinal trabecular bone density in female athletes. ***British Medical Journal, 301,*** 516-518.

Appendices

Appendix A: Review of Literature

Bone Health in Adolescent Athletes: Menstrual and Oral contraceptive Status

Section I: Introduction

The female reproductive system consists of a series of metabolic events that must occur at genetically determined intervals and thresholds in order for menstruation to repeat itself every 28-32 days. Reproductive hormones are synthesized, stored and released from the hypothalamus, the pituitary and the ovaries to act on target cells and receptors to produce a cascade of events (Yen, 1990). The hypothalamic-hypophyseal or hypothalamic-pituitary-ovarian (HPO) axis is the line of hormonal communication that is integral to a female's reproductive system. The HPO axis encompasses gonadotropin releasing hormone (GnRH), synthesized and released from the hypothalamus, follicle stimulating hormone (FSH) and luteinizing hormone (LH) released from the gonadotropin cells of the anterior pituitary, and the ovarian hormones, estrogen and progesterone. This hormonal communication system is complex as it involves positive and negative feedback loops, up-regulation and down regulation of receptors, and autocrine and paracrine control mechanisms.

As with many aspects of the human species that are fixed genetically by nature, however, the reproductive system is susceptible to change by nurture. In fact, the environment has been shown to affect the female reproductive system through stress, diet and exercise. These factors can contribute to a disruption along the HPO axis and lead to the pathogenesis of menstrual irregularities including a shortened luteal phase, anovulation and/or complete cessation of menstruation. While menstrual irregularities may seem benign, there are significant associated health implications to this condition. In a low sex steroid environment, bone modeling and remodeling process are negatively affected (Manolagas, 2000). Deficits in normal bone mineral accretion, stress fractures, failure to reach adult peak bone mass and future risk of osteopenia and osteoporosis are examples of health consequences that can result from irregular menstruation, especially during adolescence which is a crucial phase of bone mineral accrual (Warren & Stiehl, 1999; Hergenroeder, 1995; Snow-Harter, 1994).

Hormone replacement therapy (HRT) or oral contraceptive pills have been prescribed in perimenopausal and postmenopausal women as a treatment for decreased sex steroid hormone levels and associated risk for bone loss and osteoporosis in these

women (Kohrt, W., Ehsani, A. & Birge, J., 1998; Compston, 1992; Ettinger, 1988). A recent survey conducted by the University of Toronto's School of Medicine found that HRT is prescribed for adolescent patients with eating disorders in approximately 77.6% of cases (Robinson, Bachrach & Katzman, 2000). The efficacy of these hormonal treatments in adolescent females who exhibit menstrual irregularities reflecting a declination in sex steroid hormone levels and in association with vigorous exercise, psychological stress and low caloric, however, is equivocal. It is important to determine an appropriate treatment and intervention timeline in order to maximize bone mineral accretion during adolescence and prevent possible bone loss in amenorrheic or oligomenorrheic adolescent athletes.

Section II: Menstrual Irregularities in Athletic Females

1. Menstruation and Exercise

The "normal" eumenorrheic menstrual cycle is ovulatory and typically varies in length between 22 and 36 days. Average length of a cycle is approximately 28 days with the length of the follicular phase being more variable and the length of the luteal phase being more constant at 10-16 days (Vollman, 1977). When menstruation does not occur cyclically this is of clinical significance. Absence of menstruation is termed either amenorrhea or oligomenorrhea. Amenorrhea is classified further as primary, which refers to delayed menarche beyond sixteen years of age, or secondary. Secondary amenorrhea refers to the cessation of menstruation in females who have been previously menstrual and for reasons other than pregnancy (Fox, Bowers & Foss, 1993). Researchers, however, do not agree upon the length of time that menses must be absent before being characterized as secondary amenorrhea. Absence of menstruation in the three months immediately preceding the study (when pregnancy is ruled out) is one definition that has been used to describe amenorrhea (White, Hergenroeder & Klish, 1992). A more widely accepted definition describes amenorrhea as the occurrence of three periods or less in the last year and none within six months immediately prior to an investigation (Sanborn & Wagner, 1988; Moen et al., 1998; Warren et al., 1991; Hergenroeder et al., 1997).

Oligomenorrhea is also characterized by irregular menstrual cycles but periods occur more frequently with this condition. The definition of oligomenorrhea has also varied depending on the research group. Menstrual cycles occurring between 40 and 90 days (Creatsas, Salakos, Averkiou, Miras & Aravantinos, 1992); cycles occurring at a frequency between 6 weeks and 6 months (Pearce, Bass, Young, Formica, & Seeman, 1996); or 4 to 9 menstrual cycles per year (Gremion et al., 2001; Snow-Harter, 1994) defines this condition. Ten to 12 menstrual cycles per year, therefore, is considered within the range for normal menstruation or eumenorrhea (Gremion et al., 2001; Weaver et al., 2001).

Normality of the menstrual cycle is characterized not only by the time interval between menstruation and the length of menstruation, but also by hormone levels that rise and fall depending on the phase of the menstrual cycle (follicular and luteal). Aberrations of the menstrual cycle include: a shortened luteal phase, manifested by a shortened menstrual cycle length; an insufficient luteal phase which is ovulatory but involves deficient progesterone production leading to deficient corpus luteum; and anovulation wherein ovulation does not occur but withdrawal bleeding may be present in this condition as a result of low estrogen levels (Keizer & Rogol, 1990). A shortened and or insufficient luteal phase has been associated with decrements in bone mineral density possibly due to reduced secretion of progesterone (Petit, Prior & Barr, 1999). Irregularities in the menstrual cycle such as these are much more difficult to detect but may also have implications for bone health.

2. Prevalence and Etiology of Menstrual Irregularities

While the occurrence of amenorrhea may indicate disease states such as ovarian failure, a pituitary tumour, or Cushing's syndrome (Miller & Klibanski, 1999), amenorrhea or oligomenorrhea are often observed in apparently healthy athletes. Both amenorrhea and oligomenorrhea are associated with exercise frequency, intensity and type, as well as with low body fat levels, poor nutritional, hypothalamic immaturity and psychological stress (Warren & Stiehl, 1999). The multifactor etiology of amenorrhea and oligomenorrhea makes these conditions difficult to investigate. Bausenwein (1954) first observed the connection between exercise and menstrual irregularities in the 1950's

while comparing menstrual cycles of Olympic athletes to physical education students. 12.8 % of Olympic athletes, who were training to a greater extent than the education students, experienced menstrual irregularities. Following this, Erdelyi (1963) surveyed 557 Hungarian female athletes and observed "unfavorable" menstrual cycle changes in 62 subjects. Amenorrhea occurred more frequently in athletes who were participating in strenuous endurance activities such as tennis, rowing and cross-country skiing. These findings established early on that there might be a link between exercise and menstrual irregularities.

The development of amenorrhea and oligomenorrhea among some athletes and not others lead to the conclusion that intensity and type of training might be linked with menstrual cycle alterations (Keizer & Rogal, 1990). The prevalence of amenorrhea in athletes has been observed to be 24% in cross country runners (Feicht et al. (1978), 26% in marathon runners, and approximately 12% in cyclists and swimmers (Sanborn, Martin & Wagner, 1982). Incidence of menstrual irregularities in athletes has been observed to range between 2-45%, depending on the sport, compared to 2-5% in the sedentary population which favours a training induced etiology of menstrual irregularity (Keizer & Rogol, 1990). Sports involving low body weight and fat for esthetic and performance purposes (gymnastics, long distance running, figure skating, ballet) and are endurance based tend to have higher incidences of menstrual irregularities (Warren & Stiehl, 1999; Calabrese, Kirkendall, Floyd, et al., 1983; Sanborn, Martin & Wagner, 1982).

In contrast to the adult athletic population, adolescent females commonly experience irregular and anovulatory menstrual cycles during the first postmenarcheal years independent of exercise involvement. It has been reported that amenorrhea, oligomenorrhea, irregular cycles, abnormal uterine bleeding and dysmenorrhea represent 50% of their gynecologic complaints, regardless of exercise involvement (Caufriez, 1991). It has been observed that adolescent girls who begin intense exercise before the onset of menarche may experience delayed onset of menarche when compared to sedentary controls (Malina, 1983). Introduction of intense exercise at a fragile time in the development of the immature hypothalamic-pituitary-ovarian axis, therefore, may increase the incidence and duration of menstrual irregularities (Stager & Hatler, 1988).

a. 'Critical Fat' theory and menstrual irregularities

It has been proposed that a direct relationship exists between a critical amount of body fat and the onset and maintenance of menstruation. This 'critical fat' hypothesis, proposed by Frisch (1987), suggests that a minimum fat to lean mass ratio of 17% fat/body weight is necessary for menarche and maintenance of female reproductive ability requires 22% fat/body weight. Four mechanisms by which the level of body fatness may influence the menstrual cycle, ovulation, and, hence, fertility, have been proposed (Frisch, 1987): (1) Adipose tissue is a significant extragonadal source of estrogen as the conversion of androgens to estrogens takes place in adipose tissue in the breast, abdomen, and the fatty marrow of the long bones; (2) Body fat influences the direction of estrogen metabolism to more or less potent forms. For instance, leaner women make more catechol estrogens, which are the less potent form of estrogens. (3) Obese women and young girls have diminished capacity for estrogen to bind to serum sex-hormone binding globulin (SHBG), which results in an elevated percentage of free serum estradiol. Since SHBG regulates the availability of estradiol to the brain and other target tissues, changes in the proportion of body fat may influence reproductive function through the immediate effects of SHBG (Sitteri, 1981, in Frisch, 1987); (4) The female body may regulate estrogens and reproductive function by utilizing steroid hormones like estrogen, which is stored in adipose tissue; (5) Changes in body fatness may have an indirect effect on reproductive function through disturbances in regulation of body temperature and energy balance by the hypothalamus (Frisch, 1987).

The following observations have been proposed as evidence which supports this hypothesis: menarche appears to occur at a critical weight of about 48kg rather than a specific age; amenorrhea is a common marker for women with anorexia nervosa and it has also been observed in women with 'simple' weight loss (Falk & Halmi, 1982); secondary amenorrhea occurs in women when weight loss is in the range of 10-15% of normal weight for height, which is equivalent to a loss of about one-third of body fat (Frisch & McAurthur, 1974); refeeding of amenorrheic anorexic patients has resulted in the reversal of amenorrhea; and, finally, weight and body fat gains as well as decreased training in athletes experiencing amenorrhea predicates a reversal in reproductive irregularities (McAurthur et al., 1976; Keen & Drinkwater, 1997). The critical fat theory

has been discounted by some on the basis that Frisch relied on a regression equation to estimate the proportion of body weight composed of fat from individual data on height and weight (Trussell, 1980). This estimate has been shown to be especially inaccurate when estimating body fat of athletes and lean women (Loucks et al., 1984).

More recently, however, Frisch et al. (1993) used magnetic resonance imaging to measure subcutaneous fat (SF) and internal visceral fat (IF) directly in women athletes compared to non-athletic controls. Through this direct method of analysis it was found that body fat of female athletes was significantly less than those of the controls, even though their weights were significantly heavier than those of the controls. It was also found that athletes with menstrual cycle alterations had significantly decreased SF and IF overall and at all regional sites compared to controls. It was concluded that changes in regional fat deposits of both SF and IF may be involved in the menstrual cycle alterations of the athletes in addition to decreased overall fatness.

Currently researchers are investigating the association between leptin, a protein coded to the 'ob' gene that is expressed in adipocytes, and menstrual dysfunction (Conway & Jacobs, 1997). Leptin regulates eating behaviour through central neuroendocrine mechanisms and is an integral component of hypothalamic-pituitary-endocrine feedback loops. Leptin has been suggested as the missing link between fat and fertility as it plays a key role in sensing the body's fat status and energy balance. Leptin exhibits a positive correlation with weight, body fat and insulin-like growth factor-1 levels in amenorrheic anorectics (Grinspoon et al., 1996). Just as leptin levels are lower in subjects with low weight and body fat, leptin levels have been shown to increase with increases in body weight. The mechanism whereby leptin exerts is effect on the reproductive system has not been fully elucidated but possible mechanisms are that it acts on the gonadotropin releasing hormone (GnRH) neuron or that it acts directly on the ovary (Conway & Jacobs, 1997).

b. Exercise-associated menstrual irregularities

Although it is difficult to single out intense exercise training as the primary factor leading to amenorrhea, there are a couple of notable studies, which discount weight loss and low body fat as a contributor to the condition. Wakat et al. (1982) studied height,

weight, sum of five skinfolds and somatotype in 38 female national cross-country runners. Half of the participants were experiencing a form of menstrual irregularity. There were no significant anthropomentric differences between those experiencing menstrual irregularities and regularly menstruating athletes. McAurthur et al. (1980) investigated body fat in three amenorrheic athletes and five eumenorrheic sedentary controls for whom organic disease had been excluded. Estimated body fat in amenorrheics was 25.9 % compared to 25.5 % in eumenorrheics. The fact that there was little discrepancy between the body compositions of the amenorrheic athletes and the eumenorrheic controls in both of these investigations suggests that factors other than or in conjunction with body fat or weight, such as training intensity, duration, and type might mediate alterations in menstruation.

c. Exercise, critical fat and reproductive function

Athletes who engage in increased frequency, intensity and duration of training and do not match energy expenditure with adequate caloric intake are likely to demonstrate low body fat levels. Boyden, Pamenter, Stanforth, Rotkis, & Wilmore, (1983), provided evidence for this dual contribution to menstrual irregularities when they studied the effects of a 13.5 month training program on 19 regularly menstruating women. Body composition was examined at baseline, after weekly mileage increased to 30 miles above baseline and again after increases 50 miles above baseline. It was found that although total body weight did not change, subjects became leaner with mean relative body fat decreasing from 25% at baseline to 22% after an increase in weekly mileage to 50 miles above baseline. Further, it was found that all but one woman experienced menstrual alterations of some kind over the course of the study.

Bullen et al. (1985) demonstrated similar results when they evaluated the effects of a 2-month strenuous exercise program initially untrained college women with documented luteal and ovulation adequacy. Subjects were grouped by weight loss versus weight maintenance programs. A higher percentage of menstrual alterations were detectable by hormonal measures and it was found that delayed menses and loss of the luteinizing hormone surge occurred more frequently in the weight loss group than in the

weight maintenance group. It was concluded that vigorous exercise, particularly if compounded by weight loss, could disturb reproductive function in women.

Amenorrheic and oligomenorrheic females commonly exhibit lower body weight and fat, a negative energy balance, and a greater training load in terms of frequency and duration when compared to eumenorrheic athletes (Kanaley et al., 1991; Baer, 1993; Ding et al., 1988; Myerson et al., 1991). Other factors that have been associated with the development of menstrual irregularities include chronological age, age at menarche, training prior to menarche, prior menstrual irregularities, psychological stress, hormonal imbalance and specific nutrient ingestion, or lack thereof, in the diet (Snow-Harter, 1994; Bonen, 1994; Sanborn & Wagner, 1988).

3. Hormone Profiles of Irregularly Menstruating Athletes

Both primary and secondary amenorrhea in chronically exercising athletes are accompanied by depressed resting levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) when compared to chronically exercising eumenorrheic controls. Eumenorrheic chronically exercising athletes exhibit lower resting levels of these hormones than eumenorrheic, sedentary controls (Cumming & Rebar, 1985; Dale, Gerlach & Wilhite, 1979; Loucks, Mortola, Girton & Yen, 1989). Oligomenorrheic strenuously exercising adolescents exhibit significantly lower resting levels of serum LH than their eumenorrheic controls ($p<0.001$) (Creatsas et al., 1992).

In addition, amenorrheic athletes typically exhibit significantly lower resting and exercise levels of estradiol and progesterone when compared to eumenorrheic controls. When eumenorrheic athletes are compared to eumenorrheic sedentary controls, these athletes show lower levels of estrogen and progesterone (Loucks et al., 1989; Ding, Sheckter, Drinkwater, Soules & Bremner, 1988). Oligomenorrheic adolescent athletes exhibit significantly lower baseline levels of estradiol than eumenorrheic, sedentary adolescents ($p<0.01$-0.05)(Creatsas et al., 1992). The impact of a depressed sex steroid environment on bone will be discussed later in the review.

Section III: Bone

In order to understand how alterations in menstrual status and subsequent oral contraceptive use might affect bone health, it is necessary to understand the different types of bone, its components, structure, and properties, as well as the time course of bone development and mineralization in adolescence. The following is a brief review of bone physiology and development.

1. Bone Anatomy and Physiology

a. Bone content

Mature bone consists of inorganic bone material deposited in a framework of organic support material. The inorganic portion of the skeleton consists of calcium phosphate, $Ca_3 (PO_4)_2$, which interacts with calcium hydroxide, $Ca(OH)_2$, to form crystals of hydroxyapatite, $Ca_{10} (PO_4)_6(OH)_2$. As the hydroxyapatite crystals are formed, calcium salts (calcium carbonate) and ions such as sodium, magnesium, and fluoride are also incorporated into the inorganic portion of bone. The mineral portion of bone normally makes up about one quarter of the bone volume but because of its high density, the mineral volume makes up about one half of the skeleton's weight. Calcium makes up about 32.2 % of the mineral content of bone. Calcium phosphate crystals are strong and they can withstand compression but they are relatively inflexible (Martini & Timmons, 1997).

Osteoid is the organic matrix of bone and it consists of approximately 95% collagen fibers. The collagen fibers provide the framework that is necessary for the formation of mineral crystals. The hydroxyapatite crystals form small plates that lie lengthwise along the collagen fibers. Though similar to the collagen fibers present in skin and tendons, the collagen fibers in bone possess biochemical properties that give it greater mechanical strength than other types of collagen. Collagen fibers are tough, flexible and tolerate bending, twisting and stretching. The combination of collagen protein fibers and mineral crystals provides bone with unique properties that allow for maximum strength with minimum weight. The other 5% of the organic portion of the matrix is known as ground substance. Ground substance consists of a mixture of various proteoglycans that are high molecular weight compounds made up of carbohydrate and

protein. The ground substance binds the fibers and crystals into a compact unit (Martini & Timmons, 1997).

b. Bone structure

The skeleton is comprised of two types of bone, cortical (compact) bone and trabecular (spongy or cancellous). It has been estimated that about 80% of the bone in the human body is cortical while the amount of each type of bone differs depending upon the skeletal site in question (Selby, 1995). In most mature human bones there is a solid, outer portion of cortical bone with an inner core of trabecular bone. In long bones this arrangement is confined to the articulating knobs at either end of the bone, known as the epiphyses. In the diaphyses trabecular bone lines only the inner surface of the cortical bone. In a growing bone the diaphysis is separated at each end from the epiphysis by a layer of cartilage known as the epiphyseal plate (Martini & Timmons, 1997).

The matrix composition of compact bone is similar to that of trabecular bone but the two osseous tissues are different in the three dimensional arrangement of their osteocytes, canaliculi and lamellae. An osteon, or Haversian system, is the basic functional unit of mature cortical bone and lies parallel to the surface of the bone. Cortical bone, being dense and compact, is thickest where stresses arrive from a limited range of directions. Due to the fact that all of the osteons line up in the same direction, this type of osseous tissue is extremely strong when stressed along this particular axis (Martini & Timmons, 1997; Eriksen, 1992).

There are no osteons contained in trabecular bone. Concentric lamellae in this osseous tissue form a network of struts and plates called trabeculae that branch out to create an open network. Nutrients are passed to the osteocytes by diffusion along the canaliculi that open onto the surfaces of the trabeculae (Martini & Timmons, 1997; Roche, 1978). In contrast to cortical bone, trabecular bone is found where a bone is not normally heavily stressed, or where the stresses arrive from many directions. Its location at the proximal epiphysis of the femur illustrates how the trabeculae are oriented along lines of stress but with extensive cross branching. With this arrangement the trabeculae are able to transfer forces from the hip to the femoral shaft (Eriksen, 1992).

The amount of trabecular bone at a particular skeletal site is of clinical interest when determining the best site for baseline bone mineral density measurements because it has been shown that there is a higher rate of bone turnover in trabecular bone tissue (Adams, 1997). The distal forearm is estimated to contain 40-50% of its mineral within the trabecular compartment (Eastell et al., 1990). The lumber vertebrae may consist of 25% trabecular bone while the vertebral bodies are reported to contain 71% trabecular bone (Nottesad et al., 1987; Eastell et al., 1990).

c. Bone cells

Osteoblasts are responsible for the synthesis of osteoid and are considered the bone forming cells. Osteoid that is secreted from the osteoblast is deposited on the surface of the bone and bone mineral (inorganic component of bone) is then deposited on the organic osteoid matrix. The result of this process is the development of mineralized bone that will surround an osteoblast. Osteoclasts function to break down bone by secreting an acid from their lysosomes that dissolves the bony matrix and releases stored calcium and phosphate. The action of osteoclasts is coupled to the action of osteoblasts for the processes modeling and remodeling (Eriksen, 1992).

2. Bone Remodeling

During skeletal growth the skeleton is molded to achieve its shape and size by the removal of bone from one site and deposition at another site; this process is called modeling. During modeling, formation predominates over resorption and trabecular bone responds to growth or other osteogenic stimulus by increasing thickness of the plates, whereas cortical bone responds through the predominance of periosteal formation over endosteal resorption, resulting in increased cortical thickness. The greater the width of a bone, the stronger it is and the more able it is to resist bending and breaking (Bailey et al., 1996; Manolagas, 2000).

Once the skeleton has reached maturity, regeneration of bone continues in the form of a periodic replacement of old bone with new at the same location; this process is called remodeling. Remodeling maintains the mechanical integrity of the skeleton with age and provides a mechanism by which the body is able to maintain calcium

homeostasis (Manolagas, 2000). Bone remodeling allows the skeleton to adapt to loads, stresses and strains it experiences throughout life. During growth, remodeling balance is positive resulting in increased bone mineral, while later in adult hood, remodeling balance is often negative resulting in bone mineral loss. Studies on biochemical markers of bone turnover, such as osteocalcin (formation) and N-telopeptide to helix (NTX) in urine (resorption), indicate that bone turnover is highest during the longitudinal growth spurt and puberty in females (Szulc, Seeman & Delmas, 2000). A positive remodeling balance during this time is critical for optimal bone mineral accrual (Parfitt, 1994).

Remodeling occurs at specific sites on the surface of trabeculae and around Haversian systems in cortical bone. Frost (1969) put forward the hypothesis that bone remodeling took place at these sites as a result of the coupled actions of osteoclasts and osteoblasts. This unique temporary structure made up of osteoclasts in the front and osteoblasts in the rear is termed the basic multicellular unit (BMU). The BMU is approximately 1-2mm long and 0.2-0.4mm wide, has a central vascular capillary, a nerve supply and associated connective tissue. In healthy humans, 3-4 billion BMUs are initiated each year and about 1 million are operating at any moment (Manolagas, 2000).

The process of bone remodeling by the BMU consists of a resorption phase, followed by a formation phase and then a resting or quiescent phase. How the resorption phase is initiated is still not completely clear. Evidence suggests that both initiation and control of bone resorption by osteoclasts is dependent on lining cells-cells of osteoblastic lineage, which may secrete an enzyme that removes the unmineralized collagenous layer of bone to allow osteoclasts to attach to the bone's surface (Manolagas, 2000). Once "activated", osteoclasts remove bone by acidification (ATP-proton driven pump), which removes the mineral component of bone, and proteolytic digestion, which removes the protein component of bone. The resorption phase lasts for approximately 2-3 weeks (Manogolas, 2000; Compston, 1992). When the resorption phase is complete, small cavities are left in the bone surface. The sum of all cavity volumes for the whole skeleton is termed the remodeling space and the number of units active at one time, and their turnover rate, dictate the amount of bone deficit at anyone time in the skeleton.

Ensuing this, osteoblasts are recruited in order that they may replace the resorbed bone with osteoid for mineralization. Bone formation involves two processes, matrix

formation and mineralization, which occur at different points of time and space within the BMU. In cortical bone there is a longitudinal section that appears to be a cone of bone resorption traveling along the length of the bone. This cone is then filled with osteoid that is gradually mineralized. This process leads to the appearance of concentric rings at right angles to the long axis of cortical bone. In trabecular bone, however, remodeling occurs primarily on the surface of the trabecular bars and there are few Haversian systems. Instead of tunneling through the bone, the resorption phase in trabecular bone produces a relatively shallow cavity (Howships lacuna), which is then filled from the base upward with osteoid that is subsequently mineralized. The final appearance of the new bone is that of even lamellae running approximately parallel to the surface of the bone (Selby, 1995). Upon completion of the remodeling cycle, the unit is now called a bone structural unit (BSU) (Compston, 1992). The lifespan of a BMU is approximately 6-9, which is longer than the individual life spans of osteoclasts (2 weeks) and osteoblasts (3 months). This means that there must be a continuous supply of new osteoclasts and osteoblasts from respective progenitor cells in the bone marrow (Manolagas, 2000). The lower end of the range of time for a remodeling cycle is seen in adolescence where the rate of turnover is much quicker. The variation in length between the adult and adolescent remodeling cycle is the result of a faster formation phase (Parfitt, 1994).

There are many factors that can affect the number of remodeling units that are activated at any one time, as well as the rate of remodeling (bone turnover), such as a change in hormonal status, mechanical loading and nutrient intake. A change in the activation frequency of new BMUs results in a remodeling transient where the bone remodeling space is affected (Heaney, 1994). For example, biochemical markers of bone turnover have shown that oral contraceptives suppress bone turnover in 35-49 year old women (Garnero et al., 1995) and 18-39 year old women (Ott et al., 2001). Suppression of bone turnover would suppress the activation of new BMUs and result in a transient decrease in the remodeling space which would correspond to an increase in bone mass. Measurement of bone content and density during this period, therefore, would not be truly reflective of this particular intervention. This period should be separated out from the rest of the duration of an intervention and should be considered when setting the parameter for a cross sectional design as well. The remodeling transient in adolescents

was considered in the design of this investigation with oral contraceptive use being continuous for the 8 months immediately prior to the investigation.

3. Bone Mineralization in Adolescence

As the bones grow in length and width, they also grow in the density of their mineral content and, thus, their mass. In fact, the pattern of mineral acquisition follows a growth curve pattern that is similar in nature to other growth variables such as height, weight and age at menarche. Approximately 90% of bone mineral is laid down by the end of adolescence and subsequent gains to reach peak bone mass are small (McKay et al., 1998). Peak bone mass is of particular importance because this defines the amount of bone each person has at the end of the skeletal growth period. When a higher peak bone mass is obtained, there is more mineral to draw from when age and menopausal related bone loss occur. A higher peak bone mass therefore, might provide greater protection against fracture risk and osteopenia later in life (Teegarden et al., 1995; Snow-Harter, 1994).

The age at which peak bone mass is achieved is not certain and peak bone mineral content and density are site specific (Lloyd et al., 1992). The acquisition of bone mineral content increases steadily during childhood, as does height and weight, then accelerates dramatically during adolescence and slows into adulthood. Limited bone density increases appear to take place beyond the second decade (Gilsanz et al., 1988; Teegarden et al., 1995). Bone mineral content velocity occurs in females at approximately age 12.5 and is coincident with age at menarche age at which peak calcium accretion occurs (Bailey, Martin, McKay, Whiting & Mirwald, 2000). These events follow attainment of peak height velocity by one year (McKay et al., 1998). Total body bone mineral content more than doubles between the ages of 8 and 15 years; and more specifically, 30% of lumbar and total body bone mineral content and 20 % of femoral neck bone mineral content are accumulated during the three years around peak height velocity.

Theintz et al. (1992) in their longitudinal investigation of bone mass accumulation at the lumbar spine and femoral neck in healthy adolescent females also found that the increment rate in bone mineral density and content was particularly pronounced over a three-year period, from 11-14 years of age in girls. This rate decreased significantly after

16 years of age and/or 2 years post menarche in the lumbar spine and femoral neck. In addition, the mean gains in lumbar spine, femoral neck, and midfemoral shaft BMD were not statistically significant between 17 and 20 years of age. Faulkner et al. (1996) observed that there was a distinct leveling off of bone mineral content and density curves in females between the ages of 16 and 21 and there were no significant differences between bone mineral content or density at any sites (lumbar spine, proximal femur, total body) between 17 and 21 years of age. Taken together, these findings suggest that the second decade is a critical time in the achievement of peak bone mass. If amenorrhea or oligomenorrhea occur during this decade, adolescent females are at risk for deficits in bone mineral accrual, inability to attain peak bone mass, and future fractures associated with bone loss.

Recker and colleagues (1992) demonstrated an increase in bone mass at the lumbar spine, forearm, and whole body in young women between the ages of 19 and 26 years. The estimated age at which mineral acquisition ceased in this study ranged from 28.3 years to 29. 5 years at several of the skeletal sites. Teegarden et al. (1995) found that 99% of total body bone mineral density and content were achieved at 22.1 years and 26.2 years respectively. Thus, although the second decade is the period during which most of the bone mineral density is accumulated, there are small increases after this period such that peak bone mass is reached at around the third decade.

4. Measurement of Bone Mineral

Bone densitometry provides a means to non-invasively measure bone mineral density and content for clinical purposes, such as screening for osteoporosis, and for research purposes. Examples of these methods are single photon absorptiometry (SPA), dual photon absorptiometry (DPA), quantitative computed tomography (QCT), and dual energy x-ray absorptiometry (DXA). SPA and DPA are older techniques that are now used less frequently, while QCT and DXA are presently more commonly used methods (Adams, 1995). The underlying basis of these techniques is differential absorption of radiation by bone and measurement of attenuation, which is directly related to the amount of bone mineral present, by calibration against a phantom of known density (Adams, 1997). QCT involves a pulsing x-ray which circles around the entire bone so attenuation

is measured at many different angles and is able to provide a true volumetric bone density (g/cm^3) (Glastre, 1990). QCT, however also involves large doses of radiation, which make it unsuitable for research purposes, especially in children and adolescents (Adams, 1995).

DXA involves two x-ray beams of different alternating energies (70kVp and 140 kVp at 60mm/s) that are optimal for separating mineralized and soft tissue components at the region of interest and emit very low doses of radiation (Kellie, 1992). Original DXA scanners used a single beam (pencil), the scan was performed rectilinearly and the scan time was approximately 15 minutes per site. A significant development in the DXA technology was the employment of array or 'fan beam' mode. This describes a system that uses a split collimator to generate a fan beam that is able to penetrate the spine and hip from different angles. This mode results in higher resolution and faster scan times (5 minutes or less per site scanned) (Adams, 1997). The effective dose equivalent for the DXA technique is 1-6μSv per site examined in women. This is considerably less than that of a common chest x-ray, which is 60 μSv, and background environmental radiation per annum, which is 2400 μSv (Kalender, 1992). The coefficient of variation (precision) with this technique is reported to be 1% at the lumbar spine, 1-2% at the proximal femur. The accuracy of the measurement is reported to be approximately 1-6% (Lukaski, 1993; Adams, 1995). Thus there is negligible radiation exposure with this method and it has been found to be a safe, accurate and precise technology for assessing bone mineral density and the extremely low doses involved in this procedure make it a suitable tool for the investigation of skeletal development in children (Kalender, 1992).

DXA is capable of measuring bone mineral content, density and projected area of axial, appendicular, or whole skeleton. Bone mineral content is the amount of mineral (cortical and trabecular), measured in grams, in a certain projected area. Bone mineral density (g/cm^2) is the amount of bone mineral divided by the projected area. Bone mass is a term used loosely in the literature to refer to mineral content or density of bones, however bone mass cannot currently be measured in vivo (Adams, 1997). A limitation of DXA, therefore, is that it provides an areal, two-dimensional density (length x width), rather than a true volumetric density (Katzman et al., 1991). Areal density does not take into account the thickness of the bone. This can be problematic when measuring bone

density during growth with repeated measures, or when measuring subjects of different sizes. Thicker boned people would be over reported in terms of density and smaller boned (thinner) might be under estimated (Compston, 1995). Currently, computed tomography is the only technique that provides a true volumetric density.

Section IV: Menstrual Status and Bone Health in Athletic Females

1. Role of Sex Steroids and Bone Health

Depressed levels of estrogen and progesterone observed in irregularly menstruating females are similar to those observed in postmenopausal women (Chen & Brzyski, 1999). Prolonged hormonal deficiency in adolescence and young adulthood, therefore, can result in a similar pattern of bone loss as seen in postmenopausal women, or deficits in bone accretion and an inability to attain adult peak bone mass, depending on the age at which menstrual irregularities are experienced (Keen & Drinkwater, 1997; Biller et al., .1991; Miller & Klibanski, 1999). It is widely accepted that estrogen deficiency plays a key role in the pathogenesis of bone mineral loss (DeCherney, 1993). The effects of estrogen deficiency on bone are characterized by an acceleration of bone turnover with a disproportionate augmentation of resorption compared to formation (Manolagas, 2000). The precise mechanism by which estrogen deficiency causes increased bone turnover and ingestion of exogenous estrogen (HRT) inhibits bone turnover remains unclear. Possible mechanisms include a direct effect on osteoblasts via estrogen receptors, increased osteoclastic activity due to local bone resorbing cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF), and an alteration in the skeletal vulnerability to the effects of parathyroid hormone (PTH) (Compston, 1992; Turner, Riggs & Spelsberg, 1994). Recently it has also been demonstrated that estrogen exerts pro-apoptotic effects on osteoclasts, thereby promoting their death, and antiapoptotic effects on osteoblasts and osteocytes, thereby promoting a longer lifespan in these cells. This results in a positive remodeling balance in a normal estrogen environment and a negative remodeling balance in a low estrogen environment, such as with amenorrhea and oligomenorrhea, leading to a net bone mineral loss (Manolagas, 2000).

2. Bone Mineral in Amenorrheic Athletes

Cann, Martin, Genant & Jaffe (1984) were initial researchers who observed bone loss in amenorrheic athletes. Females 16-49 years of age with hypothalamic amenorrhea perceived to be related to vigorous exercise exhibited spinal bone content that was lower in amenorrheic subjects by 22-29% when compared to control groups ($p<0.001$). Peripheral bone mineral content of the radius was decreased by 5-10%. Subjects who were amenorrheic for less than one year exhibited greater spinal bone content than those with longer durations of amenorrhea.

Biller, Coughlin, Saxe, Schoenfeld, Spratt & Klibanski, (1991) investigated the spinal trabecular bone content (QCT) of women 19-42 years of age with primary and secondary hypothalamic amenorrhea. Comparisons were made to age matched eumenorrheic controls at 0 months, 6 months and two years later. Amenorrheic subjects showed significantly decreased bone mineral when compared to the controls and osteopenia was most severe in those subjects with greater duration of amenorrhea. Prospective evaluation at 6 months and 2 years later indicated a further decline in spinal bone mineral content only in subjects who had been amenorrheic for less than 5 years. Subjects with amenorrhea for longer than 5 years exhibited more stability in spinal bone mineral content though it remained significantly lower than control subjects. This finding suggests that there is a critical period at the onset of hypothalamic amenorrhea during which bone mineral loss occurs at rate of approximately 3-5% per year. These data illustrate the need for early intervention when amenorrhea results from physical and psychological stresses such as vigorous exercise.

While lower bone mineral density in adult amenorrheic athletes has been observed (Keen & Drinkwater, 1997; Snead et al., 1992; Myburgh, Hutchins, Fataar, Hough & Noakes, 1990) there have been fewer studies focused on this condition and its implications to the bone health of adolescents who are at a different stage of bone mineralization and accrual. Dhuper, Warren, Brooks-Gunn & Fox (1990) studied the relationship between sex hormone concentrations and lumbar bone mineral density in adolescent amenorrheic ballet dancers and non dancers (average age 18 years) using length of amenorrhea as one factor in developing an estrogen score for each subject. Lumbar bone mineral density (Lunar-DPA) was found to be lower in the amenorrheics

than in the eumenorrheic dancers and controls of similar ages. Total estrogen score (the combined effects of estradiol concentration, age at menarche, months of amenorrhea, Tanner breast stage, and use of oral contraceptives or estrogen therapy) correlated positively with lumbar bone mineral density. These researchers attributed a lower bone mineral density in late adolescence to a low pubertal hormonal milieu, anthropometric factors such as thinness, increased fiber intake, and significant physical training.

White, Hergenroeder & Klish (1992) investigated lumbar spine bone mineral density (DXA-Lunar DPX) in eumenorrheic and amenorrheic (absent menstrual periods for at least 3 months) subjects, 15-21 year of age. While amenorrheic subjects exhibited lower mean lumbar bone mineral density than eumenorrheic subjects, after controlling for weight influences on bone mineral density these differences were not significant. Similar results were obtained in dancers 13-29 years of age at the lumbar spine, wrist and metatarsal (Warren et al., 1991). These data indicate that while there is a significant effect by the presence of amenorrhea and lack of hormonal support on bone density, these effects may be weight dependent. Moen et al. (1998) compared lumbar spine bone mineral density (DXA-Lunar DPX) between amenorrheic (< 5 menstrual periods in the last year) runners and eumenorrheic runners (15.1-18.8 years) who averaged 59 km per week in the 9 months prior to the bone mineral testing. Although expected trends were observed with amenorrheic lumbar bone mineral exhibiting lower density than that of eumenorrheics, there was no significant difference between these menstrual status groups on this measure.

Resumption of normal menstruation appears to provide little guarantee in terms of halting or even reversing bone mineral losses to bone mineral gains. Although amenorrheic woman who regain normal menstruation after a decrease in training and an increase in body weight may exhibit some increases in lumbar bone mineral density, 6.2% (equivalent to 0.071 g/cm^2) in 14.4 months, there appears to be a net loss in bone mineral density when compared to normally menstruating controls (Drinkwater et al., 1990). A more recent 8-year follow-up study of amenorrheic and oligomenorrheic athletes (mean age at time 1, 30 years; mean age at time 2, 38 years) indicated that vertebral bone mineral density remained significantly lower in the amenorrheic athletes when compared to regularly menstruating counterparts (Keen & Drinkwater, 1997).

These results occurred despite the fact that eighty per cent of the amenorrheic subjects had resumed menses within one year following the first study. These data suggest that complete normalization of vertebral bone mineral density in former amenorrheic athletes is not likely. This puts these women at greater risk for fractures, osteopenia and osteoporosis, especially in conjunction with age and postmenopause-related bone mineral losses.

Jonnavithula, Warren, Fox, & Lazaro (1993) confirmed this finding that resumption of menses may not lead to a return of normal bone mineral levels in a 2 year prospective study. Amenorrheic dancers exhibited a mean increase of 9.6% in spine bone mineral density in the first year. This increase demonstrated a significant positive correlation with changes in caloric intake and energy expenditure. This group also had a significant increase in mean weight over the first year. Spine and wrist bone mineral density demonstrated a high negative correlation with age ($p<0.05$ and $p< 0.06$ respectively) in the first year which indicates that the older the subject, the smaller the increase in bone mineral density. Despite this increase, amenorrheic dancers exhibited significantly lower bone mineral density of the spine, wrist and foot ($p<0.05$) compared to eumenorrheic dancers and sedentary controls, even though menses resumed in over half of the amenorrheic dancers over the two-year period. These data show that while increases in bone mineral might occur in young amenorrheic individuals before the return of normal menses and coinciding with the return of menses, absolute bone mineral density of specific sites is likely to remain below that of eumenorrheic counterparts matched for weight and maturation. Considering that peak bone mass of the proximal femur has been observed to occur around 16 years of age and most of lumbar bone mineral density is accrued before the age of 20 (Theintz et al., 1992; McKay et al., 1998; Katzman et al, 1991), such young amenorrheic athletes are at risk for not achieving their genetically predetermined peak bone mass, despite return of menses. This could have a significant impact on lifelong health.

2. Bone Mineral in Oligomenorrheic Athletes

Oligomenorrheic athletes, with their low levels of circulating reproductive hormones, are also at risk of skeletal demineralization and related injuries to bone such as

stress fractures and vertebral compression fractures. Lloyd, Myers, Buchanan, & Demers (1988) were the first to confirm this negative relationship between oligomenorrhea and bone mineral density. These researchers investigated L1 and L3 spinal bone mineral density in six eumenorrheic and 13 oligomenorrheic athletes aged 18-19 years (QCT). Mean bone density of the severely oligomenorrheic group (missed > 50% of their expected menses but had at least 3 menses per year) was 69% that of the eumenorrheic group. These researchers suggested that while the eumenorrheic women in this study had already reached a mean bone mineral density of 197 mg/mL, the severely oligomenorrheic women in the study would reach the age of 20 with a peak bone density of <150 mg/mL, and, therefore, these women would be at a substantially greater risk for the development of osteoporosis later in life.

Micklesfield and colleagues (1995) demonstrated similar results when they compared mean lumbar spine and proximal femur bone mineral density between oligomenorrheic athletes and eumenorrheic athletes (29-39 years of age; ultramarathon runners). Lumbar spine bone mineral density of the oligomenorrheic group was significantly lower than eumenorrheic subjects, regardless of resumption of menses. Considering the maturity of the women, they had likely reached or were close to their peak adult bone mass. These data imply that the peak adult bone mass of the lumbar spine of women with a history of oligomenorrhea may never reach that of women who have always had regular menstrual cycles. It could not be determined whether skeletal demineralization was due to bone loss or lack of bone accretion.

Pearce et al., (1996) investigated the impact of oligomenorrhea on bone mineral density during the critical bone development years. Oligomenorrheic ballet dancers (17 years of age) were grouped based on length of oligomenorrhea. Dancers with oligomenorrhea of less than 40 months duration had higher bone densities (than age predicted mean) at weight bearing sites but not at non-weight bearing sites. These results suggest a benefit of loaded exercise at weight bearing sites when oligomenorrhea is less than 40 months. Dancers with greater than 40 months oligomenorrhea exhibited similar bone mineral density to age predicted mean at weight bearing sites and lower bone mineral density at non-weight bearing sites. Bone density did not remain stable at weight bearing sites with increased duration of oligomenorrhea likely because exercise did not

completely offset the bone loss associated with oligomenorrhea as it did in the groups with less than 40 months oligomenorrhea. The cross sectional design of this investigation, as well as the fact that the exercise level of the control group was not described, make it difficult to interpret the results. However, this research illustrates a significant relationship between oligomenorrhea and bone mineral loss from non-weight bearing sites such as the arms, and ribs. This is of interest because these regions are potential future fracture sites for these women.

Section V: Oral Contraceptives and Bone Health

Fuller Albright first recognized the association between estrogen deficiency and osteoporosis in 1941 in postmenopausal women (Albright, Smith & Richardson, 1941). Albright observed that osteoporosis tended to occur mostly in women after menopause and that there was a beneficial effect of estrogen therapy (thought to be mediated alterations in calcium metabolism) on the bone density of these postmenopausal osteoporotic women. Subsequent to this, many investigations have illustrated the effectiveness of utilizing estrogen as a 'hormone replacement therapy' for the prevention of menopause related bone loss at a number of skeletal sites including the metacarpals, radius, spine, femoral shaft, and femoral neck (Nachtigall, Nachtigall, Nachtigall, & Beckman, 1979; Ettinger, Genant & Cann, 1985; Christiansen & Riis, 1990). Dose-response studies of postmenopausal women have demonstrated that the minimum daily oral dose of estrogen required for the prevention of bone loss is 0.6 mg conjugated equine estrogen (equivalent to 0.02 mg of ethinyl estradiol)(Christiansen et al., 1982), 1 mg of 17β-estradiol (Ettinger et al., 1987), or 0.015 mg of ethinylestradiol (Horsman et al. 1983). This leads to the hypothesis that OCs have the potential to positively impact bone mineral content in adolescent athletes experiencing irregular menstruation since OCs commonly contain 0.035 mg of ethinyl estradiol.

1. Retrospective Research

Initial research involving the therapeutic use of OCs for the protection of bone mineral content was epidemiological, cross sectional or retrospective in nature and involved mainly women who were premenopausal or postmenopausal. Table 7 presents a

number of retrospective studies that examined prior oral contraceptive use and its relationship to bone mineral density. Primarily, these studies suggest that prior use of oral contraceptives may provide a protective effect on bone mineral density (Lindsay, Tohme & Kanders, 1986; Kleerekoper et al., 1991). The degree of protection from bone mineral loss in these investigations was positively related to increased duration of exposure to OCs. Subjects with six or more years of exposure had significantly higher bone mineral densities of the femoral neck and lumbar spine when compared to age matched controls (Kritz-Silverstein & Barrett-Connor, 1993; Tupparainen et al., 1994). Timing of this exposure may also be important. Grainge et al. (2001) demonstrated that oral contraceptive use before the age of 23 years was significantly associated with increased bone density in early postmenopausal women. Lindsay, Tohme & Kanders (1986) found that the magnitude of the relationship between bone mineral content and OC use was 1% per year of exposure. Interestingly, these researchers also demonstrated that the beneficial effect of OC use on vertebral bone mineral density might be dampened after exposure to the menopausal state for two years.

A more recent retrospective study found dissimilar results and concluded that prior OC use had a negative impact on current bone mineral density (Prior et al., 2001). These researchers suggested that lower bone mineral densities in OC users may have been a result of: 1) OC users having had more menstrual cycle disturbances than those who had never used OCs; 2) The week long withdrawal period from "high-dose" steroids every month has the potential to trigger increased rates of bone resorption similar to those documented within one week following premenopausal ovariectomy; 3) Discontinuation of OC use might lead to ovulatory disturbances for the first 6-12 months (Bracken, Hellenbrand, & Holford, 1990) that may be associated with accelerated bone loss (Prior, Vigna, Schechter, & Burgess, 1990).

While many of the findings in the retrospective literature are encouraging, there were specific limitations to these studies. OC type and formulation, which may have contributed significantly to the to these findings, were rarely considered. It is unknown whether or not the lower doses of estrogen that exist in OCs today would provide similar results in a retrospective examination of bone mineral density (Tuppurainen et al., 1994).

Table 7

Retrospective Studies Examining the Association Between Past Oral Contraceptive Use and Current Bone Mineral Measures

Reference	N	Age and Reproductive Status	OC Formulation	Average Duration OC Use	*Site(s) Measured	Findings
Lindsay, Tohme & Kanders, 1986	17 14	Premenopausal Postmenopausal (x 42 & 55 yrs)	0.030 –0.050 mg ethinyl estradiol w/ norgestrel	7.9 yrs 10.2 yrs	LS (DPA)	(+) BMD in OC users 12% higher than controls ($p<0.01$)
Kleerekoper et al. 1991	548 1747	Premenopausal Postmenopausal (x 54 years)	Not considered	4.7 yrs	LS (DPA) F (SPA)	(+) OC users significantly less likely to have low BMD (+) 10 or more years OC use = greatest protection to BMD
Kritz-Silverstein & Barrett-Connor, 1993	239	Postmenopausal (55-60 years)	Not considered	4.1 yrs	LS, FN (DXA)	(+) 6 or more years of OC use = significantly higher bone mineral at both sites ($p\leq0.05$)
Tupparainen et al., 1994	3222	Peri & post menopausal (48-60 years)	0.050 mg or less of ethinyl estradiol (not considered)	<1 year 1-5 years >6 years	LS (DXA)	(+) Significantly higher BMD in those who use OCs for 6 or more years
Prior et al., 2001	524	Premenopausal (25-45 years)	Not reported	6.8 yrs	LS, FT (DXA)	(-) OC users exhibited significantly lower BMDs at these sites

* LS Lumbar Spine
F Forearm
FN Femoral Neck
FT Femoral Trochanter

In addition, these studies often presented specific limitations such as differences between OC-user and non-user groups that were not controlled or specific factors that are known to affect bone mineral density were not considered or measured. Confounding variables included lifestyle factors such as smoking, exercise frequency and type, diet, familial history, menstrual history, alcohol intake, number of pregnancies and childbirths, HRT use, marital and educational status.

2. Prospective Research

Prospective research examining the influence of OC use on bone mineral measures has also yielded conflicting results. No effect (Lloyd et al., 2000; Gremion et al, 2001; Mazess & Barden, 1991; Volpe, Silferi, Genazzani & Genazzani, 1993) positive effects with bone gain or bone maintenance in healthy and disease cases (Hergenroeder et al., 1997; Recker et al., 1992), and negative effects with relative bone mineral loss have been observed (Polatti, Perotti, Filippa, Gallina & Nappi, 1995; Weaver et al., 2001; Burr et al., 2000; Hartard et al., 1997) in varying age groups. The potential for OCs to influence bone mineral appears to be region specific with the lumbar spine being more susceptible to hormonal influences than the proximal femur and its femoral neck sub regions (Parfitt, 1994).

a. Oral contraceptive use-No effect

Lloyd et al. (2000) compared 28 healthy OC users who had been using them for at least 6 months and continuously until the age of 20 with 34 healthy non OC users. Subjects were followed from 12 to 20 years of age in this eight-year prospective design. The youngest age for beginning OC use was 14 while 54 % of the OC users had used them for at least one year or more and 46 % had used them for 6-12 months. The groups were similar in anthropometric measures, sport exercise history score, and age at menarche. Total body bone mineral density and content as well as proximal femur density (QDR Hologic 1000 and 2000 DXA) remained indistinguishable between these two groups at all ages up to and including the age of 20. These data indicate that initiating use of OCs during adolescence does not affect acquisition of peak bone mass as measured at age 20.

Volpe, Silferi, Genazzani & Genazzani (1993) studied the effects of using an OC containing 0.020 mg ethinylestradiol and 0.150 mg of desogestrel on bone mineral density. Subjects were 61 non-smoking women aged 40 and over with 46 of them between the ages of 45-48 at the time of completion of the study. The duration of the study was 48 months during which time subjects were examined at baseline and after 6, 12, 24, 36, 48, and 60 cycles (a cycle being 21 days of OC use followed by 7 days medication free). Bone density measurements were made at baseline, 1 and 2 years in only 37 of the 61 patients at L2-L4 of the lumbar spine using dual photon absorptiometry. Although there was a trend toward increases in bone mineral density in these subjects (1.02 ± 0.15 g/cm^2 at baseline, 1.09 ± 0.08 g/cm^2 after 1 year, and 1.10 ± 0.12 g/cm^2 after 2 years), the researchers concluded that bone mineral density was not modified during estro-progestinic administration.

Mazess & Barden (1991) studied the effect of OC use on bone mineral density in a 2-year prospective study of 300 healthy women between the ages of 20 and 39 years. These researchers controlled for such potentially confounding variables as calcium intake, exercise and smoking. No association was observed between OC use and bone density.

b. Oral contraceptive use-Positive effect

Recker, Davies, Hinders, Heaney, Stegman & Kimmel (1992) followed 156 healthy college aged women for five years to determine whether various self chosen levels of physical activity, nutrient intake, or use of OCs influenced skeletal consolidation in the third decade. OC use was recorded as the number of interviews wherein the subject was currently using OCs divided by the total number of interviews. Regression equations relating change in total body bone mineral to OC use suggested that use of OCs would be expected to result in a gain in total body bone mineral of approximately 11% during the third decade. There was no relationship between OC use and bone density of the spine and forearm, however, which suggests that the effects of OCs on bone are site specific in this age group and are only detected with total body bone mineral measurements. This investigation did not account for differences in type and dose of OCs, age at first use of OCs, or length of time for OC use.

Hergenroeder, Smith, Shypailo, Jones, Klish, & Ellis (1997), in a one year prospective design, examined the impact of OCs compared to medroxyprogesterone and placebo on bone mineral density in 15 young women (18-21 years) who were experiencing hypothalamic amenorrhea. Amenorrhea was associated with eating disorders and subjects were both athletes and non-athletes. After 12 months of treatment with 0.035 mg ethinyl estradiol/0.5-1.0 mg norethindrone per day, the OC group had significantly greater bone mineral density and content of the lumbar spine than the medroxyprogesterone ($p \leq 0.003$) and placebo groups ($p \leq 0.05$). There were no significant differences in femoral neck bone mineral density and content between the three subject groups at 12 months although this measure increased in the OC group and decreased in the placebo group after a year. These researchers suggest that an effect of OC use observed in the lumbar spine and not in the femoral neck because of differences in percentage of trabecular bone mineral at each of these sights (71% of lumbar vertebral body is trabecular versus 43% at the femoral neck). Trabecular bone is more metabolically active (increased rate of bone turnover) due to its larger surface area and, therefore, alterations in mineral content and density might be observed in this region before the femoral neck. Alternately, bone mineral accretion in the hip may have plateaued and the inference is that OCs may have had a greater impact on density and content at the femoral neck if ingested at an earlier age. Exercise and calcium intake could have been confounding variables in this investigation.

c. Oral contraceptive use-Negative effect

Polatti, Perotti, Filippa, Gallina & Nappi (1995) evaluated the effect of a low dose OC (0.020 mg ethinyl estradiol combined with 0.150 desogestrel) on the lumbar spine bone mineral density of 200 women, 19-22 years of age over 5 years. The OC group showed no significant changes in lumbar spine bone mineral density from baseline to 5 years while the non OC group significantly increased lumbar bone mineral density by 7.8 % over five years. Other lifestyle determinants of peak bone mass were not considered in this investigation such as the physical activity levels, diet and nutrient intakes, smoking and alcohol use within each of the groups. In addition, groups were not compared on factors that have been shown to correlate with bone mineral measures, such as height and

weight, in order to detect differences between the groups at baseline. Such limitations in research design make these results difficult to interpret and extend to the population in general. A more recent cross sectional study by this group Perotti, Bahamondes, Petta, & Castro (2001) found no effect of OC use (2 years) on the bone mineral density of the non dominant forearm distal and ultradistal sections in women 30-34 years of age. These results confirm the retrospective findings of Kritz-Silverstein & Barrett-Connor (1993).

Any effects that OCs may or may not have on bone mineral content and density in young women may be modified by exercise intensity and type (Weaver et al., 2001; Burr et al, 2000; Hartard et al, 1997). Gremion, Rizzoli, Slosman, Theintz and Bonjour (2001) found that when exercise was similar in intensity and type between oligo-amenorrheic, eumenorrheic and OC groups, there were no differences evident between eumenorrheic and OC groups in areal bone mineral density at axial or appendicular measurement sites. Although menstrual status of the OC athletes prior to OC use was not gathered, the subjects had been taking OCs for a minimum of four years and if the OCs were correcting exercise associated menstrual irregularities, the OCs may have been protecting these subjects from bone mineral loss, particularly at the axial skeleton, which was observed in the oligo-amenorrheic athletes in this investigation. If this was not the case, then the OCs appeared to have no effect on any of the bone measures when compared to eumenorrheic counterparts.

In an effort to detect the impact of exercise and OC use combined on bone mineral content, density and strength (bending rigidity of the femoral neck), Burr et al. (2000) used a longitudinal design that included OC and non OC users who were then randomized into exercise and non exercise groups. Women were healthy, aged 18-31 and not involved in more than 2 hours per week of "formal" exercise for at least a year prior to the study (n=123). Exercise included 30-second alterations of universal weights and cycle ergometer for a total of 45 minutes 3 times per week as well as a total of 60 minutes of rope skipping per week. Prior OC use was not discussed. Total body bone mineral content increased significantly in the exercise group while this measure decreased in the non exercising group resulting in net loss of total body bone mineral content. There were no improvements due to exercise in specific bone sites (spine radius and hip), however. At the femoral neck, non-exercising, non-OC users had the greatest percentage increase

in bone mineral density while this measure declined in both exercising and non-exercising OC users. The exercising, non-OC users exhibited the lowest bone mineral density at this site. These data suggested that either exercise or OC use was associated with a suppression of the normal increase in bone mass of the femoral neck.

OC use resulted in a lower total body bone mineral content compared to nonusers (Weaver et al., 2001). There was no exercise by OC use effect on total body bone mineral content, however. At the lumbar spine (bone mineral content and density) there was an exercise by OC use interaction with these measures remaining significantly lower in this group for the two-year intervention. Markers of bone turnover in this group did not indicate an imbalance between bone formation and resorption as a possible reason for the loss of bone mineral at this site. It is suggested that mineralization was inadequate due to calcium intakes of less than 1200 mg/d in this group and that adequate calcium must be present for bone modeling to occur under the stimulus of mechanical loading. This investigation reflects similar findings by Hartard et al., (1997) who observed no effect of physical activity on bone mineral density measures in a long term exercise (approximately 10 years) and long term OC group (approximately 8 years) while significantly higher bone mineral density of the lumbar spine and femoral neck were found in a long term exercise and short term (approximately 2 years) OC use group.

3. Oral Contraceptives and Markers of Bone Turnover

If oral contraceptives confer an effect on bone mineral density, this effect would be mediated by changes in the rate of bone resorption and/or formation, which together reflect bone turnover. Biochemical markers of bone resorption include: hydroxyproline, deoxypyridinoline, pyridinoline and peptides containing these crosslinks such as N-telopeptide to helix in urine (NTX) and C-telopepdide-2 in urine and serum (CTX); tartrate resistant acid phosphatase; hydroxylysine and its glycosides. Markers of bone formation include: osteocalcin (bone Gla protein); alkaline phosphatase and its skeletal isoenzyme; procollagen I extension peptides (Szulc, Seeman & Delmas, 2000). Concentrations of these markers depends on the amount of marker secreted during growth and bone formation and released from bone during bone resorption, on the peripheral catabolism of the molecule, on biological variations such as circadian and

diurnal variation, on the specificity of the marker for bone and on the specificity and precision of assays. Examination of these markers provides an expanded model of the pathophysiology of skeletal mineralization and demineralization under various conditions of growth and intervention such as OC use (Ott et al., 2001; Garnero, Sornay-Rendu & Delmas, 1995).

Garnero et al. (1995), in a cross sectional design, investigated the effect of OCs on the resorption markers NTX and CTX, and formation markers osteocalcin, bone specific alkaline phosphatase and C-terminal propeptide of type I collagen. OC users (average age 40 years) were taking between 0.030 and 0.050 mg of ethinylestradiol in the form of progesterone combination pills and average pill use was approximately 7 years. It was found that OC users exhibited significantly lower levels of both resorption and formation markers when compared to the control group. Bone mineral density of the lumbar spine, total hip and distal radius were similar between pill users and the control group, however. Thus OC use was associated with a moderate but significant decrease in bone mineral turnover, which, in this cohort, may be associated with a beneficial influence on bone mass after long-term OC use. A benefit from decreased bone mineral turnover would be derived from a decrease in the remodeling space as active BMUs continue to fill in previously resorbed cavities (Ott et al., 2001). Decreased osteocalcin and NTX levels were found also in OC users when compared to non-users (18-39 years of age) in a recent study (Ott et al., 2001). Bone mineral density at all anatomic sites (spine, femur, total body) was not different, however, between users and non-users of OCs (0.035 mg combined with progestins norethindrone or levonorgestinel). This suggests that although these markers of bone turnover were suppressed, the net balance of bone formation and resorption was similar between users and non-users of OCs and there was no increase in bone mineral density.

Burr et al. (2000) measured osteocalcin, serum alkaline phosphatase, serum tartrate resistant acid phosphatase and urinary hydroxyproline in OC users and non-users aged 18-31 years. OC users had a lower rate of bone turnover at baseline than non-users as depicted by osteocalcin and alkaline phosphatase (formation) and urinary hydroxyproline (resorption) markers. Tartrate-resistant acid phophatase levels were similar in users and non-users. Unique to this investigation was that the OC use had a

negative impact on total body bone mineral density and combined OC use and exercise resulted in lower spine and femoral neck bone mineral density. The markers of bone turnover suggest that the OC group began the study at a different state of bone remodeling that may have lead to a differential response to exercise. If this baseline level of reduced turnover persisted, it could have suppressed age related increases in bone mass and increases in bone strength that would normally occur with a positive formation/resorption balance.

Section VI: Other Factors Influencing Bone

1. Exercise and Mechanical Loading

Weight bearing physical activity places a mechanical load on muscle and bone that is integral to the development and maintenance of bone mineral (Chillibeck, Sale & Webber, 1995; Bailey, McHay, Mirwald, Crocker, & Faulkner, 1999; Weaver et al., 2001). The relationship between physical activity and bone mass varies depending on exercise type (loading mode), intensity (frequency and strain of loading), skeletal site and timing of physical activity relative to maturation levels (Snow-Harter, 1994). Adaptation of bone to mechanical loading is described by Frost's mechanostat theory: a minimum effective strain (biomechanical term for the amount of deformation of bone per unit length of the bone under load, and is therefore unit-less or sometimes described as micro strain and abbreviated με) is needed for bone formation to occur. Bone cells detect any increased strain and form more bone, which then reduces the strain response to a particular load. Progressively higher strains are therefore required to stimulate continued formation of bone (Frost, 1993). The theory ensured that species-specific bones would adapt to sustain their characteristic lifetime activity levels and patterns. This suggests that in humans, appropriate levels of activity must be maintained throughout childhood and adolescence to benefit from this bone adaptation (Parfitt, 1994). In addition to strain magnitude, strain distribution and number of loading cycles might also be important factors influencing bone adaptation (Skerry, 1997). Research in loading animal limbs has shown that functional strains (those that produce an osteogenic response) are strains that have uneven distribution, are novel or different, and are of a high magnitude and rate (Lanyon, 1996). Only a few loading cycles producing these types of strains are required

to saturate the osteogenic response, but loading must be regular (daily or every other day) in order to maintain any level of bone mass (Lanyon et al., 1984). These researchers concluded that load bearing could be the most important functional influence on bone mass and architecture (Lanyon, 1996).

These animal studies have also shown that the positive effects of loading on bone in childhood are maintained into adulthood and that adult bone responds differently to loads than growing bone (Chillibeck, Sale & Webber, 1995). When cross sectional moment of inertia (CSMI-a measure of the distribution of material around a given axis and is proportional the bone's bending rigidity about the axis) was compared in young and adult rats subjected to mechanical loading, adult rats showed no change in CSMI while young rats did and also had greater periosteal formation which would have a profound positive effect on the bending strength of bone (Burr et al., 2000; Chillibeck, Sale & Webber, 1995). It appears from animal studies that bone is able to adapt to mechanical loading much more readily during growth as opposed to after maturity (Parfitt, 1994).

Weight bearing activity in adults generally has a positive effect on bone mineral density at weight bearing sites and total body bone mineral content (Weaver et al., 2001; Friedlander, Genant, Sadowsky, Byl & Gluer, 1995; Recker et al, 1992) but the increase in bone mass at any skeletal site is generally not more than 6% and usually results in an increase of bone mass of about 2%-3% (Friedlander et al., 1995; Pruitt, Taafe, & Marcus, 1995). Physical activity in the adult skeleton that has reached peak bone mass allows conservation of bone mass, but doesn't always lead to significant bone acquisition (Forwood & Burr, 1993). In adults, exercise suppresses the formation of new remodeling sites and allows the remodeling space to refill which can result in small increases in bone mass (Burr et al., 2000). As well as reducing activation frequency of remodeling units, the force of generalized muscle contraction invokes an osteogenic stimulus in bone as a result of the pull of the muscle tendons at the insertion site on a specific skeletal site. The muscle tendon pull stimulates local osteoblasts (bone formation)(Lanyon, 1992).

Changes in measures of bone strength as reflected by bone geometry and architecture may also be significant predictors of future fracture risk in conjunction with bone mineral density (Bell et al., 1999). Burr et al. (2000) observed that a 2 year exercise

regimen of aerobic and non aerobic resistance training producing peak strains of 1000-6000 με 3 times per week with the addition of 60 minutes of skipping significantly increased total body bone mineral content but did not significantly increase either bone mineral density or bending rigidity of the femoral neck in women 18-31 years of age. In another investigation which included intermittent bouts of skipping and jumping into an exercise class and daily jumping (50 repetitions) at home resulted in a significant increase of 3.4% in femoral trochanter bone density in young women over a 6 month period compared to those who just participated in an exercise class with no high intensity bouts and daily walking or swimming (Bassey & Ramsdale, 1994). Friedlander et al. (1995) found significant increases in femoral neck and trochanter as well as spine and calcaneous bone mineral density in women 20-35 years of age who participated in an aerobic plus weight-training program compared to a stretching group. Methodological differences such as bone sites measured, technology used (SPA, DPA, DXA, QCT), exercise intervention modes and intensities (different loading strains), and duration of training make it difficult to make conclusions regarding threshold intensity or duration of exercise needed to elicit significant positive results on bone, or about different effects at specific bone sites. It has been found, however, that impact exercises such as squash, aerobic dance and gymnastics result in a greater osteogenic response than low impact exercises such as swimming, endurance running and weight training (Burr et al., 2000).

Exercise in children and adolescence has also have been observed to have a positive impact on bone mineral accrual and bone mineral content/density. Bailey et al. (1999) found a 17% increase in total body bone mineral content in active girls compared to inactive girls (mean age 12 years). This study also demonstrated a greater peak bone mineral accrual rate and a greater bone mineral accumulation for the 2 years around peak for children in the highest activity quartile for physical activity over those in the lowest quartile within a group of normally active children (boys and girls). Slemenda et al. (1994) reported a 4-7% greater increase in bone mineral density for prepubertal children in the uppermost quartile of physical activity compared with those in the lowest quartile. Monitoring of bone mineral measures during seasonal training and detraining in female gymnasts (average 18 years) showed a consistent pattern of bone density increases over the training followed by clear declines in the off seasons. Increases in the spine were

3.5% and 3.7% followed by declines of 1.5% and 1.3% in the off seasons. Total hip bone mineral density increased by 2.3% and 1.9% during the competitive season followed be decreases of 1.5% and 1.2% in the off seasons. Overall there was a 4.3% increase in spine bone mineral density in 2 years (Snow, Williams, LaRiviere, Fuchs & Robinson, 2001).

A weight training intervention study (Blimkie et al., 1996) in postmenarcheal girls who were matched for age, body weight and level of physical activity and who were randomly assigned to an exercise or control group indicated that after 26 weeks of 4 sets of 13 exercises of progressive resistance training there were no significant increases in total body and lumbar spine bone mineral density and content. This was despite significant gains in strength. There was a trend for greater increases in lumbar spine bone mineral density and content in the first 13 weeks in the training group over the control group. The authors suggested that the intake of calcium might not have been enough to support growth related and training related bone mineralization. In addition the duration of the study may not have been long enough to see an effect of exercise considering the approximate time of the bone remodeling transient (six months).

Though weight-bearing exercise appears to have a positive effect on bone mineral, it is not clear whether it can offset the deleterious effects of menstrual irregularities on bone mineral. Gremion et al. (2001) observed that in oligo-amenorrheic 19-37 year olds, bone mineral density of the appendicular (peripheral) skeleton was protected by weight bearing exercise. There were no differences in femoral neck, trochanter or shaft areal bone mineral densities (QDR-Hologic 1000 DXA) between oligo-amenorrheic athletes compared to eumenorrheic athletes. Both groups were long distance runners whose frequency and duration of training were similar as well as dietary intake and anthropometric characteristics. There were, however, significant differences in the areal bone mineral density of the lumbar spine between these groups when adequate dietary intakes were insured which suggests that weight-bearing exercise does not protect against sex hormone deficient bone mineral loss in the axial skeleton. Pearce et al. (1996) found that adolescent female dancers who had experienced oligomenorrhea for greater than 40 months exhibited bone mineral density no higher than the age predicted mean at weight bearing sites and was lower at non weight bearing sites.

Exercise may have been protecting the weight bearing sites from further losses of bone mineral in this group but these individuals would need to be followed further to their peak bone mass in order to determine the significance of these results.

2. Calcium

The most metabolically active phase in the pathway to peak bone mass is the pubertal growth period (9-17 years of age). During this phase, calcium intake is the most influential factor on calcium balance (calcium absorption versus calcium excretion) and adolescents must maintain a sufficiently positive calcium balance in order to increase skeletal mass and accumulate bone mineral density. It is also during this phase that there is the highest retention of calcium when compared to children and young adults (Matkovic & Ilich, 1993). Matkovic, Fontana, Tominac, Goel, Chesnut (1990) investigated calcium as a potential influence on peak bone mass in a calcium balance study and a two-year intervention calcium supplementation study. Adolescent females in the calcium balance study retained 200-500 mg of calcium (Ca) per day. When calcium intake was increased, calcium absorption increased and urinary Ca output did not change. There was a more pronounced increase in bone accretion over time in the calcium-supplemented group (1640mg Ca/day) over the control group (750mg Ca/day). Thus, calcium intake during adolescence influences skeletal calcium retention, calcium balance and possibly accumulation of bone mineral density and peak bone mass (Matkovic et al., 1990; Matkovic and Ilich, 1993).

Johnston et al. (1992) provided further evidence of the effect of calcium intake on skeletal calcium retention. This was a three-year double blinded, placebo controlled trial of calcium supplementation in 22 pairs of twin pairs aged less than 14 years. There was significantly greater bone gain in the forearms and lumbar spines of prepubertal twins who received a daily supplement of 1 g of calcium compared to those receiving the placebo. In addition, Lloyd and colleagues (1993) observed significant increases in total body and spinal bone density when daily calcium intake was increased in adolescent girls from 80% of the recommended dietary allowance (RDA-1200 mg in the USA) to 110% through calcium supplementation. The increase of 24g of bone gain per year in these

subjects would translate into an additional 1.3% skeletal mass increase per year during adolescent growth and, possibly, lead to a higher peak bone mass.

Calcium nutrition appears to play an important role in the development of peak bone mass and ironically, calcium intake starts to decline in girls as they reach puberty and adolescent girls are less likely to meet the recommended dietary levels of calcium intake than are teenage boys (US Department of Agriculture, 1978). Chan (1991) investigated the bone mineral status of 164 children aged 2 to 16 years and found that children older than 11 years had low dietary calcium intake and only 15% met the RDA. Children ingesting more than 1000mg of Ca^{++}/day had higher bone mineral densities than those ingesting less. This calcium neglect in adolescence could have serious ramifications on the skeletal mineralization of adolescent females.

3. Heredity

Genetic factors are major determinants of bone mass accumulation throughout childhood and adolescence, of peak bone mass, and of age related bone mineral loss (Armamento-Villareal, Villareal, Avioli & Civitelli, 1992). It is speculated that genetic factors contribute to about 60-80% of the variance in bone mass while environmental factors contribute to the remaining 20-40% of the variance (Johnston et al., 1992; Slemenda et al., 1994). Exactly how the genetic message controls bone mineral density is thus far unknown but twin models have been used to illustrate the genetic relationship to bone mineral density. It has been observed that bone mineral density is significantly better correlated in monozygotic than dizygotic twins at the radius, lumbar spine, and the hip (Slemenda et al, 1991a; Pocock et al., 1987). Slemenda et al. (1991a) investigated the genetic determinants of bone mass in pre and postmenopausal women (47 dizygotic pairs and 124 monozygotic pairs, Caucasian, 25-80 years of age) using dual photon absorptiometry. At all skeletal sites, monozygotic intraclass correlations exceeded dizyogotic correlations for both pre and postmenopausal women. These findings lead to highly significant estimates of heritability for bone mass (i.e. H^2 = .0.88, femoral bone mineral density, H^2 = 0.84, Ward's triangle bone mineral density). Adjustments for age, height and environmental characteristics did not reduce the heritability estimates. The genetic effect on bone mass diminished with increased age in this investigation, as

evidenced by increasing intraclass monozygotic pair variability in older women. This finding points to the importance of increasing the accumulation of bone mass in early adolescence and adulthood. Limitations of the twin model for studying the effect of genetics on bone mineral density include a greater monozygotic environmental similarity and the probability of gene interaction, which may lead to an upward bias in the results (Slemenda et al., 1991a).

Armamento-Villareal et al. (1992) studied the bone mineral density of a cross sectional sample of 84 premenopausal women. Quantitative computed tomography was used to measure bone density from the 12th thoracic vertebrae to the 3rd lumbar vertebrae. Measurements indicated that premenopausal women with low bone mass had a positive maternal family history of osteoporosis more frequently than subjects with normal bone mass. While this investigation supports the strong governing role of genetics on bone mineral density, the paternal family history of osteoporosis was not included. Matkovic et al. (1990) included the fathers in a comparative investigation of the bone mineral densities of adolescent females to the parents' bone mineral densities. It was found that the highest correlation between measured variables of the daughters and parents occurred when the mean values for parents (mother + father/ 2) and daughters were compared. The correlation coefficient between daughters and parents for distal forearm bone mineral density was 0.72 ($p< 0.00001$). This suggests that the father may have a possible genetic role in the bone densities of adolescent daughters as well as the mothers. Matkovic et al. (1990) demonstrated that by age 14 years, bone size, mass and density in adolescent females approached the corresponding values of their mothers. By 16 years of age the girls had accumulated 97% of the bone mass of their premenopausal mothers.

Conclusions

It is apparent from this review that the area of bone health and menstruation in adolescent athletes is complex and involves several considerations within each research design. Much of the research that is available in this area is based on young adult samples who are at stages of bone development that differ from adolescents. More research investigating bone mineral in eumenorrheic, amenorrheic and oligomenorrheic athletic adolescents that uses a longitudinal design and follows adolescents to peak bone

mass and requires hormonal in addition to bone mineral measures is needed. This would provide a better understanding of the mechanisms surrounding potential deficits in bone mineral accretion with irregular menstruation as well as the timing and nature of these effects relative to bone development. Currently it appears that there is a critical period during skeletal development wherein the greatest amount of bone accretion occurs in females. This peak level of bone accretion is exhibited during adolescence with limited increases in bone mineral density beyond the second decade. Sex steroid levels, which are necessary for normal bone mineral development, are depressed in association with menstrual irregularities attributed to vigorous exercise and disordered eating. Irregular menstruation in adulthood leads to bone loss. Irregular menstruation during adolescence may have a substantial impact on the attainment of genetic peak bone mass and lead to subsequent acute and chronic bone health problems such as osteoporosis later in life. The effects of menstrual irregularities on bone may be prevented with oral contraceptive use but more research is needed to determine the efficacy of this treatment in this cohort.

References

Adams, J.E. (1995). Quantitative measurements in osteoporosis. In F.I. Tovey & T.C. Stamp (Eds.), ***The measurement of metabolic bone disease*** (pp.107-142). New York: Parthenon.

Adams, J.E. (1997). Single and dual energy x-ray absorptiometry. ***European Radiology, suppl. 2,*** S20-S31.

Albright, F., Smith, P., & Richardson, A. (1941). Postmenopausal osteoporosis. ***Journal of the American Medical Association, 116,*** 2465-2474.

Armamento-Villareal, R., Villareal, D., Avioli, L., & Civitelli, R. (1992). Estrogen status and heredity are major determinants of premenopausal bone mass. ***Journal of Clinical Investigation, 90,*** 2464-2471.

Baer, J.T. (1993). Endocrine parameters in amenorrheic and eumenorrheic adolescent female runners. ***International Journal of Sports Medicine, 14***(4), 191-195.

Baer, J.T., Taper, L., Gwazdaukas, F., Walber, J., Novascone, M., Ritchy, S., & Thye, F. (1992). Diet, hormonal and metabolic factors affecting bone mineral density in adolescent amenorrheic and eumenorrheic female runners. ***The Journal of Sports Medicine and Physical Fitness, 32,*** 51-58.

Bailey, D.A., Faulkner, R., & McKay, H.A. (1996). Growth, physical activity, and bone mineral acquisition. ***Exercise and Sport Sciences Reviews, 24,*** 233-266.

Bailey, D.A., Martin, A.D., McKay, H.A., Whiting, S., & Mirwald, R. (2000). Calcium accretion in girls and boys during puberty: A longitudinal analysis. ***Journal of Bone and Mineral Research, 15***(11), 2245-2250.

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***(10), 1672-1679.

Bassey E.J., & Ramsdale, S.J. (1994). Increase in femoral bone density in young women following high impact exercise. ***Osteoporosis International, 4,*** 72-75.

Bausenwein, I. (1954). On the question of sport and menstruation. ***Deutsche Medizinishe Wochenschrift, 79***, 1526-1532.

Bell, K., Loveridge, N., Power, J., Stanton, M., Lunt, M., Meggitt B.F., & Reeve, J. (1999). Structure of the femoral neck hip fracture: Cortical bone loss in the

inferoanterior to superoposterior axis. ***Journal of Bone and Mineral Research, 14,*** 111-119.

Biller, B., Coughlin, J., Saxe, V., Schoenfeld, D., Spratt, D., & Klibanski, A. (1991). Osteopenia in women with hypothalamic amenorrhea: A prospective study. ***Obstetrics and Gynecology, 78***, 996-1001.

Blimkie, C.J.R., Rice, S., Webber, C., Martin, J., Levy, D., & Gordon, C.L. (1996). Effects of resistance training on bone mineral content and density in adolescent females. ***Journal of Physiology and Pharmacology, 74,*** 1025-1033.

Bonen, A. (1994). Exercise-induced menstrual cycle changes: A functional, temporary adaptation to metabolic stress. ***Sports Medicine, 17***(6), 373-392.

Boyden, T., Pamemter, R., Stanforth, P., Rotkis, T., & Wilmore, J. (1983). Sex steroids and endurance running in women. ***Fertility and Sterility, 39***, 629-632.

Bracken, M.B., Hellenbrand, K.G., Holford, T.R. (1990). Conception delay after oral contraceptive use: The effect of estrogen dose. ***Fertility and Sterility, 53***(1), 21-27.

Bullen, B., Skrinar, G., Beitins, I., von Mering, G., Turnbull, B., & McArthur, J. (1985). Induction of menstrual disorders by strenuous exercise in untrained women. ***New England Journal of Medicine, 312***, 1349-1353.

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), 855-863.

Calabrese, L.H., Kirkendall, D.T., Floyd, M., Rapoport, S., Williams, G.W., & Weiker, G.G. (1983). Menstrual abnormalities, nutritional patterns and body composition in female classical ballet dancers. ***Physician and Sports Medicine, 11(2)***, 86-98.

Cann, C., Martin, M., Genant, H., & Jaffe, R. (1984). Decreased spinal mineral content in amenorrheic women. ***Journal of the American Medical Association, 251***(5), 626-629.

Chan, G.M. (1991). Dietary calcium and bone mineral status of children and adolescents. ***American Journal of Diseases in Children, 145,*** 631-634.

Chillibeck, P.D., Sale, D.G., & Webber, C.E. (1995). Exercise and bone mineral density. ***Sports Medicine, 19,*** 103-122.

Christiansen, C., Christiansen, M., & Larsen, N. (1982). Pathophysiological mechanisms of estrogen effect on bone metabolism: Dose response relationships

in early postmenopausal women. ***Journal of Clinical Endocrinology and Metabolism, 55***(6), 1124-1130.

Christiansen, C., & Riis, B.J. (1990). 17β-estradiol and continuous norethisterone: A unique treatment for established osteoporosis in elderly women. ***Journal of Clinical Endocrinology and Metabolism, 71***(4), 836-841.

Compston, J.E. (1992). HRT and osteoporosis. ***British Medical Bulletin, 48***(2), 309-344.

Compston, J.E. (1995). Bone density: BMC, BMD, or corrected BMD? ***Bone, 16***(1), 5-7.

Conway, G.S. & Jacobs, H.S. (1997). Leptin: A hormone of reproduction. ***Human Reproduction, 12,*** 633-655.

Creatsas, G., Salakos, N., Averkiou, M., Miras, K., & Aravantinos, D. (1992). Endocrinological profile of oligomenorrheic strenuously exercising adolescents. ***International Journal of Gynecology and Obstetrics, 38***, 215-221.

Cumming, D., & Rebar, R. (1985). Hormonal changes with acute exercise and with training in women. ***Seminars in Reproductive Endocrinology, 3***(1), 55-64.

Dale, E., Gerlach, D.H., & Wilhite, A.L. (1979). Menstrual dysfunction in distance runners. ***Obstetrics and Gynecology, 54***, 47-53.

DeCherney, A. (1993). Physiologic and pharmacologic effects of estrogen and progestins on bone. ***Journal of Reproductive Medicine, 38***(12), 1007-1014.

Dhuper, S., Warren, M.P., Brooks-Gunn, J., & Fox, R. (1990). Effects of hormonal status on bone density in adolescent girls. ***Journal of Clinical Endocrinology and Metabolism, 71***(5), 1083-1088.

Ding, J-H., Sheckter, C., Drinkwater, B., Soules, M., & Bremner, W. (1988). High serum cortisol levels in exercise-associated amenorrhea. ***Annals of Internal Medicine, 108***(4), 530-534.

Drinkwater, B., Bruemner, B., & Chesnut, C. (1990). Menstrual history as a determinant of current bone density in young athletes. ***Journal of the American Medical Association, 263,*** 545-548.

Eastell, R., Mosekilde, L., Hodgson, S., & Riggs, B. (1990). Proportion of human vertebral body bone that is cancellous. ***Journal of Bone and Mineral Research, 5,*** 1237-1241.

Erdelyi, G.J. (1963). Gynecological survey of female athletes. ***Journal of Sports Medicine and Physical Fitness, 2,*** 174-179.

Eriksen, E. (1992). ***Osteoporosis: Pathogenesis and treatment therapy manual.*** Denmark: Novonordisk.

Ettinger, B. (1988). Prevention of osteoporosis: Treatment of estradiol deficiency. ***Obstetrics and Gynecology, 72***(5), 12S-17S.

Ettinger, B., Genant, H., & Cann, C. (1985). Long-term estrogen replacement therapy prevents bone loss and fractures. ***Annals of Internal Medicine, 102***, 319-324.

Ettinger, B., Genant, H., & Cann, C. (1987). Postmenopausal bone loss is prevented by treatment with low dosage estrogen with calcium. ***Annals of Internal Medicine, 106***, 40-45.

Falk, J.R., & Halmi, K.A. (1982). Amenorrhea in anorexia nervosa: Examination of the critical body weight hypothesis. ***Biological Psychiatry, 17***, 799.

Faulkner, R.A., Bailey, D.A., Drinkwater, D.T., McKay, H.A., Arnold, C. & Wilkinson, A.A. (1996). Bone densitometry in Canadian children 8-17 years of age. ***Calcified Tissue International, 59,*** 344-351.

Feicht, C.B., Johnson, T.S., Martin, B.J., Sparks, K.E., & Wagner, W.W., Jr. (1978). Secondary amenorrhoea in athletes. ***Lancet, 2***, 1145-1146.

Forwood, M.R. & Burr, D.B. (1993). Physical activity and bone mass: Exercises in futility? ***Bone and Mineral, 21,*** 89-112.

Fox, E.L., Bowers, R.W., & Foss, M.L. (1993). ***The physiological basis for exercise and sport*** (5^{th} ed.). Dubuque, IA: Wm. C. Brown.

Friedlander, A.L., Genant, H.K., Sadowsky, S., Byl, N.N., & Gluer, C. (1995). A two-year program of aerobics and weight training enhances bone mineral density of young women. ***Journal of Bone and Mineral Research, 10***(4), 574-585.

Frisch, R.E. (1987). Body fat, menarche, fitness, and fertility. ***Human Reproduction, 2***(6), 521-533.

Frisch, R.E., & McArthur, J.W. (1974). Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. ***Science, 185,*** 949.

Frisch, R.E., Snow, R., Johnson, L., Gerard, B., Barbieri, R., & Rosen, B. (1993). Magnetic resonance imaging of overall and regional body fat, estrogen

metabolism, and ovulation of athletes compared to controls. ***Journal of Clinical Endocrinology and Metabolism, 77,*** 471-477.

Frost, H.M. (1969). Tetracycline-based histological analysis of bone remodelling. ***Calcified Tissue International, 3***, 211-237.

Frost, H.M. (1987). Bone mass and the mechanostat: A proposal. ***Anat Rec, 219,***1-9.

Frost, H.M. (1993). Suggested fundamental concepts in skeletal physiology. ***Calcified Tissue International, 52,*** 1-4.

Garnero, P., Sornay-Rendu, E., & Delmas, P.D. (1995). Decreased bone turnover in oral contraceptive users. ***Bone, 16***(5), 499-503.

Gilsanz, V., Gibbens, D., Roe, T., Carlson, M., Senac, M., Boechat, M., Huang, H., Schulz, E., Libanati, C., & Cann, C. (1988). Vertebral bone density in children: Effect of puberty. ***Radiology, 166,*** 847-850.

Glastre, C., Braillon, P., David, L., Cochat, P., Meunier, P., & Delmas, P. (1990). Measurement of bone mineral content of the lumbar spine by dual energy x-ray absorptiometry in normal children: Correlations with growth parameters. ***Journal of Clinical Endocrinology and Metabolism, 70***(5), 1330-1333.

Gremion, G., Rizzoli, R., Slosman, D., Theintz, G., & Bonjour, J. (2001). Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. ***Medicine and Science in Sports and Exercise, 33***(1), 15-21.

Grinspoon, S., Gulick, T., & Askari, H. (1996). Serum leptin levels in women with anorexia nervosa. ***Journal of Clinical Endocrinology and Metabolism, 81,*** 3861-3863.

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,*** 87-90.

Heaney, R.P. (1994). The bone-remodeling transient: Implications for the interpretation of clinical studies of bone mass change. ***Journal of Bone and Mineral Research, 9,*** 1515-1523.

Hergenroeder, A.C. (1995). Bone mineralization, hypothalamic amenorrhea, and sex steroid therapy in female adolescents and young adults. ***Journal of Pediatrics, 126***(5), 683-689.

Hergenroeder, A.C., Smith, E., Shypailo, R., Jones, L., Klish, W., & Ellis, K. (1997). Bone mineral changes in young women with hypothalamic amenorrhea treated

with oral contraceptives, medroxyprogesterone, or placebo over 12 months. ***American Journal of Obstetrics and Gynecology, 176***(5), 1017-1025.

Horseman, A., Jones, M., Francis, R., & Nordin, C. (1983). The effect of estrogen dose on postmenopausal bone loss. ***The New England Journal of Medicine, 309,*** 1405-1407.

Johnston, C., Miller, J., Slemenda, C., Reister, T., Hui, S., & Christian, J. (1992). Calcium supplementation and increases in bone mineral density in children. ***New England Journal of Medicine, 327,*** 82-87.

Jonnavithula, S., Warren, M., Fox, R.P., & Lazaro, M.I. (1993). Bone density is compromised in amenorrheic women despite return of menses: A two year study. ***Obstetrics and Gynecology, 81***(5), 669-674.

Kalender, W. (1992). Effective dose values in bone mineral measurements by photon absorptiometry and computed tomography. ***Osteoporosis International, 2,*** 82-87.

Kanaley, J., Boileau, R., Bahr, J., Misner, J., & Nelson, R. (1991). Cortisol levels during prolonged exercise: The influence of menstrual phase and menstrual status. ***International Journal of Sports Medicine, 13***(4), 332-336.

Katzman, D.K., Bachrach, L.K., & Carter, D.R. (1991). Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. ***Journal of Clinical Endocrinology and Metabolism, 72(6),*** 1332-1339.

Keen, A., & Drinkwater, B. (1997). Irreversible bone loss in former amenorrheic athletes. ***Osteoporosis International, 7,*** 311-315.

Keizer, H., & Rogol, A. (1990). Physical exercise and menstrual cycle alterations. ***Sports Medicine, 10***(4), 218-235.

Kellie, S. (1992). Measurement of bone density with dual energy x-ray absorptiometry (DEXA). ***Journal of the American Medical Association, 267***, 286-294.

Kleerekoper, M., Brienza, R., Schultz, L., & Johnson, C. (1991). Oral contraceptive use may protect against low bone mass. ***Archives of Internal Medicine, 151,*** 1971-1976.

Kohrt, W.M., Ehsani, A.A., & Birge, S.J. (1998). HRT preserves increases in bone mineral density and reductions in body fat after a supervised exercise program. ***Journal of Applied Physiology, 84***(5), 1506-1512.

Kritz-Silverstein, D., & Barrett-Connor, E. (1993). Bone mineral density in postmenopausal women as determined by prior oral contraceptive use. ***American Journal of Public Health, 83,*** 100-102.

Lanyon, L.E. (1984). Functional strain as a determinant for bone remodeling. ***Calcified Tissue International, 36,*** S56-S61.

Lanyon, L.E. (1992). Control of bone architecture by functional load bearing. ***Journal of Bone and Mineral Research, 7(Suppl.2),*** S369-S375.

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,*** 13S-37S.

Lindsay, R., Tohme, J., & Kanders, B. (1986). The effect of oral contraceptive use on vertebral bone mass in pre and post menopausal women. ***Contraception, 34***(4), 333-340.

Lloyd, T., Andon, M., Rollings, N., Martel, J., Landis, R., Demers, L., Eggli, D., Kieselhorst, K., & Kulin, H. (1993). Calcium supplementation and bone mineral density in adolescent girls. ***Journal of the American Medical Association, 270***(7), 841-844.

Lloyd, T., Meyers, C., Buchanan, J.R., & Demers, L.M. (1988). Collegiate women athletes with irregular menses during adolescence have decreased bone density. ***Obstetrics and Gynecology, 72***(4), 639-642.

Lloyd, T., Rollings, N., Andon, M., Demers, L., Eggli, D., Kieselhorst, K., Kulin, H., Landis, J., Martel, J., Orr, G., & Smith, P. (1992). Determinants of bone density in young women. ***Journal of Clinical Endocrinology and Metabolism, 75***(2), 383-387.

Lloyd, T., Taylor, D.S., Lin, H.M., Matthews, A.E., Eggli, D.F., & Legro, R.S. (2000). Oral contraceptive use by teenage women does not affect peak bone mass: A longitudinal study. ***Fertility and Sterility, 74***(4), 734-738.

Loucks, A.B., Horvath, S.M., & Freedson, P.S. (1984). Menstrual status and validation of body fat prediction in athletes. ***Human Biology, 56***, 383-392.

Loucks, A.B., Mortola, J., Girton, L., & Yen, S. (1989). Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axes in athletic women. ***Journal of Clinical Endocrinology and Metabolism, 68,*** 402-411.

Lukaski, H.C. (1993). Soft tissue composition and bone mineral status: Evaluation by dual energy x-ray absorptiometry. ***Journal of Nutrition, 123,***438-443.

Manolagas, S.C. (2000). Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. ***Endocrine Reviews, 21***(2), 115-137.

Martini, F.H., & Timmons, M.J. (1997). ***Human anatomy*** (2nd ed.). New Jersey: Prentice Hall.

Matkovic, V., Fontana, D., Tominac, C., Goel, P., & Chesnut, C.H. (1990). Factors that influence peak bone mass formation: A study of calcium balance and the inheritance of bone mass in adolescent females. ***American Journal of Clinical Nutrition, 52,*** 878-888.

Matkovic V., & Ilich, J. (1993). Calcium requirements for growth: Are current recommendations adequate? ***Nutrition Reviews, 51***(6), 171-180.

Mazess, R., & Barden, H. (1991). Bone density in premenopausal women: Effects of age, dietary intake, physical activity, smoking, and birth control pills. ***American Journal of Clinical Nutrition, 53,*** 132-142.

McArthur, J.W., Bullen, B.A., Beitins, I.Z., Pagano, M., Badger, T.M., & Klibanski, A. (1980). Hypothalamic amenorrhea in runners of normal body composition. ***Endocrine Research Communications, 7***, 13-25.

McArthur, J.W., Johnson, L., Hourihan, J., & Alonso, C. (1976). Endocrine studies during the refeeding of young women with nutritional amenorrhea and infertility. ***Mayo Clinic Proceedings, 51***, 607-616.

McKay, H., Bailey, D., Mirwald, R., Davison, K.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***, 682-687.

Micklesfield, L., Lambert, E., Fataar, A., Noakes, T., & Myburgh, K. (1995). Bone mineral density in mature, premenopausal ultramarathon runners. ***Medicine and Science in Sports and Exercise, 27***(5), 688-696.

Moen, S., Sanborn, C., Dimarco, N., Gench, B., Bonnick, S., Keizer, H., & Menheere, P. (1998). Lumbar bone mineral density in adolescent female runners. ***Journal of Sports Medicine and Physical Fitness, 38,*** 234-239.

Myburgh, K., Hutchins, J., Fataar, A., Hough, S., & Noakes, T. (1990). Low bone density is an etiologic factor for stress fractures in athletes. ***Annals of Internal Medicine, 113,*** 754-759.

Myerson, M., Gutin, B., Warren, M., May, M., Contento, I., Lee, M., Pi-Sunyer, F., Pierson, R., & Brooks-Gunn, J. (1991). Resting metabolic rate and energy

balance in amenorrheic and eumenorrheic runners. ***Medicine and Science in Sports and Exercise, 23***(1), 15-22.

Nachtigall, L.E., Nachtigall, R.H., Nachtigall, R.D., & Beckman, E.M. (1979). Estrogen replacement therapy I: A 10-year prospective study in the relationship to osteoporosis. ***Journal of The American College of Obstetricians and Gynecologists, 53***(3), 277-281.

Nelson, M.E., Fisher, E.C., Catsos, P.D., Meredith, C.N., Turksoy, R.N., & Evans, W.J. (1986). Diet and bone status in amenorrheic runners. ***American Journal of Clinical Nutrition, 43***, 910-916.

Nottestad, S., Baumel, J., Kimmel, D., Recker, R., & Heaney, R. (1987). The proportion of trabecular bone in human vertebrae. ***Journal of Bone and Mineral Research, 2***, 221-229.

Ott, S.M., Scholes, D., Lacroix, A.Z., Ichikawa, L.E., Yoshida, C.K., & Barlow, W.E. (2001). Effects of contraceptive use on bone biochemical markers in young women. ***Journal of Clinical Endocrinology and Metabolism, 86***(1), 179-185.

Parfitt, A.M. (1994). The two faces of growth: Benefits and risks to bone integrity. ***Osteoporosis International, 4,*** 382-398.

Pearce, G., Bass, S., Young, N., Formica, C., & Seeman, E. (1996). Does weight-bearing exercise protect against the effects of exercise induced oligomenorrhea on bone density? ***Osteoporosis International, 6,*** 448-452.

Perrotti, M., Bahamondes, L., Petta, C., & Castro, S. (2001). Forearm bone density in long-term users of oral combined contraceptives and depot medroxyprogesterone acetate. ***Fertility and Sterility, 76***(3), 469-473.

Petit, M.A., Prior, J.C., & Barr, S.I. (1999). Running and ovulation positively change cancellous bone in premenopausal women. ***Medicine and Science in Sports and Exercise, 31***(6), 780-787.

Pocock, N., Eisman, J., Hopper, J., Yeates, M., Sambrook, P., & Eberi, S. (1987). Genetic determinants of bone mass in adults. ***Journal of Clinical Investigation, 80,*** 706-710.

Polatti, F., Perotti, F., Filippa, N., Gallina, D., & Nappi, R.E. (1995). Bone mass and long-term monophasic oral contraceptive treatment in young women. ***Contraception, 51***, 221-224.

Prior, J.C., Kirkland, S.A., Joseph, L., Kreiger, N., Murray, T.M., Hanley, D.A., Adachi, J.D., Vigna, Y.M., Berger, C., Blondeau, L., Jackson, S.A., & Tenenhouse, A. (2001). Oral contraceptive use and bone mineral density in premenopausal

women: Cross-sectional, population-based data from the Canadian multicentre osteoporosis study. ***Canadian Medical Association Journal, 165***(8), 1023-1029.

Prior, J., Vigna, Y., Barr, S., Rexworthy, C., & Lentle, B. (1994). Cyclic medroxyprogesterone treatment increases bone density: A controlled trial in active women with menstrual cycle disturbances. ***The American Journal of Medicine, 96***, 521-530.

Prior, J., Vigna, Y., Schechter, M., & Burgess, A. (1990). Spinal bone loss and ovulatory disturbances. ***New England Journal of Medicine, 323***, 1221-1227.

Pruitt, L.A., Taafe, D.R., & Marcus, R. (1995). Effects of a one year high intensity versus low intensity resistance training program on bone mineral density in older women. ***Journal of Bone and Mineral Research, 10,*** 1788-1795.

Recker, R., Davies, K., Hinders, S., Heaney, R., Stegman, M., & Kimmel, D. (1992). Bone gain in young adult women. ***Journal of the American Medical Association, 268***(17), 2403-2408.

Rhoades, R. & Pflanzer, R. (1996). ***Human physiology*** (3rd ed.). Toronto: Saunders College Publishing.

Robinson, E., Bachrach, L.K., & Katzman, D.K. (2000). Use of hormone replacement therapy to reduce the risk of osteopenia in adolescent girls with anorexia nervosa. ***Journal of Adolescent Health, 26***(5), 343-348.

Roche, A. (1978). Bone growth and maturation. In F. Falkner & J. Tanner (Eds.) ***Human growth: vol 2 postnatal growth***. (pp. 317-355). New York: Plenum press.

Sanborn, C., Martin, B., & Wagner, W. (1982). Is athletic amenorrhea specific to runners? ***American Journal of Obstetrics and Gynecology, 143(8),*** 859-61.

Sanborn, C., & Wagner, W. (1988). The female athlete and menstrual irregularity. In J. Puhl, C.H. Brown, & R. Voy (Eds.) ***Sport science perspectives for women*** (pp. 111-130). Champaign, IL: Human Kinetics.

Seeman, E., Karlsson, K., & Duan, Y. (2000). On exposure to anorexia nervosa, the temporal variation in axial and appendicular skeletal development predisposes to site-specific deficits in bone size and density: A cross-sectional study. ***Journal of Bone and Mineral Research, 15***(11), 2259-2265.

Selby, P.L. (1995). Anatomy and physiology of normal bone turnover. In F.I. Tovey, & T.C.B. Stamp (Eds.), ***The measurement of metabolic bone disease*** (pp.3-11). New York: Parthenon Publishing Group.

Skerry, T.M. (1997). Mechanical loading and bone: What sort of exercise is beneficial to the skeleton? ***Bone, 20,***179-181.

Slemenda, C., Christian, J., Williams, C., Norton, J., & Johnston, C. (1991a). Genetic determinants of bone mass in adult women: A reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. ***Journal of Bone and Mineral Research, 6***(6), 561-567.

Slemenda, C., Miller, J., Hui, S., Reister, T., & Johnston, C. (1991b). Role of physical activity in the development of skeletal mass in children. ***Journal of Bone and Mineral Research, 6,*** 1227-33.

Slemenda, C., Reister, T., Hui, S., Miller, J., Christian, J., & Johnston, C. (1994). Influences on skeletal mineralization in children and adolescents: Evidence for varying effects of sexual maturation and physical activity. ***Journal of Pediatrics, 125,*** 201-207.

Snead, D.B., Weltman, A., Weltman, J.Y., Evans, W.S., Veldhuis, J.D., Varma, M.M., Teates, C.D., Dowling, E.A., & Rogol, A.D. (1992). Reproductive hormones and bone mineral density in women runners. ***Journal of Applied Physiology, 72***(6), 2149-2156.

Snow, C.M., Williams, D.P., LaRiviere, J., Fuchs, R.K., & Robinson, T.L. (2001). Bone gains and losses follow seasonal training and detraining in gymnasts. ***Calcified Tissue International, 69,*** 7-12.

Snow-Harter, C. (1994). Bone health and prevention of osteoporosis in active and athletic women. ***Clinics in Sports Medicine, 13***(2), 389-404.

Szulc, P., Seeman, E., & Delmas, P.D. (2000). Biochemical measurements of bone turnover in children and adolescents. ***Osteoporosis International, 11***, 281-294.

Teegarden, D., Proulx, W.R., Martin, B.R., Zhao, J., McCabe, G.P., Lyle, R.M., Peacock, M., Slemenda, C., Johnston, C.C., & Weaver, C.M. (1995). Peak bone mass in women. ***Journal of Bone and Mineral Research, 10***(5), 711-715.

Theintz, G., Buchs, B., Rizzoli, R., Slosman, D., Clavien, H., Sizonenko, P., & Bonjour, J.P.H. (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*(4),** 1060-1065.

Trussell, J. (1980). Statistical flaws in evidence for the Frisch hypothesis that fatness triggers menarche. ***Human Biology, 52,*** 711-720.

Tuppurainen, M., Kroger, H., Saarikoski, S., Honkanen, R., & Alhava, E. (1994). The effect of previous oral contraceptive use on bone mineral density in perimenopausal women. ***Osteoporosis International, 4,*** 93-98.

Turner, R.T., Riggs, B.L., & Spelsberg, T.C. (1994). Skeletal effects of estrogen. ***Endocrine Reviews, 15***(3), 275-300.

US Department of Agriculture (1978). Nationwide food consumption survey. ***Nutrient Intakes: Individuals in 48 States, 1977-78. Report No. 1-2.*** Consumer Nutrient Division, Human Nutrition Information Service, USDA, Hyattsville, MD, 1984: 439.

Vollman, R.F. (1977). The menstrual cycle. In Friedman (Ed.), ***Major problems in obstetrics and gynecology (vol. 7).*** Toronto: W.B. Saunders.

Volpe, A., Silferi, M., Genazzani, A.D., & Genazzani, A.R. (1993). Contraception in older women. ***Contraception, 47,*** 229-239.

Wakat, K., Sweeney, K., & Rogol, A. (1982). Reproductive system function in women cross-country runners. ***Medicine and Science in Sports and Exercise, 14***, 263-269.

Warren, M.P., Brooks-Gunn, J., Fox, R.P., Lancelot, C., Newman, D., & Hamilton, W.G. (1991). Lack of bone accretion and amenorrhea: Evidence for a relative osteopenia in weight-bearing bones. ***Journal of Clinical Endocrinology and Metabolism, 72***(4), 847-853.

Warren, M.P. & Stiehl, A. (1999). Exercise and female adolescents: Effects on the reproductive and skeletal systems. ***Journal of the American Medical Women's Association, 54,*** 115-120.

Weaver, C., Teegarden, D., Lyle, R.M., McCabe, G.P., McCabe, L.D., Proulx, W., Kern, M., Sedlock, D., Anderson, D.D., Hillberry, B.M., Peacock, M., & Johnston, C.C. (2001). Impact of exercise on bone health and contraindication of oral contraceptive use in young women. ***Medicine and Science in Sports and Exercise, 33***(6), 873-880.

White C.M., Hergenroeder, A.C., & Klish, W.J. (1992). Bone mineral density in 15 to 21 year old eumenorrheic and amenorrheic subjects. ***American Journal of Diseases in Children, 146,*** 31-35.

Yen, S.S. (1990). Clinical endocrinology of reproduction. In E. Baulieu & P. A. Kelly (Eds.), ***Hormones: from molecules to disease.*** (pp. 445–481). New York: Chapman and Hall.

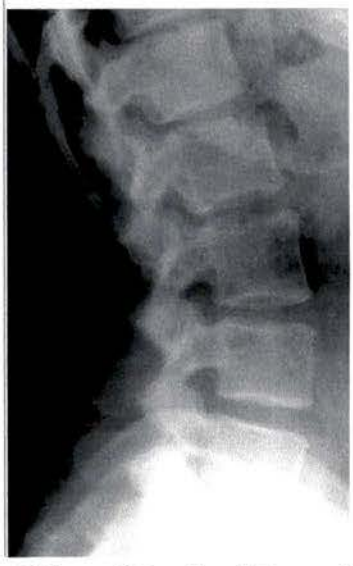
Appendix B: Recruitment Poster

University of Victoria

Learn About Your Bone Health & Nutrition By Being a Volunteer!

Shana Ooms
MSc Candidate
Exercise Physiology

Committee Members:
Dr. Catherine Gaul
Dr. Richard Backus
Dr. Howard Wenger
Dr. Marie Hoskins



Are You...

- ✓ interested in **learning** about your bone density & nutrient intake?
- ✓ female, **15-20 years old**?
- ✓ involved in vigorous, weight bearing **exercise at least 3x/wk for 45 min** each time?

Why Study Your Bones?

Bone development during adolescence is critical as the greatest amount of bone mass is acquired before the age of 20! Bone mass is related to menstruation and females who engage in physical activity on a regular basis may be more susceptible to absence of menstruation. This absence of menstruation and it's relationship to estrogen levels may increase the risk for not attaining your genetic peak bone mass. This, in turn, increases the potential for bone related health problems such as fractures and premature loss of bone. Low dose hormones found in oral contraceptives may offer some protection, however, much more research is needed! Osteoporosis is our responsibility, young and old, and your participation in this health research is invaluable.

What Does The Research Involve?

Health & Nutrition Questionnaires
Body Composition • Height/ Weight
Time: 1.5 hrs at ***University of Victoria***

DXA Bone Density Measurements
Sites: Spine & Hip
Time: 30 min at ***Royal Jubilee Hospital***

HERBONES RESEARCH

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

HerBones Research
Shana Ooms
Phone: (250) 370-0759
Email: shanad@uvic.ca

Appendix C: Informed Consent

Menstrual Irregularities, Low Dose Hormone Therapy and Bone Density in Adolescent Athletes

---

INFORMED CONSENT FORM

Purpose of the Research:

The purpose of this research project is to investigate the effect of menstrual status and low-dose hormone therapy on bone density in adolescent athletes. Adolescent athletes experiencing menstrual irregularities associated with exercise are at risk for having a lower bone mineral content than their regularly menstruating counterparts. The link between menstrual irregularities and bone health is estrogen. Lowered estrogen levels associated with menstrual irregularities may negatively impact bone health at any age. Adolescents are particularly vulnerable, however, because they are experiencing a critical period of bone development between the ages of 11 and 20 years with limited increases in bone mineral density beyond this point. Thus, exercise associated menstrual irregularities experienced by this cohort may inhibit them from attaining their genetically predetermined peak bone masses. The result is that these athletes would be at risk for developing bone health problems such as stress fractures and premature osteopenia. Low dose hormone therapy has a beneficial effect on bone density in older women. It has the potential, therefore, to also protect younger women from the detrimental effect menstrual irregularities have on bone density during the critical period of bone accretion. Thus, this research may lead to early recognition and intervention of menstrual-related bone health problems in athletes.

I understand that I will not be placed on oral contraceptives by the researchers of this investigation and that if I am placed in one of the oral contraceptive groups it is because I am already taking oral contraceptives prescribed by my physician.

During this investigation I will participate in an information session, and two laboratory sessions, one at the **UVic Sport and Fitness Centre** and one at the **Royal Jubilee Hospital Nuclear Medicine Department**.

While at the **UVic Sport and Fitness Centre** I will be asked to complete a General Health Questionnaire, the Eating Attitudes Test (EAT) and I will have already been given a 3-day Dietary Record and Food Frequency Questionnaire (FFQ) to complete on my own before this laboratory session. The General Health Questionnaire will provide the researchers with details about factors that may affect my bone density. These include: oral contraceptive and menstrual histories, physical activity levels, caffeine and alcohol consumption, smoking history, individual medical history and family history of osteoporosis, medication and vitamin intake as well as my age. The 3-Day Dietary Record and FFQ will provide information about my diet that will be used to determine my calcium intake and its potential influence on my bone density. The EAT will provide the researchers with information about my eating habits in general. Also at this time I

will have my height, weight and skin folds measured at five sites. This laboratory session will take approximately 1 hour.

During the other laboratory session, which will take place at the **Royal Jubilee Hospital's Nuclear Medicine Department,** I will have my bone mineral density measured by a qualified medical imaging technician. Dual energy x-ray absorptiometry (DEXA) will be used as the measurement technique and the lumbar spine (lumbar 1-4) and hip (femoral neck) will be the measurement sites. For this procedure I will wear clothing with no metal around my waist and I will be lying in a supine position on a bench. My weight will be measured before the procedure begins for inclusion in the DEXA technique. During the procedure I will be at no risk as radiation absorbed by this technique is negligible at 1-6 μSv per site examined. (A chest x-ray is 10 times this amount!) The procedure will take approximately 20 minutes.

I am aware that any data collected in this study will remain confidential and my anonymity will be protected. A code number will be assigned to me that will be used to identify me on all raw data sheets and questionnaires. The key to the code numbers will be kept separate from the data and it's location will be known only to the primary researcher, Shana Ooms (250 370-0759) and her graduate supervisor, Dr. Kathy Gaul (250 721-8380) and only these researchers will have access to the data. All raw data will be stored in a secured office and it will be destroyed within five years of the study. A computer database of results using only code numbers to identify subjects will be archived and used for writing scientific papers. My name will not be attached to any published results and they will be described in terms of group response only. Furthermore, I am aware that I am free to withdraw from the study at any time without negative repercussions or influences on my participation in my sport. Should I choose to withdraw prior to the completion of the study, the primary researcher will destroy my data immediately. If I have any questions regarding my rights as a research subject, I may contact Dr. Ernie Higgs (Capital Health Region) at 250 727-4110.

Having read and understood the above, I agree to participate in the study entitled '***Menstrual Irregularities, low-dose hormone therapy and bone density in adolescent athletes***'

_____	_____	_____
Name of participant	Signature	Date
_____	_____	_____
Name of parent/guardian	Signature	Date

Address of participant: _____

_____

Telephone number: _____day _____evening

Appendix D: Three Day Dietary Information and Record

HERBONES RESEARCH

UVic UNIVERSITY OF VICTORIA

3-Day Dietary Record and Analysis

Project Title:

The Effect of Exercise Associated Menstrual Irregularities and Low-dose Hormone Therapy on Bone Mineral Density in Adolescents

The University of Victoria's Human Ethics Committee as well as the Capital Health Region's Research and Ethical Approval Committe have approved this project.

Investigators:

Shana Ooms (MSc Candidate) ph: 370-0759 email: shanad@uvic.ca
Dr. Kathy Gaul (PhD, Supervisor) ph: 721-8380 email: kgaul@uvic.ca

Nutritional Consultant:
Susan Boegman (BSc Dietetics)

Committee:
Dr. Richard Backus (MD)
Dr. Howie Wenger (PhD)
Dr. Marie Hoskins (PhD)

This analysis is necessary to accurately assess calcium intake as well as any other dietary practices that may affect bone mineral density. It will take 10-15 minutes each day for three days to complete. The analysis will be seen only by the principal investigators and will be kept completely confidential.

Instructions

Please record the exact foods, brands, flavours, nutritional content etc. and the quantities of each food consumed with the record sheets provided. Also record the approximate time that they were eaten. Your record will be for two typical days during the week and one typical day on the weekend. (One day MUST BE a WEEKEND day!). Break down the combination of foods as well as possible, and include any condiments (i.e. mayonnaise, mustard, ketchup etc.). Indicate whether or not the quantity reflects before or after cooking where necessary. Use your zip-lock to collect wrappers and cut out any nutritional information that you can from what you eat and save it. The more accurate your information is, the more precisely we can assess your nutritional intake and its contribution to your bone mineral density.

Example:

Time	Food	Quantity/Description	NI
7am	skim milk yogurt	½ cup (125ml), Olympic NonFat, Vanilla	y
	multigrain bagel	1, 11cm diameter, Olafson's	y
	peanut butter	2tsp, light, Squirrel	n
10:00	licorice	5 pieces, Nibs	y
	orange	1 med, sunripe	n
12:30	sourdough bread	2 pieces (med size)	y
	cheddar cheese	4pcs,light, Cracker Barrel, 5x 5x 0.5cm (see picture for size)	y
	tomato	2 slices	n
	mayonnaise	2 tsp, Kraft, light	n

Thank you for your efforts in filling out this questionnaire!

HERBONES RESEARCH

UVIC UNIVERSITY OF VICTORIA

Guidelines for Keeping a Dietary Record

A dietary record is a detailed description of each food or beverage item taken over 24 hours. An accurately completed food record can provide valuable information about the nutritional content of an individual's diet. To assess your diet record correctly, we must be able to clearly picture the foods and beverages that you record. The guidelines below will help you in recording accurate quantities and portion sizes of foods that you eat/drink.

****Include all beverages (pop, juice, coffee/tea etc.) and toppings (sauces, gravy, spreads etc.)****

Portion Size (Quantity)

Measure if you can!!!! Helpful hints for measuring:

- **Measure** how much your regular glasses, cups and bowls hold
- Use a small ruler to measure your food
- Use your hand as a fist = approximately 1 cup of food
- Size of an egg = approximately an ounce
- Deck of cards (LxHxW) = approximately 3 ounces
- Tennis ball size = a medium sized piece of fruit
- **Draw** a one dimensional outline and describe the 3rd dimension-hand in with your record
- Use volume or size or weight to describe your food:

Volume

1 cup or 8oz or 250ml of 2% milk
1 tablespoon (Tbsp) or 15 ml of peanut butter or cream cheese
1 teaspoon (Tsp) or 5ml of sugar or honey

Size

5cm x 5cm x ½ cm piece of cheese (or use inches too!)
1 medium egg, poached
1 small apple
1 digestive cookie, 2 inches in diameter
1 medium bran muffin

Weight

1 ounce = approx 30 grams = approx an egg
2 oz or 60 grams of lean hamburger meat/chicken/fish
(Use label on packages to help you, and then save the label for me!)

****Use the drawings provided to help you determine some sizes and weights****

Description

- Before or after cooking?
- Method of cooking used? (Steamed, boiled, fried, BBQ vs. Raw etc.)
- Wet or dry? With skin/without skin?
- Fresh or frozen or canned? (i.e. veggies, fruit juices)
- Brand names?
- Peeled or unpeeled? Dried / Raw?
- Fortified? (This is where it's good to keep the label)
- "LITE"/"LIGHT"/REDUCED FAT/NON-FAT etc.?
- Sweetened/unsweetened?
- White or whole-wheat or multigrain etc.?
- Keep ***wrappers!***
- Keep any ***nutritional label information!***

Supplements/Vitamins

Keep track of any vitamins or supplements that you ingest on the three days that you are recording. For example: 1 calcium carbonate (500mg elemental calcium) at 7:30am. Write down the nutritional information from the bottle if it is a multivitamin.

3-Day Dietary Record and Analysis

Dietary Record: (Please circle below) Subject Number_____

Record (1) (2) (3); Day [M] [T] [W] [Th] [F] [Sa] [Su] Date:_____

How much Vitamin D did you get today? (Check one choice only)

- 0-10 min of sun on face and hands ☐
- 10-20 min of sun on face and hands ☐
- >20 min of sun on face and hands ☐

Time	Food	Quantity & Description

Appendix E: General Health Questionnaire

HERBONES RESEARCH

UVIC UNIVERSITY OF VICTORIA

General Health Questionnaire

Subject Number_____ **Age**_____ **Date**_____

Please answer the following questions as completely as possible. These questions will provide valuable information about factors that may affect your bone mineral density and they will allow researchers to assign you to the correct research group. **Your individual answers are considered confidential and will not be shared with other participants, parents and/or coaches, and you have the right to refuse answering any question.** If you need further clarification on any of the questions please do not hesitate to ask.
If the question does not apply to you, please indicate this with N/A (not applicable).

General Information

1. Do you consume caffeine in the form of coffee or cola products? If yes, include how many cups per day (or per week) you drink of products that contain caffeine?

   _____

2. Do you **currently** smoke? If yes, how often do you smoke? (per day/week/month)

   _____

3. Have you smoked in the past? If yes, indicate how much you smoked (per day or week) and for how long (months/years).

   _____
   _____

Medical History

1. Have you recently (in the last year) had a complete physical examination by your doctor?
   Yes or No _____ If no, how long ago was your last exam? _____
   Who is your doctor? (General Practitioner)

   _____

2. Do you have a history of bone related health problems in your family, such as stress fractures or osteoporosis (hip fractures)? Describe.

   _____
   _____
   _____

3. Have you ever had a stress fracture? If yes, where was the fracture (i.e. leg), when did you have it and what caused it?

   _____
   _____
   _____

4. Have you ever had your bone mineral density measured? If so, when and where was the procedure done?

   _____

5. Are you **currently** on any medication(s)? If yes, please indicate the name of the medication(s) and **for how long** you have been taking it.

_____

_____

_____

6a. Do you have or have you had any medical conditions that may affect your bone mineral density directly or indirectly? If you are unsure, please list any medical conditions that you have currently (i.e. asthma, epilepsy, hyperparathyroidism, liver/renal disease etc).

_____

_____

6b. Do you currently have or have you had an eating disorder (undiagnosed or diagnosed) or experienced weight cycling? If yes, please explain the time frame surrounding this behaviour in terms of your age (i.e. began when I was 16 and continues today; began when I was 16, stopped when I was 18, began again at 20 and continues today etc.)

_____

_____

_____

7. To your knowledge, have you used any of the following medications in the past 12 months?

- ☐ Corticosteroids (**see list provided)
- ☐ Glucocorticoids (Types of corticosteroids)
- ☐ Anticonvulsants
- ☐ Methotrexate
- ☐ Heparin
- ☐ Gonadotropin Releasing Hormone (GnRH)
- ☐ Thyroid Hormones (for hyperparathyroidism or hyperthyroidism)

8. To your knowledge, have you used any of the above medications **in the last 5 years**?
Yes or No _____
If yes, which medication(s) _____

_____

9. Have you ever taken Estrogen Replacement Therapy (**not an oral contraceptive**) or any other type of hormone therapy? If yes, include the type of therapy and for how long it was taken and the age you were at the time of ingestion?

_____

_____

_____

Menstrual History

1. Are you premenarcheal, meaning that you have never menstruated, or postmenarcheal, meaning that you have experienced your first menstruation at some point.

_____

2. If you are postmenarcheal, how old were you when you had your first menstrual period? (Try to record this as accurately as possible (i.e. to the year and month; or 14 ¼ yrs; 14 ½ yrs; 14 ¾ yrs etc.)

_____

3. Since your first menstrual cycle, have your periods been relatively consistent in terms of cycle length (i.e. usually about 28-32 days or approximately once a month) or inconsistent (i.e. one period every 3 months)? Please describe.

_____

_____

_____

4. When was your last period (Month/Year)?

_____

5. How many days do your periods usually last for?
   - ☐ 1-3 days
   - ☐ 4-7 days
   - ☐ 7+ days
   - ☐ It's never the same length

6. How many menstrual periods have you had **in the last year**?
(i.e. from Dec 1st, 2000 to Dec 1st, 2001)

☐ none	☐ 4-6	☐ 10-12
☐ 1-3	☐ 7-9	☐ >12

7. How many periods have you had in **the last six months?**
(i.e. from July 1st, 2001 to Nov 30th , 2001)

☐ none	☐ 2	☐ 4	☐ 6
☐ 1	☐ 3	☐ 5	☐ >6

8. Are you **currently taking** oral contraceptives prescribed by your physician?
Yes or No _____ (If yes,go to #9; If no, go to #10)

9. **How old** were you when you began taking them? _____ years old?

From today's date, how long have you been taking them for **continuously** (months, years)? _____

If you took them, went off them, and now are on them again, what is **the total amount** of **time** you have been taking oral contraceptives and at what **ages** were you taking them?

In the last year (months)?

_____

In the past (total years/months)? Ages?

_____

Indicate the **type** that you are taking (i.e. Synphasic, Lo-estrin, Brevicon, Marvalon, Alesse etc.) and the **level of estrogen.**

_____

10. If you are **not** currently taking oral contraceptives, have you in the past?
Yes or No _____
If yes, **how old** were you when you **began** taking them? _____
When did you stop taking them (Date and age that you were)_____

If yes, how many months did you take them for **in the last year in total**?
_____

If yes, how many months/years did you take them for **in the past in total**?
_____

11. Are you now or have you ever been pregnant? Yes____ No_____

Physical Activity

1. How often are you involved in **vigorous weight bearing** physical activity (activity which elevates your heart rate and increases your breathing rate) **per week**?

☐ 1-3 times per week	☐ 5 x per week	☐ 7 x per week
☐ 4 x per week	☐ 6 x per week	☐ >7 x per week

2. Approximately how do long do you do this activity each time?

☐ 0-30 min	☐ 45-60min	☐ 75-90min
☐ 30-45 min	☐ 60-75min	☐ >90min

3. What is the total amount of hours per week that you spend doing vigorous weight bearing activity?_____

4. What type of vigorous weight bearing physical activities do you do? Please describe. (i.e. skating, cross-country running, highland dance etc.)
_____
_____

5. Please describe your level of activity prior to the last year (i.e. gymnastics from age 12-17, 3 times per week etc.).
_____
_____

6. Do you participate in a regular weight-training (resistance) program? If yes, describe how long you have been participating in the training program, how many times per week you weight train, and for how long each time.
Weight training since _____
Times per week _____
Duration of each training session_____

Thank you for taking the time to participate and to answer these questions as completely as possible! Remember, your answers are **confidential** and they will not be shared with other participants, parents and/or coaches and you do have the right to refuse to answer any of the above questions.

Appendix F: EAT-26

Eating Attitudes Test-26 (EAT-26)

Subject Number: _____ Age: _____ Date: _____

Current Weight: _____ (lbs) Highest Weight: _____ (lbs) Lowest (Adult) Weight: _____ (lbs)

Please check a response for each of the following questions	Always	Usually	Often	Sometimes	Rarely	Never
1. Am terrified about being overweight.	☐	☐	☐	☐	☐	☐
2. Avoid eating when I am hungry.	☐	☐	☐	☐	☐	☐
3. Find myself preoccupied with food.	☐	☐	☐	☐	☐	☐
4. Have gone on eating binges where I feel that I may not be able to stop.	☐	☐	☐	☐	☐	☐
5. Cut my food into small pieces.	☐	☐	☐	☐	☐	☐
6. Aware of the calorie content of foods that I eat.	☐	☐	☐	☐	☐	☐
7. Particularly avoid food with high carbohydrate content (ie bread, rice, potatoes, etc.)	☐	☐	☐	☐	☐	☐
8. Feel that others would prefer if I ate more.	☐	☐	☐	☐	☐	☐
9. Vomit after I have eaten.	☐	☐	☐	☐	☐	☐
10. Feel extremely guilty after eating.	☐	☐	☐	☐	☐	☐
11. Am preoccupied with a desire to be thinner.	☐	☐	☐	☐	☐	☐
12. Think about burning up calories when I exercise.	☐	☐	☐	☐	☐	☐
13. Other people think that I am too thin.	☐	☐	☐	☐	☐	☐
14. Am preoccupied with the thought of having fat on my body.	☐	☐	☐	☐	☐	☐
15. Take longer than others to eat my meals.	☐	☐	☐	☐	☐	☐
16. Avoid foods with sugar in them.	☐	☐	☐	☐	☐	☐
17. Eat diet foods.	☐	☐	☐	☐	☐	☐
18. Feel that food controls my life.	☐	☐	☐	☐	☐	☐
19. Display self-control around food.	☐	☐	☐	☐	☐	☐
20. Feel that others pressure me to eat.	☐	☐	☐	☐	☐	☐
21. Give too much time and thought to food.	☐	☐	☐	☐	☐	☐
22. Feel uncomfortable after eating sweets.	☐	☐	☐	☐	☐	☐
23. Engage in dieting behaviour.	☐	☐	☐	☐	☐	☐
24. Like my stomach to be empty.	☐	☐	☐	☐	☐	☐
25. Enjoy trying new rich foods.	☐	☐	☐	☐	☐	☐
26. Have the impulse to vomit after meals.	☐	☐	☐	☐	☐	☐

Adapted from Garner, Olmsted, Bohr, & Garfinkel (1982, p. 875) and Garner & Garfinkel (1979, p. 278). Copyright 1979 and 1982. Note: Cutoff score = 20.

Scoring System: Responses are weighted 0-3, with a score of 3 assigned for the response farthest in the symptomatic direction ("always" or "never" depending on whether the item is keyed in the positive or negative direction-item 25 is the only negatively keyed item), a sore of 2 for the immediately adjacent response, 1 for next adjacent response, and 0 for the three responses farthest in the asymptomatic direction.

VITA

Surname: DeNeef Ooms Given Names: Shana Lorraine

Place of Birth: Kamloops, British Columbia, Canada

Educational Institutions Attended:

University of Victoria	1990 to 2002

Degrees Awarded:

B.Sc. Kinesiology (Honours)	University of Victoria	1996

Honours and Awards:

Canadian Academy of Sport Medicine Research Grant	2000
Mike Latoski Memorial Award for Excellence in Human Physiology	1993
T.S. McPherson Scholarship	1990 to 1992
Commemorative Medal for the 125th Anniversary of the Confederation of Canada	1992
Premier's Award of Excellence	1990

UNIVERSITY OF VICTORIA PARTIAL COPYRIGHT LICENSE

I hereby grant the right to lend my thesis to users of the University of Victoria Library, and to make single copies only for such users or in response to a request from the Library of any other university, or similar institution, on its behalf or for one of its users. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by me or a member of the University designated by me. It is understood that copying or publication of this thesis for financial gain by the University of Victoria shall not be allowed without my written permission.

Title of Thesis:

Bone Health in Adolescent Athletes: The Influence of Menstrual and Oral Contraceptive Status on Bone Mineral Density and Content of the Lumbar Spine and Proximal Femur

Author 

Shana L. DeNeef Ooms

April 22, 2002