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CYTOKINES, DEPRESSION, AND BONE HEALTH: EXPLORING THE ASSOCIATIONS
IN HEALTHY ADOLESCENT FEMALES

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ABSTRACT

Background: Cytokines such as interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α are cellular messengers related to immune and inflammatory responses in the body. Cytokines have been implicated as being involved in both depression, a mental health condition affecting over 350 million people worldwide, and bone breakdown, a disease process leading to increased risk for osteoporosis and in turn, potential fracture. Specifically, these pro-inflammatory markers have been associated with increased depressive symptoms in adolescent females and have also been identified as osteoclastogenic factors in bone resorption in both animals and humans. However, the nature of the role each factor plays is unclear. Given the inter-developmental stage variability and lifetime trajectory of bone growth and breakdown, it is important to identify potential contributing factors in order to improve health promotion during key periods of development in females. The purpose of this thesis is to address the following clinical question in healthy adolescent girls: What are the associations of baseline cytokine levels with depressive symptoms and bone mass one year later?

Methods: This is a secondary analysis of an existing cross-sequential, longitudinal dataset collected (2003-2006) in a three-year study of 262 female adolescents. Data related to levels of cytokines IL-1 β , IL-6, and TNF- α , measured via bioassay, from Time 1 (initial clinic visit) were analyzed in connection with levels of depressive symptoms measured by the Children's Depression Inventory (CDI). Then measures of bone mass (Whole Body Bone Mineral Content; BMC, Hip Bone Mineral Density; BMD, and Lumbar Spine BMD), via dual energy x-ray absorptiometry, from Time 1 and Time 2 (one year later) were used to consider change in bone mass across time. Multiple covariates were included: (e.g., gynecological age, serum 25-hydroxy Vitamin D, weight). The association of depressive symptoms and bone-specific cytokines on

bone mass was then analyzed. Data analysis involved descriptive statistics, correlations, and three separate stepwise regression models.

Results: The majority of the sample was white (61.80%); averaged 5.25 (SD = 0.26) feet in height and 137.02 (SD = 40.12) pounds in weight. Subjects reported age at menarche (N = 253, M = 12.40, SD = ±1.24) in years, and gynecological age in years was subsequently calculated (N = 209, M = 3.39, SD = 1.90). None of the observed independent variables (Time 1 IL-1 β , IL-6, TNF- α , CDI) were significantly correlated with any observed dependent variables (Time 1 Whole Body BMC, Lumbar Spine BMD, and Hip BMD). Only IL-1 β was significantly related to Lumbar BMD (B = -.0002, SE = .0001, $p < .01$) upon stepwise regression analysis. Multiple covariates such as weight, Vitamin D intake, and gynecological age exhibited significant effects on bone health.

Conclusions and Implication: Psychological assessment during adolescence remains a crucial consideration for females, but proof of the impact of depressive symptoms on bone health was not reflected in these particular analyses. With one exception, the specific cytokines also showed no association with bone health. Future research should capitalize on the longitudinal changes in these factors and if cytokines may play a mediating role in how depressive symptoms may influence bone health. Health promotion and teaching regarding behaviors promoting long-term bone health continues to be a significant area of interest for the adolescent female population and those primary care providers involved in their care.

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Chapter 1

Introduction

Cytokines are cellular products that exert specific effects on various processes of health and disease in the human body (Wilson & Warise, 2008). As cellular messengers, they play a role in regulating immune and inflammatory responses as well as contributing to development and maintenance of tissues and organs. Cytokines can be anti-inflammatory, pro-inflammatory, chemotactic, or growth-promoting. Pro-inflammatory cytokines, part of the current study's focus, act in the body by promoting systemic inflammation (Clough & Roth, 1998). Systemic inflammation is consistent with the development or progression of a wide variety of diseases. Decreases in cytokine levels can negatively impact cellular immune responses, further potentiating disease processes, and recent evidence has supported that elevated pro-inflammatory cytokine levels correlate with obesity (Schmidt, et al., 2013), depression (Byrne, et al., 2013), and other inflammatory and autoimmune disease processes as well (Riis, et al., 2013).

The relationships between cytokines, depression, and bone health are varied and involve a wide range of body processes. Since adolescence is a time of rapid physical and mental growth and development dependent on many biological factors, research on cytokine levels across this time in the lifespan may be crucial in delineating tangible mechanisms regarding inflammatory body processes and potential presence of disease or disorder. Depression is a mood disorder characterized by persistent feelings of sadness, hopelessness, and loss of interest in daily activities, but depressive symptoms can occur in the absence the diagnosed disorder (World Health Organization, 2012). There are an estimated 350 million people affected by depression

worldwide, with women being two times more likely than men to develop the disorder or its related symptoms (World Health Organization, 2012). The prevalence difference between the sexes becomes evident beginning at adolescence, indicating a need for further analysis of the mechanisms associated with this sex discrepancy (Cyranski, et al., 2000). Bone health is measured by bone mineral density (BMD), or how much mineral fills a given space, and bone mineral content (BMC), a measure of mass. One key aspect of physical development during adolescence involves achievement of peak bone mass, which is the highest point of bone density in the lifespan achieved by the end of skeletal maturation (Cashman, 2007). Skeletal development accelerates and is largely completed by the end of adolescence, but bone build-up and breakdown is a continuous process occurring across the lifespan (Cashman, 2007). Bone build-up slows down later in life, so it is crucial to maximize healthy accrual during development to minimize the severity of bone loss after aging (Cashman, 2007). Lack of sufficient peak bone mass accrual can lead to poor bone health later in life. Subsequently, weakened bone structure puts individuals at an increased risk for developing osteoporosis (NIH, 2011). The dynamics of the process of bone formation requires optimal development in peak bone mass during adolescence in order to best minimize debilitating effects of resorption later in life. Pro-inflammatory cytokines IL interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α , which have been associated with increased depressive symptoms in adolescent females, have also been associated to bone density as osteoclastogenic factors in bone resorption (Abuhashish, et al., 2014; Kahl, et al., 2005; Moieni, et al., 2015). This indicates higher depressive symptoms and associated increased serum levels of IL-1, IL-6, and TNF- α could potentiate the ongoing process of bone breakdown.

It is evident that certain deviations in “normal” adolescent development, including incidence of depression or depressive symptoms, can contribute to physiological dysfunction with potentially long term effects like compromised bone health. The dynamic of these abnormalities indicates an urgent need to pinpoint the contributions to dysfunction and stop or reverse resulting negative developmental outcomes.

Problem

Inflammation has been identified to play a role in physical and mental health (Dantzer, O’Connor, Freund, Johnson, & Kelley, 2008). The same inflammatory markers associated with depressive symptoms have been linked with bone breakdown and osteoporotic risk (Abuhashish, et al., 2014; Kahl, et al., 2005; Moieni, et al., 2015). However, methods of measurement, subject population, whether studies are longitudinal or cross-sectional, and associations between variables have been inconsistent across studies. Few studies incorporate longitudinal data, and none have focused data on all three variables (cytokines, depression, and bone health) within one specific age and gender stratification. It is important to further investigate the topic to identify potential contributors to associations between depressive symptoms and bone health.

Significance

The study will be significant in identifying possible inflammatory contributions to bone health, specifically with inflammation related to presence of depressive symptoms. Systemic inflammation evidenced by increases in levels of cytokines IL-1, IL-6, and TNF- α could

correlate to decreased bone health beginning as early as adolescence. Prevalence of poor bone health among adolescents indicates a need for early intervention regarding contributors to systemic inflammation, such as depression. Narrowing the population of interest will help control for potentially extraneous and confounding variables, such as hormone levels and gender differences. Collecting data longitudinally will strengthen the significance of findings by allowing for identification of changes within individuals over a one-year period. In determining what disease processes might influence inflammatory responses in the body, this study may guide possible treatment or prevention strategies for depression and osteoporosis, two very prevalent and debilitating disorders.

Nursing implications. Practically speaking, osteoporosis is a highly prevalent and also preventable disease process largely affecting postmenopausal women over the age of sixty. It is characterized by increased bone breakdown and subsequent weakening of bone structure (NIH, 2011). Health promotion is imperative in minimizing risk for osteoporosis and future fracture in developing female adolescents because such a significant majority of bone growth occurs by the end of adolescence. Further evidence on the correlations between depressive symptoms and subsequent bone health could reinforce a need for patient education over the course of pediatric well child visits. It would be practical to put an emphasis on bone density screening in high-risk candidates based on modifiable and non-modifiable risk factors, as well as nutritional and lifestyle guidelines for all ages to promote bone health. This type of teaching is highly time-sensitive due to peak bone mass accrual during adolescence; therefore, well-established guidelines need to be reinforced or reassessed based on this study and the current body of nursing research on the subject.

Objective. The aim of this secondary analysis is to address the following clinical question in healthy adolescent girls: (1) What are the associations of baseline cytokine levels with depressive symptoms and bone mass one year later? The research process will involve analyzing data related to levels of cytokines IL-1 β , IL-6, and TNF- α in connection with levels of depressive symptoms in adolescent females at Time 1, initial visit. Second, it will utilize measures of bone mass (Whole Body Total BMC, Hip BMD, and Lumbar BMD) at Time 1 and Time 2 (one year later) to consider change in bone mass across time. The influence of depressive symptoms and bone-specific cytokines on bone mass will be analyzed.

Chapter 2

Literature Review

The cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α , have been identified as playing a role in both bone breakdown and depression (Kahl et al., 2005; Wilson & Warise, 2008). Specifically, these pro-inflammatory markers have been linked to increased depressive symptoms in adolescent females (Moieni, et al., 2015) and have also been identified as osteoclastogenic factors in bone resorption in both animals and humans (Abuhashish, et al., 2014; Kahl, et al., 2005). Given the inter-developmental stage variability and lifetime trajectory of bone growth and breakdown, it is important to identify potential contributing factors in order to improve health promotion during key periods of development in females. This particular chapter will review the role and significance of the relevant cytokines by examining research findings on these cytokines and their separate relationships to depression and bone health, and identify implicative connections between depression and bone health in literature. As evidenced by Figure 1, current research in adults suggests that the association between depression and inflammation is bidirectional, inflammation correlates with low bone mineral density (BMD), and depression could therefore have a direct association on BMD as well. This study will attempt to confirm or solidify the dynamics of these relationships within a sample of adolescent females.

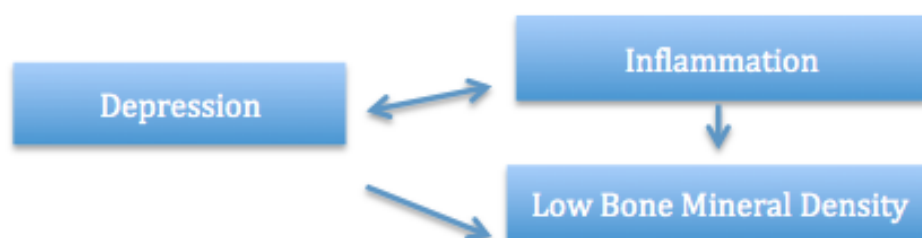


Figure 1. Identifying potential relationships among variables.

The literature search was conducted in PubMed and PsycInfo databases using the following search terms: cytokines, inflammatory markers, depression, depressive symptoms, bone health, bone density, and bone mass (Figure 2). Articles were limited to those published in English between 2005 and 2015. They included randomized controlled trials, systematic reviews, meta-analyses, and longitudinal studies, all of which were in vivo studies. These search criteria produced a wealth of relevant articles, in addition to a few extending somewhat beyond the scope of this particular study but still providing clinically significant information.

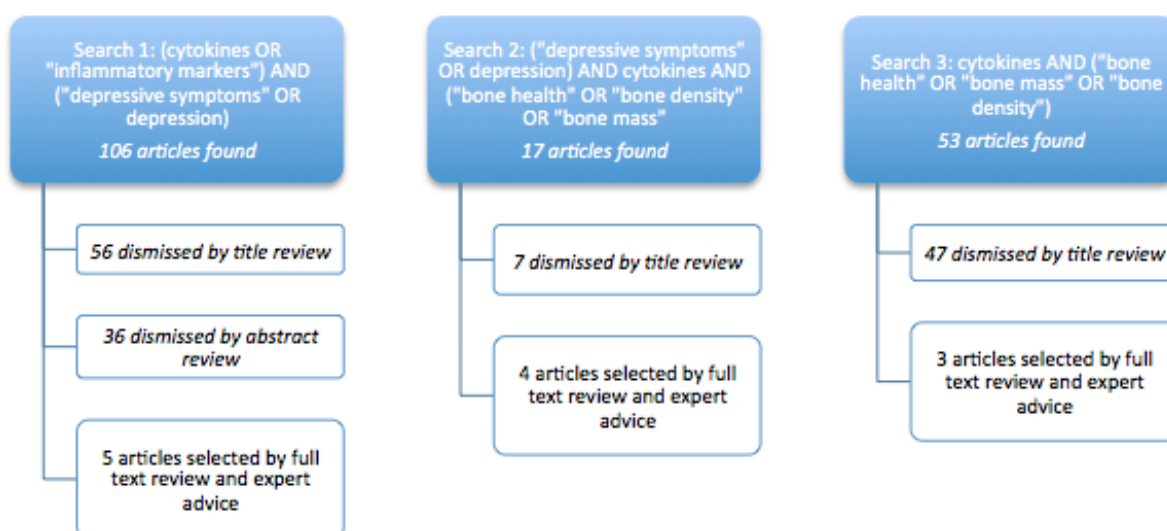


Figure 2. Process of article selection.

Role and Significance of Cytokines

As stated, this study will focus on levels of three pro-inflammatory cytokines (IL-1, IL-6, and TNF- α) and their potential roles in systemic inflammation, whether stemming from or contributing to depressive symptoms and bone breakdown. These three particular cytokines were chosen based on their prevalence in literature and tendency to be referenced in studies deal with both depression and bone health. The pro-inflammatory nature is significant because these types of cytokines promote systemic inflammation (Wilson & Warise, 2008). IL-1, IL-6, and TNF- α are categorized as interleukins, which are important mediators and enhancers of the inflammatory response. Stemming primarily from leukocytes and macrophages, interleukins have been found to interact with various cells in the body to regulate immune and, consequently, other physiologic processes (Wilson & Warise, 2008). These three inflammatory modulators are not always studied together, but it is important to note that they are the cytokines of focus in this study.

Cytokines and Depression

The general consensus regarding depression is that the immune system plays a role in the pathogenesis of the disorder, and that levels of many cytokines are elevated in subjects with major depressive disorder or depressive symptoms (Dannehl et al., 2014; Moieni et al., 2015; Schmidt et al., 2014). Research to be discussed supports this hypothesis, with many studies incorporating both psychological and somatic dimensions in finding pro-inflammatory cytokine elevations. It will be important to identify any differences that may occur within and among age and gender stratifications.

Cytokines and depression in adults: gender differences. Studies on inflammatory-depressive interactions are largely focused on the adult population, frequently highlighting gender differences. Two studies found significantly increased levels of TNF- α in depressed male and female subjects (Dannehl et al., 2014; Schmidt et al., 2014), and one (Schmidt et al., 2014) reports additional elevations in IL-5, IL-12, IL-13, GM-CSF, IFN- γ , and TNF- α in depressed versus non-depressed total sample. When analyzing data in terms of predictability of depressive symptoms on cytokine changes, no significance was found using hierarchical regression analysis (Dannehl et al., 2014). However, when a sex-stratified regression analysis was performed, higher somatic and depressive symptoms during a two-year window significantly predicted an increase in TNF- α in only women with major depressive disorder (Dannehl et al., 2014). In one randomized-controlled trial, male and female subjects were injected with an *e. coli* derived endotoxin to measure immune, depressive, and socioemotional response to an inflammatory challenge (Moieni et al., 2015). Results indicated that in those receiving the endotoxin (versus placebo), an increase was observed in TNF- α , IL-6, depressed mood, and social disconnectedness; the latter of the two were found to be higher in females than males (Moieni et al., 2015). Additionally, increased TNF- α and IL-6 correlated with increased social disconnection in females, but not in males (Moieni et al., 2015). Serum TNF- α elevations contributed the most conclusive evidence for cytokine elevations in relation to depression. These results support a need for gender-specific analysis of depression and inflammatory response. Differences in inflammatory and depressive correlations exist, but the intricacies of these variations are unclear.

Cytokines and depression in youth. Evidence regarding inflammation and depression in children and adolescents is less robust, but some early findings have been reported. In a study

measuring acute phase protein and cytokine levels in serum and saliva in male and female adolescents (Byrne et al., 2013), no significant differences were found between depressed and non-depressed clinical groups. This could be attributed to a small sample size (n=35). However, this same study found that adolescent salivary samples yielded higher cytokine detection rates, with significant serum and salivary correlation of CRP (C-reactive protein), IL-2, IL-12p70, and IFN- γ when non-detectable in serum samples. When non-detectable serum levels were excluded, however, only CRP was found to have significant correlation between serum and saliva. (Byrne et al., 2013). These results indicate that salivary cytokine levels may not be as reliable an indicator as serum levels. In a longitudinal analysis of female adolescents identified as being at high risk for depression, diagnostic interviews and serum cytokine analyses were performed every 6 months for 2.5 years. Results indicated that in subjects determined to have experienced increased childhood adversity, increased depressive symptoms were accompanied by increases in CRP and IL-6. Additionally, higher IL-6 levels were predictive of depression 6 months later. In subjects without childhood adversity, depression and inflammation did not appear to correlate (Miller & Cole, 2012). No current research exists highlighting gender differences in depressed youth.

Cytokines and Bone Health

Bone health is determined by measures of BMD or how much mineral fills a given space, and bone mineral content (BMC), a measure of mass (PubMed Health, 2015). Cytokines play a role in bone remodeling by osteoblasts and bone resorption by osteoclasts across the lifespan. When the physiologic balance between the two processes becomes unbalanced in favor of

osteoclasts a number of disorders can occur, such as osteoporosis (Takayanagi, Kim, & Taniguchi, 2002). The purpose of the current study involves understanding physiology of females and potential contributing factors to osteoporotic risk, as evidenced by lower bone mass in adolescence. For this reason, and because literature is largely focused on bone breakdown through certain diseases, it is important to look at cytokine interactions in disease-specific osteoclast activity.

Cytokines and osteoporotic risk. Two particularly salient studies have observed and analyzed inflammation and bone breakdown as they relate to risk for osteoporosis in postmenopausal women. Estrogen, which is a regulator of inflammatory activity, has decreased production after menopause meaning that estrogen deficiency can increase cytokine levels (Gertz et al., 2010). For this reason, postmenopausal women are particularly at risk for decreased BMD or resultant osteoporosis. In healthy postmenopausal women, BMD, BMC, and serum cytokine levels were measured at baseline, 6 months, and 12 months. Analysis of longitudinal data found significant associations between IL-6 and TNF- α with the percentage change in BMD and BMC of lumbar spine, hip, and total body (Gertz et al., 2010). In fact, inflammatory markers accounted for 1.1%-6.1% of variance in the bone density changes over one year (Gertz et al., 2010). These findings are significant in identifying possible associations, but it is unclear whether these links can describe any cause and effect.

In a more complex study on overweight and obese postmenopausal women, a somewhat similar association was found. Change in a TNF- α receptor, soluble TNF receptor 1 (sTNFR1), was significantly associated with change in femoral neck BMD in obese women participating in a weight loss program combined with aerobic exercise (Silverman, Nicklas, & Ryan, 2009). It is difficult to draw any conclusive associations regarding inflammation from this study, however,

due to the confounding effects of obesity and associated systemic inflammation. The mechanisms of cytokines on bone health are likely far more complex than what the study controlled for.

While neither of these studies involved adolescent females certain questions are suggested. If variations in estrogen levels (falling estrogen in the menopausal populations in the previous studies) impact cytokine levels one could hypothesize that rising estrogen levels in adolescents may also impact cytokine levels. This, however, is currently unknown.

Depression and Bone Health

Depression, a psychological disorder with some physiological implications, has been tied to bone health. Research to be discussed suggests that major depressive disorder and depressive symptoms precipitate low BMD with potential cytokine involvement, but studies tend to be heterogeneous (Cizza, et al., 2012; Eskandari et al., 2007; Kahl et al., 2005; Spangler et al., 2008). Samples typically consist of female subjects (Cizza, et al., 2012; Eskandari et al., 2007; Kahl et al., 2005; Spangler et al., 2008). Clinical implications mostly center around the role of menopause on osteoporosis risk in depressed subjects (Cizza, et al., 2012; Eskandari et al., 2007; Spangler et al., 2008). Aims and hypotheses do not tend to stray from this general theme in the aforementioned body of research.

Depression and bone mineral density. Recent research has found that BMD is lower in subjects with major depressive disorder (MDD). In a 36-month prospective study of premenopausal women with MDD (n=92) and healthy controls (n=44), the women with MDD tended to have lower BMD over the three years in which the study was conducted (Cizza, et al.,

2012). In another cohort of healthy subjects (comparison group - patients with bipolar disorder (BPD), some with MDD), the group with MDD had the lowest BMD (Kahl et al., 2005). There were no deficits in BMD found in the subjects with BPD alone (Kahl et al., 2005). Several studies examined bone mass at specific skeletal regions. One study found that in women with MDD, there was a higher incidence of low BMD at the femoral neck, total neck, and lumbar spine, but not at the radius of the arm (Eskandari et al., 2007). In a more recent study, Estonian women with MDD were found to have lower BMD at the lumbar spine only and not the femur (Sommerhage, Kull, Schweiger, & Rudolf, 2013). Earlier research in the same dataset as the current study indicates that in adolescent female subjects aged 11-17, an increase in depressive symptoms correlated with lower total body BMD and BMC (Dorn et al., 2008).

Cytokines, depression, and BMD. Research is lacking on the particular three-part frontier encompassing the relationships between cytokines, depression, and bone health. There are fewer studies and a weaker body of evidence, and it is unclear what role each variable plays. It is possible that cytokines mediate the effects of depression on bone health (e.g.: depression influences cytokine levels, which in turn influence bone health). In Kahl's 2005 study, subjects with comorbid MDD and BPD were found to have lower BMD accompanied by increased levels of TNF- α and IL-6. Additionally, BPD patients without comorbid MDD did not see the same alterations in inflammatory cytokines (Kahl et al., 2005). Similarly, the 2007 study by Eskandari reporting lower BMD in women with MDD found that these women also had increased levels of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α , and lower levels of anti-inflammatory cytokines. More recently, the study on women with MDD in Estonia reported no signs of elevated cytokine levels, including IL-6 and TNF- α (Sommerhage et al., 2013). This could be

attributed in part to lack of power in the study, but it still indicates a need for further research in this area.

Controlling for medications. Antidepressant medications such as selective serotonin reuptake inhibitors (SSRIs), along with some anti-seizure drugs, chemotherapies, contraceptives, proton pump inhibitors (PPIs), and steroids among others can be harmful to bones (National Osteoporosis Foundation, 2015). It is important to note that in each of the aforementioned studies on cytokines, depression, and bone health, medications were not controlled for in the sample or data analysis (Eskandari et al., 2007; Kahl et al., 2005; Sommerhage et al., 2013). Controlling for medication use among sample subjects is important in producing results that may clarify the actual impact of medications on bone health. The study by Dorn, 2008, of adolescent females with depressive symptoms did, in fact, control for medication use, but did not include any data on cytokine levels. One cohort study of postmenopausal women, however, found varied results when controlling for antidepressant therapy (Spangler et al., 2008). In the total sample, no significant associations were found between depressive symptoms or antidepressant therapy and 3-year change in BMD. In women not using antidepressants, however, there was a significant difference in whole body BMD change over three years in women with depressive symptoms compared to those without (Spangler et al., 2008). Lack of control for medication is problematic because different medications or doses could potentially exert varying influence on bone resorption. These factors make it difficult to have confidence in findings on the associations and indicate a need for controlled studies encompassing all relevant factors.

Conclusion

Existing literature on this particular comprehensive topic indicates a serious need for more research on the relationships between cytokines, depression, and bone health. Studies are heterogeneous in terms of the population of focus, variables of interest, methods of collection, and results. Furthermore, it can be concluded that building current knowledge has been difficult and even inefficient, given this heterogeneity. In bone health, very little research exists specific to adolescents due to a lack of concern since osteoporosis is not a disorder in youth. However, there is a pressing need for more evidence pertaining to adolescent bone health and future outcomes in order to best foster health promotion and future risk reduction. Because adolescence is a time of crucial physical and psychological development, and females are more likely to develop major depressive disorder compared with males (WHO, 2012), it would be highly beneficial to specifically examine longitudinal changes in cytokine levels in conjunction with occurrence of bone accrual (or breakdown) and depression. Fortification of this realm of research is necessary, and this study will work to bring new findings to the table and also build on what has been previously theorized.

Chapter 3

Methods

Cytokines, depression, and bone health have a dynamic relationship with inconclusive mechanisms of influence on each other. Cytokines, or markers of inflammation in the body, have been connected to disease processes related to both depression and bone resorption. By studying all three variables, potentially causative associations may become clearer (although the current study will not work to prove causation, only correlation). This analysis examined the following question in adolescent females: What are the associations of baseline cytokine levels with depressive symptoms and bone mass one year later?

Design

This is a secondary analysis of an existing dataset. The current study draws from data that has previously been collected in a three-year accelerated longitudinal study focusing on the impact of smoking and depressive symptoms on bone health in adolescent females (Dorn et al., 2013). The design is cross-sequential. Subjects were recruited in the following age cohorts: age 11, 13, 15, and 17. There are data from each subject's three annual visits in addition to phone interviews at 3-month intervals (Dorn et al., 2013). This design allows for data to be collected at every age across adolescence in only three years. Relevant data was extracted and analyzed according to the aims of this current study which, however, did not take advantage of the cross-

sequential design. This secondary analysis uses data at Time 1 and Time 2 (one year later) to analyze the four cohorts.

Sample

The parent study consists of 262 healthy adolescent girls in four age cohorts: age 11, 13, 15, and 17. The reason for enrolling girls starting at age 11 was that the population of interest is healthy, typically developing adolescent females and their bone accrual. These ages allowed for examining bone accrual and obtaining peak bone mass as the ages of adolescents ranged from 11-19 years by the end of the parent study (Dorn et al., 2013).

Selection and screening of sample. Subjects for the parent study were recruited from a teen health clinic in a large Midwestern children's hospital as well as from the surrounding community. Screening interviews were performed on all subjects after parental consent to the screen. This information was used to determine preliminary eligibility for the study. Sample recruitment was carried out in order to accurately represent the proportions of smokers within each age cohort based on national statistics, but this is relevant only in the parent study (Dorn et al., 2013). When eligible, parents provided consent for the study and adolescents provided assent. The study was approved by the Institutional Review Board of the associated children's hospital.

Inclusion and exclusion criteria. Certain criteria needed to be met for inclusion in the parent study. Inclusion criteria were: (1) female; (2) age 11, 13, 15, or 17; (3) fluent in English; (4) parental permission; and (5) adolescent assent. Smoking status criteria and depressive symptoms criteria also needed to be met, although smoking history does not play a role in the

current study. Exclusion criteria included: (1) known or suspected pregnancy; (2) primary amenorrhea if 16 or older; (3) secondary amenorrhea; (4) body mass index (BMI) <5th percentile or >95th percentile; (5) use of any medication altering bone health; (6) medical disorder influencing reproductive or bone health; (7) psychological disabilities preventing comprehension of the study protocol; and (8) unwillingness to remove jewelry for x-rays (Dorn et al., 2013).

Data Collection

Plan. Data was drawn from the parent study's dataset, including all 262 study participants, on all primary variables – cytokines, depression, and bone health – among other relevant covariates such as gynecological age, contraceptive use, and physical activity. Cohorts were based on age of the subjects at Time 1, or age at recruitment – either age 11, 13, 15, or 17. Then, Time 2 data came from assessment of the same subjects within each cohort one year later.

Measures and instruments. Measures included at Time 1 and Time 2 were as follows: cytokine (interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)- α) levels via bioassay, depressive symptoms via Children's Depression Inventory (CDI), and bone composition measurements via dual energy x-ray absorptiometer (DXA). Multiple covariates were also measured, including: race by parent report (recoded as 1 = black and 0 = non-black); pubertal stage by trained clinician exam with the Tanner breast stage scale (stages I-V); height measured with a wall-mounted Holtain Ltd. stadiometer; weight measured with a Scaletonix digital scale; BMI computed from height and weight values ($BMI = \text{weight (kg)} / \text{height (m)}^2$); age at menarche by clinician interview in years and months; gynecological age by computation based on age at menarche in years and months; lifetime duration of hormonal contraceptive use versus other

contraceptive use by clinician interview; weight-bearing physical activity in the week prior to assessment measured with the Physical Activity Questionnaire for Older Children (1 [low; did not do] to 5 [high; 7+ times]); daily calcium intake measured with a modified food frequency intake questionnaire; and Vitamin D status at baseline by radioimmunoassay of serum 25-hydroxy Vitamin D (Dorn et al., 2013).

Bioassay: Time 1. Serum cytokine levels (including IL-1, IL-6, and TNF- α) were collected by intravenous blood sampling procedures. Samples were drawn at approximately 1-2 p.m. in all participants. Cytokines were measured using the LincoplexTM methodology and the Luminex platform. Samples were centrifuged, frozen at -70° C, and assayed in batches based on time of measurement to minimize interassay variability. Accuracy is reported as 93-112%, inter-assay coefficients of variation are 2.16-14.27%, and intra-assay coefficients of variation are 3.11-5.86% (Riis et al., 2013).

Children's Depression Inventory (CDI). CDI is a 27-item, self-rating, symptom oriented scale used to determine the presence and severity of depressive symptoms in children aged 7-18. In each item, subjects choose statements which best reflect their thoughts and feelings in the past two weeks, with each statement being associated with a rating from 0-2. Results are scored 0-54. Scores above 19 are considered to suggest clinically significant depression (Kovacs, 1992). Internal consistency reliability ranged from $\alpha=0.71$ to $\alpha=0.89$ (Kovacs, 1992). The parent dataset contains the CDI Total Score (T-score). When analyzing T-score values, guidelines suggest that scores ranging from 45-55 are average. Higher scores, specifically those above 65, are indicative of potentially clinically depressed individuals (Kovacs, 2004).

Dual energy x-ray absorptiometry (DXA). DXA is a technology measuring bone mineral composition to provide representation of bone strength and fracture risk through a 2-dimensional

representation of the 3-dimensional properties of bone. Bone sites observed include total body, left proximal femur or “hip” (total proximal femur and femoral neck), and lumbar spine (L1-L4) using a Hologic QDR4500 bone densitometer. Bone mineral content (BMC) is measured in grams, and bone mineral density (BMD) in grams per square centimeter. The current study limited analyses to whole body total BMC, and hip and lumbar BMD. The same categories of data were collected at Time 1 and Time 2 (one year later). DXA body scans are highly precise and accurate. In repeated DXA for BMD, the coefficient of variation is $1.15 \pm 0.83\%$ (Dorn et al., 2013).

Statistical Analysis

Research Question. *What are the associations of baseline cytokine levels and depressive symptoms with bone mass one year later?* Descriptive statistics were analyzed at Time 1 compared to Time 2 to determine changes in bone mass, and how they may correlate to Time 1 cytokine levels. The type of statistical analysis was correlational. IBM SPSS was used for all data analyses (IBM Corp., 2015).

Data analyses were conducted in a series of steps. First, descriptive statistics were reported for all relevant primary variables and covariates. Some descriptive statistics for covariates collected at multiple time points were computed, including physical activity and calcium intake. Next Pearson correlations were computed involving all relevant independent variables, dependent variables, and covariates. Finally, hierarchical regression was used. This means that three separate linear regression analyses were performed, each successively adding the independent cytokine variables. This method was chosen to distinguish between levels of

influence when comparing covariates and independent variables. Regression analyses were split based on one of three dependent variables. Two separate regression models were reported for each dependent variable: Whole Body Total BMC, HIP BMD, and Lumbar Spine BMD. Each Model 1 used covariates, CDI, and the respective dependent variable only, and Model 2 incorporated each of the three cytokines as well. Levels of significance were set at $p < 0.05$ and $p < 0.01$.

Conclusion

In conclusion, this section introduced the analytic plan, described the study design, sample, data collection procedures, and statistical analysis. This secondary analysis of longitudinal data explored and described relationships among cytokines, depression, and bone health in adolescent females. The sample of subjects were representative of a population requiring improved health promotion strategies. Data was collected on the following measures and their respective instruments: cytokine (IL-1, IL-6, TNF- α) levels via bioassay, depressive symptoms via CDI, and bone composition measurements via DXA. Correlational data analysis and regression analysis were used to analyze associations between variables in the two time points.

Chapter 4

Results

Introduction

In this chapter, the results of the analysis of associations of baseline cytokine levels with depressive symptoms and bone mass one year later are presented. Descriptive statistics are provided, along with Pearson correlations and multiple linear regressions.

Sample Demographics

Data were collected from a sample of 262 healthy female adolescents aged 11-17 at time of recruitment. Mean age was 14.94 years (SD = 2.17), with cohort distributions provided in Table 1. The majority of the sample was white (61.80%). The sample averaged 5.25 (SD = 0.26) feet in height and 137.02 (SD = 40.12) pounds in weight. Subjects reported age at menarche (N = 253, M = 12.40, SD = ±1.24) in years, and gynecological age in years was subsequently calculated (N = 209, M = 3.39, SD = 1.90). Only 37.80% (N=99) of the sample reported using hormone related contraception, including Depo-Provera (N=41, 15.8%) and oral contraceptive pills (OCP) (N=56, 21.5%). Vitamin D status of participants was 20.45 ng/mL (SD = 9.22) via serum radioimmunoassay of 25-hydroxy vitamin D. Average daily calcium intake from food via self-report from Time 1 (baseline) to Time 2 (1 year later) was computed as 765.59 mg (SD = 417.72), compared to Time 1 value of 1062.96 (SD = 828.98). The computed average Physical Activity Questionnaire (PAQ) score from data collected at 5 time points 3 months apart from Time 1 to Time 2 was 2.62 (SD = 0.73).

Key Variable Descriptive Information

Descriptive information for the key study variables is as follows: mean serum level of the cytokine interleukin-1 β (IL-1 β) via bioassay at Time 1 was 10.17 (SD = 38.71), interleukin-6 (IL-6) was 16.58 (SD = 40.82), and tumor necrosis factor- α (TNF- α) was 10.14 (SD = 21.03). The mean Time 1 Children's Depression Inventory (CDI) Total Score (T-score) was 46.27 (SD = 10.77), and the computed average CDI T-score from Time 1 to Time 2 was 45.07 (SD = 9.66). Mean Whole Body bone mineral content (BMC) at Time 1 was 1912.04 g (SD = 416.24), compared to 1996.11 (SD = 385.47) at Time 2. Mean Hip bone mineral density (BMD) at Time 1 was 0.96 g/cm² (SD = 0.16), and 0.98 g/cm² (SD = 0.15) at Time 2. Mean Lumbar Spine BMD at Time 1 was 0.97 g/cm² (SD = 0.17), and 1.00 g/cm² (SD = 0.16) at Time 2.

Research Question: What are the associations of baseline cytokine levels and depressive symptoms with bone mass one year later?

Correlations. A comprehensive correlational analysis was conducted among all independent variables, clinically relevant covariates, and dependent variables (see Table 2). This analysis provided preliminary indication of possible linear relationships and significant trends among and between variables. For the purpose of answering the research question it was important to examine the relationships between independent cytokine and depressive symptom variables and dependent bone variables. The results of the correlational analysis indicate that a number of statistically significant relationships exist between covariates and dependent bone variables in this dataset.

Independent and dependent variables. Time 1 cytokine values for IL-1 β , IL-6, and TNF- α were all significantly correlated with each other (see Table 2). IL-1 β was negatively correlated with the PAQ, measuring weight bearing activity, in the previous week ($r = -.165, p < .05$) and positively correlated with OCP use ($r = .139, p < .05$). IL-1 β was not significantly correlated with any dependent variables. IL-6 was not significantly correlated with any other variable. TNF- α was positively correlated with OCP use ($r = .215, p < .01$), but not significantly correlated with any other variables including dependent variables.

CDI T-scores measuring depressive symptoms were not significantly correlated with any dependent variables ($p \geq -.06$), nor were they correlated with any other independent variable.

Time 1 Whole Body BMC, Time 1 Lumbar BMD, and Time 1 Hip BMD measured by DXA were all significantly positively correlated with each other and with Time 2 bone measures (see Table 2). Independent variables saw numerous positive correlations with many covariates, as outlined in Table 2.

Covariates and dependent variables. In addition to widespread correlations seen among all Time 1 and Time 2 bone measures, whole Body BMC at Time 2 was also significantly positively correlated with height ($r = .768, p < .01$), weight ($r = .758, p < .01$), gynecological age ($r = .266, p < .01$), Depo-Provera use ($r = .152, p < .05$), and OCP use ($r = .205, p < .01$), and significantly negatively correlated with PAQ ($r = -.221, p < .01$) and Vitamin D ($r = -.146, p < .05$). Lumbar BMD at Time 2 was significantly positively correlated with height ($r = .597, p < .01$), weight ($r = .676, p < .01$), gynecological age ($r = .237, p < .01$), and OCP use ($r = .198, p < .01$), and was significantly negatively correlated with PAQ ($r = -.191, p < .01$) and Vitamin D ($r = -.144, p < .05$). Finally, Hip BMD at Time 2 was significantly positively correlated to height ($r = .492, p < .01$), weight ($r = .664, p < .01$), and OCP use ($r = .139, p < .05$), and significantly

negatively correlated to Vitamin D ($r = -.197, p < .01$). There were no statistically significant correlations found between calcium intake and Time 2 bone measures.

Independent, covariate, and dependent variables. Multiple regression analyses were conducted to evaluate how well cytokine levels, depressive symptoms, and covariates at Time 1 predicted Time 2 bone measures (Whole Body BMC, Hip BMD, and Lumbar Spine BMD). In the three separate regression analyses for each respective Time 2 bone measure, one model included covariates only and the second model included covariates and cytokines.

As outlined in Table 3, values of Time 2 bone measures could be largely attributed to the covariates included in the study, but with minimal changes in the significance of these relationships seen with Time 1 cytokine values taken into account (Whole Body BMC Model 1 $R^2 = .957$, Model 2 $R^2 = .957$; Hip BMD Model 1 $R^2 = .951$, Model 2 $R^2 = .952$; Lumbar BMD $R^2 = .925$, Model 2 $R^2 = .930$). Only IL-1 β was significantly related to Lumbar BMD ($B = -.0002$, $SE = .0001, p < .01$). Weight and gynecological age were consistently significant contributors to variance in Time 2 bone values. Vitamin D, OCP use, and PAQ tended to show significance as well.

Table 1. Descriptive characteristics of study sample.

| Demographic Information | Total Sample (N=262) Frequency [n (%)] or Mean (\pmSD) |
|---|---|
| Age (yrs) | 14.94 (2.17) |
| Age (cohorts) | |
| 11 years | 52 (19.80%) |
| 13 years | 52 (19.80%) |
| 15 years | 87 (33.20%) |
| 17 years | 71 (27.10%) |
| Race | |
| White | 162 (61.80%) |
| African American | 86 (32.80%) |
| Other | 14 (5.30%) |
| Height (ft) | 5.25 (0.26) |
| Weight (lbs) | 137.02 (40.12) |
| Body Mass Index | |
| BMI | 24.05 (6.21) |
| Gynecological age (yrs) | 3.39 (1.90) |
| Age at menarche (yrs) | 12.40 (1.24) |
| Use of hormone related contraception? | |
| Yes | 99 (37.80%) |
| No | 161 (61.50%) |
| Total # of hormone meds used | |
| 0 | 161 (61.50%) |
| 1 | 64 (24.40%) |
| 2 | 28 (10.70%) |
| 3 | 7 (2.70%) |
| Use of Depo-Provera | |
| Yes | 41 (15.8%) |
| No | 219 (84.2%) |
| Use of oral contraceptive pills (OCP) | |
| Yes | 56 (21.5%) |
| No | 204 (78.5%) |
| Vitamin D status (ng/mL) | 20.45 (9.22) |
| Daily calcium intake from food (mg) | |
| Time 1 | 1062.96 (828.98) |
| Computed Average (between Time 1 and Time 2) | 765.59 (417.72) |
| Physical Activity Questionnaire | |
| Computed Weekly Average (between Time 1 and Time 2) | 2.62 (0.73) |

Table 1 cont.

| Key Variable Descriptive Information | Total Sample (N=262) Frequency [n (%)] or Mean (\pmSD) |
|--|--|
| CDI (T score) Time 1 Computed Average | 46.27 (10.77) 45.07 (9.66) |
| DXA: Time 1 Whole body BMC (g) Hip BMD (g/cm ²) Lumbar BMD (g/cm ²) DXA: Time 2 Whole body BMC (g) Hip BMD (g/cm ²) Lumbar BMD (g/cm ²) | 1912.04 (416.24) 0.96 (0.16) 0.97 (0.17) 1996.11 (385.47) 0.98 (0.15) 1.00 (0.16) |
| Cytokine bioassays: Time 1 IL-1 β IL-6 TNF- α | 10.17 (38.71) 16.58 (40.82) 10.14 (21.03) |

Key: Vitamin D status (ng/mL), serum 25-hydroxy vitamin D (ng/mL); CDI, Children's Depression Inventory; Whole Body BMC, Whole Body Bone Mineral Content (g) via dual energy x-ray absorptiometry (DXA); Lumbar BMD, Lumbar Bone Mineral Density (g/cm²) via DXA; Hip BMD, Hip Bone Mineral Density (g/cm²) via DXA; IL-1 β , cytokine interleukin-1 β ; IL-6, cytokine interleukin-6; TNF- α , cytokine tumor necrosis factor α

Table 2. Correlations Among All Covariate, Independent, and Dependent Variables in Analysis.

| | Height | Weight | PAQ | Calcium Intake | Vitamin D (ng/ml) | Gynecological Age | Depo-Provera Use | OCP Use | CDIT | IL-1 β Time 1 | IL-6 Time 1 | TNF- α Time 1 | Whole Body BMC Time 1 | Lumbar BMD Time 1 | Hip BMD Time 1 | Whole Body BMC Time 2 | Lumbar BMD Time 2 |
|------------------------------|---------|---------|---------|----------------|-------------------|-------------------|------------------|---------|-------|---------------------|-------------|----------------------|-----------------------|-------------------|----------------|-----------------------|-------------------|
| Covariates | | | | | | | | | | | | | | | | | |
| Weight | .494** | | | | | | | | | | | | | | | | |
| PAQ | -.189** | -.228** | | | | | | | | | | | | | | | |
| Calcium Intake | .023 | -.205** | .259** | | | | | | | | | | | | | | |
| Vitamin D | .077 | -.198** | -.030 | .113 | | | | | | | | | | | | | |
| Gynecological Age | .021 | .263** | -.244** | -.243** | .020 | | | | | | | | | | | | |
| Depo-Provera Use | .069 | .088 | -.073 | -.104 | -.111 | .220** | | | | | | | | | | | |
| OCP Use | .124* | .188** | -.274** | .015 | .256** | .310** | -.031 | | | | | | | | | | |
| Independent Variables | | | | | | | | | | | | | | | | | |
| CDIT | .124 | .202** | -.264** | -.116 | .012 | .072 | .117 | .054 | | | | | | | | | |
| IL-1 β Time 1 | .012 | .038 | -.165* | -.061 | -.028 | -.025 | -.062 | .139* | -.087 | | | | | | | | |
| IL-6 Time 1 | .058 | .047 | -.117 | -.072 | -.001 | -.053 | .041 | .064 | -.072 | .442** | | | | | | | |
| TNF- α Time 1 | .042 | .045 | -.130 | -.034 | .042 | .038 | -.034 | .215** | -.061 | .658** | .379** | | | | | | |
| Whole Body BMC Time 1 | .747** | .749** | -.258** | -.085 | -.084 | .367** | .132* | .252** | .131 | .030 | -.016 | .037 | | | | | |
| Lumbar BMD Time 1 | .606** | .689** | -.266** | -.100 | -.095 | .346** | .061 | .244** | .118 | .020 | -.022 | .001 | .908** | | | | |
| Hip BMD Time 1 | .531** | .689** | -.158* | -.083 | -.161** | .235** | .078 | .173** | .091 | .010 | -.059 | -.022 | .866** | .859** | | | |
| Dependent Variables | | | | | | | | | | | | | | | | | |
| Whole Body BMC Time 2 | .768** | .758** | -.221** | -.076 | -.146* | .266** | .152* | .205** | .119 | .014 | .018 | .043 | .971** | .884** | .865** | | |
| Lumbar BMD Time 2 | .597** | .676** | -.191** | -.087 | -.144* | .237** | .032 | .198** | .103 | -.045 | -.063 | -.007 | .861** | .959** | .845** | .877** | |
| Hip Bone BMD Time 2 | .492** | .664** | -.090 | -.065 | -.197** | .110 | .082 | .139* | .081 | -.010 | -.050 | -.014 | .793** | .800** | .960** | .840** | .834** |

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Shading indicates main variables of interest based on research question.

Key: PAQ, Physical Activity Questionnaire computed average; Vitamin D, serum 25-hydroxy vitamin D (ng/mL); OCP Use, frequency of oral contraceptive pill use; CDI T, Children's Depression Inventory T score; IL-1 β , cytokine interleukin-1 β ; IL-6, cytokine interleukin-6; TNF- α , cytokine tumor necrosis factor alpha; Whole Body BMC, Whole Body Bone Mineral Content (g) via dual energy x-ray absorptiometry (DXA); Lumbar BMD, Lumbar Bone Mineral Density (g/cm²) via DXA; Hip BMD, Hip Bone Mineral Density (g/cm²) via DXA.

Table 3. Stepwise regression analyses using T1 cytokines to predict T2 bone mass and density.

| | Whole Body Total BMC Time 2 | | Hip BMD Time 2 | | Lumbar BMD Time 2 | |
|---------------------------------------|-----------------------------|-----------------|----------------|----------------|-------------------|----------------|
| | Model 1 | Model 2 | Model 1 | Model 2 | Model 1 | Model 2 |
| | B (SE) | B (SE) | B (SE) | B (SE) | B (SE) | B (SE) |
| Intercept | 47.91 (162.02) | 90.80 (165.45) | .14 (.07) | .15 (.07) | .12 (.08) | .15 (.08) |
| Height | 132.86 (112.27) | 96.01 (115.70) | .14 (.07) | -.05 (.05) | -.03 (.05) | -.05 (.05) |
| Weight | 1.95 (.46)** | 1.88 (.47)** | -.04 (.04)** | .001 (.00)** | .001 (.00)** | .001 (.00)** |
| Vitamin D | -1.23 (.58)* | -1.19 (.59)* | .00 (.00) | .00 (.00) | .00 (.00) | .00 (.00) |
| Calcium Intake | .004 (.01) | .005 (.01) | .00 (.00) | .00 (.00) | .00 (.00) | .00 (.00) |
| PAQ | 3.68 (8.31) | 4.46 (8.51) | .00 (.004) | .001 (.004) | .009 (.004)* | .008 (.004) |
| Gynecological Age | -24.36 (3.29)** | -24.35 (3.32)** | -.005 (.001)** | -.005 (.001)** | -.007 (.002)** | -.008 (.002)** |
| OCP Use | -3.11 (3.74) | -2.78 (3.91) | -.004 (.002)* | -.004 (.002)* | .00 (.002) | .001 (.002) |
| Depo-Provera Use | .35 (3.70) | .59 (3.72) | -.002 (.002) | -.001 (.002) | -.001 (.002) | -.001 (.002) |
| CDI T | -.78 (.55) | -.75 (.56) | .00 (.00) | .00 (.00) | .00 (.00) | .00 (.00) |
| Whole Body BMC Time 1 | .91 (.03)** | .91 (.03)** | --- | --- | --- | --- |
| Hip BMD Time 1 | --- | --- | .92 (.02)** | .92 (.02)** | --- | --- |
| Lumbar BMD Time 1 | --- | --- | --- | --- | .92 (.03)** | .92 (.03)** |
| IL-1β Time 1 | --- | -.17 (.16) | --- | .00 (.00) | --- | .00 (.00)** |
| IL-6 Time 1 | --- | .32 (.24) | --- | .00 (.00) | --- | .00 (.00) |
| TNF-α Time 1 | --- | -.01 (.29) | --- | .00 (.00) | --- | .00 (.00) |
| R Square | 0.957 | 0.957 | 0.951 | 0.952 | 0.925 | 0.930 |

** . Indicates significance at the 0.01 level.

* . Indicates significance at the 0.05 level.

Note: Model 1: regression with covariates only. Model 2: regression with covariates and cytokines.

Key: PAQ, Physical Activity Questionnaire computed average; Vitamin D, serum 25-hydroxy vitamin D (ng/mL); OCP Use, frequency of oral contraceptive pill use; CDI, Children's Depression Inventory computed average; IL-1 β , cytokine interleukin-1 β ; IL-6, cytokine interleukin-6; TNF- α , cytokine tumor necrosis factor alpha; Whole Body BMC, Whole Body Bone Mineral Content (g) via dual energy x-ray absorptiometry (DXA); Lumbar BMD, Lumbar Bone Mineral Density (g/cm²) via DXA; Hip BMD, Hip Bone Mineral Density (g/cm²) via DXA.

Chapter 5

Discussion

The purpose of this secondary analysis of longitudinal data was to examine the associations of baseline cytokine levels with depressive symptoms and bone mass one year later. The results found no significant correlations between baseline serum cytokine levels or depressive symptoms and bone mass at Time 2. Based on the stepwise regression analyses, baseline cytokines provided minimal contribution on Time 2 bone measures. Covariates used in the analysis showed significance which was of interest in terms of clinical application, to be discussed further in this chapter.

Associations Among All Observed Variables

Key variables. Cytokines did not correlate significantly with any other independent variable, nor with any dependent variable. The only significant key variable relationship found in the regression analyses was between interleukin (IL)-1 β and Time 2 Lumbar bone mineral density (BMD) ($B = -.0002$, $SE = .0001$, $p < .01$). It is unclear why only IL-1 β showed significance, and IL-6 and tumor necrosis factor (TNF)- α did not. All three of these pro-inflammatory cytokines have been identified as being involved in osteoclast formation (Manolagas & Jilka, 1995). IL-1 β has also been reported to be associated with decreased BMD in women with major depressive disorder (MDD), however that same study reported similar associations with both IL-6 and TNF- α (Eskandari, 2007). It is possible that since bone loss

occurs over a period of decades typically later in life, observable associations do not become evident until later in life (NIH, 2011).

Covariates. Some unexpected associations were found upon correlational analysis of the data. Calcium intake and all bone mineral content (BMC)/BMD measures were negatively correlated. This finding does not align with typical trends seen in literature where higher calcium intake is associated with stronger, more dense bone during growth, therefore affecting peak bone mass achieved in early adulthood (Zhu, 2012). The computed average of calcium intake among the sample between Time 1 and Time 2 was 765.59 mg/day (SD = 417.72), which does fall short of the recommended minimum daily intake of 1,300 mg/day to achieve maximal retention (Institute of Medicine, 2011). The unexpected correlation could potentially be attributed to calcium intake being a self-reported measure, or it could indicate that there are other factors involved in the relationship between calcium and bone health that were not measured.

A similar phenomenon was seen in the findings where the correlation between BMD/BMC measures and serum vitamin D. Time 1 serum 25-hydroxy vitamin D was significantly negatively correlated with all Time 2 bone measures. Although the sample again compared poorly to recommended serum vitamin D levels of approximately 30-40 ng/mL (20.45 ng/mL (SD = 9.22)), a positive correlation would still be expected due to existing literature supporting vitamin D's role in optimal bone health (Dawson-Hughes, 2005). Perhaps the observed digression from typical trends could be attributed to the relationship between bone health and vitamin D not being generalizable to those falling so significantly below recommended standards.

Yet another covariate exhibiting different than expected trends in the correlational analysis was the Physical Activity Questionnaire (PAQ). Literature indicates that increased

physical activity is related to decreased loss of bone mineral density (Bailey, 1996). However, the current study showed significant negative correlations between PAQ score and Time 2 Whole Body BMC and Time 2 Lumbar BMD. This is opposite to what is typically observed in literature, even in childhood (Gunter, 2012). Since the PAQ is self-reported data, there is a potential for inaccuracy of results. Additionally, PAQ scores reflect occurrences of physical activity within one week prior to taking the questionnaire. It is possible that the weeks assessed by the questionnaire are not representative of each subjects' actual exercise habits. Regardless, it could be important to consider other factors such as weight-bearing status of subjects' physical activity and lifetime history of physical activity, for example.

Study Limitations

One major limitation of the current study lies in the fact that the sample of 262 healthy adolescent females did not show a particularly high level of depressive symptoms at baseline. In fact, only 39 subjects fell in the "above average" range, and only 20 of these subjects fell within range for clinically significant depressive symptoms (Kovacs, 2004). Mean Children's Depression Inventory (CDI) Total Score (T-score) of the sample at Time 1 was 46.27 (SD = ± 10.77), which falls on the low side of "average" depressive symptomatology (Kovacs, 2004). It is possible that the lack of substantial evidence of depressive symptoms skewed results, as one of the major predicting variables was at low levels in the sample. Therefore using a largely non-depressed sample is a limitation for the current study, and limits potential for analysis of observed trends associated with depressive symptoms in adolescence. However, these results might reflect more accurately on the general population since a majority of individuals in the intended population do not show evidence of clinically significant depression.

Additionally, the CDI is a self-report questionnaire. In filling out the CDI, subjects may not accurately perceive depressive symptoms within themselves. Therefore, clinically significant depressive symptoms could be present in subjects but not accurately reflected in CDI scores (and vice versa). Erroneous self-report on the CDI would decrease the likelihood that depressive symptoms would be physiologically reflected in the data.

Clinical Significance

Although serum cytokine levels were not significantly associated with bone health at Time 2, the current study still holds clinical significance in terms of health promotion for adolescent females. Analysis of data from the 262 female study subjects showed statistically significant positive relationships between oral contraceptive pill (OCP) use and bone health, as evidenced in Table 2. Depo-Provera use did not show similar trends in the data. Literature reports OCPs (containing estrogen) as being helpful to bone formation and maintenance, and Depo-Provera (not containing estrogen) as being detrimental to bone (Lopez, et al., 2014). Estrogen contributes to higher BMD, so oral hormonal contraception could be seen as a potential medical supplement for adolescents with an increased osteoporotic risk. Gynecological age, a measure indicative of length of increased estrogen exposure, was another major contributing factor to increased bone mass at both Time 1 and Time 2. This reaffirms the positive impact estrogen has on bone. Longer estrogen exposure, either from hormonal pubertal changes or initiation of oral hormonal contraceptives, tends to lead to more optimal bone accrual in adolescent females (Bonjour & Chevalley, 2014).

The most significant long-term clinical implication of the current study lies in potential for increased osteoporotic risk for female adolescents who fail to reach sufficient peak bone mass

during critical periods of growth. Health promotion and education should be stressed during adolescence, before the chance to accrue peak bone mass has passed and bone resorption begins dominating the bone breakdown cycle. The findings of the current study do not support associations between depressive symptoms and decreased bone health. However, according to the current study it would still be important to stress healthy behaviors beginning or continuing into the onset of puberty due to the significance of gynecological age and estrogen exposure on increased bone mass. Similarly, it is important for health care providers and adolescents to consider the benefits of OCP use on bone health when making decisions regarding hormonal contraceptive use.

Although the current study did not show expected significant statistical findings upon analysis of data for cytokines, depression, and bone health, this does not provide enough evidence to completely disregard previously supported dietary and lifestyle choices that enhance bone health. Physical activity and adequate calcium and vitamin D intake should still be considered healthy behaviors supportive of overall physical health. When considering the potential impact of depressive symptoms, no conclusive decisions can be made based on the results shown in the current data. As with any traits or disorders with potential inhibitory effects on physical or psychological development, individuals showing increased depressive symptoms should be monitored and treated as needed. However, based on the current study, no specific recommendations can be made regarding the relationships among pro-inflammatory cytokines, depression, and bone health.

Conclusion

The current study examined the associations of baseline cytokine levels with depressive symptoms and bone mass one year later through descriptive statistics, correlational analysis, and stepwise regression analyses. Results did not show statistically significant associations among the three variables of interest: cytokines, depression, and bone health. Multiple covariates exhibited significant effects on bone health, however. Psychological assessment during adolescence remains a crucial consideration for females, but proof of the impact of depression on bone health was not reflected in this particular dataset. Health promotion and teaching regarding behaviors promoting long-term bone health continues to be a significant area of interest for the adolescent female population and those primary care providers involved in their care.

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ACADEMIC VITA

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EDUCATION

May 2016 **Bachelor of Science in Nursing with Honors**
The Pennsylvania State University, College of Nursing, Schreyer Honors College
Dean's List Fall 2012 – Spring 2015
Thesis Title: Cytokines, Depression, and Bone Health: Exploring the Associations in Healthy Adolescent Females
Thesis Supervisor: Lorah D. Dorn, Ph.D., CPNP

EMPLOYMENT

May 2015-Present **Patient Safety Extern**
Thomas Jefferson University Hospital, Philadelphia, PA
Patient safety 1:1 observation, full unit vital signs, AccuChecks, intake and output collection and charting, patient ambulation and transport, various independent and supervised nursing interventions.
Worked on many different units, primarily on those involving Nursing care of patients with neurological alterations.
Supervisor: Nora Kramer, MSN, CNRN

May-August 2014 **Volunteer**
Shore Medical Center, Somers Point, NJ
Worked with team of volunteers to clean and restock patient rooms, coordinate patient check-in, transport patients, and organize patient charts on the Same Day Surgery Unit.
Supervisor: Lisa DiTroia

CLINICAL EXPERIENCE

2013-2016

Student Nurse

The Pennsylvania State University, University Park, PA

Fundamentals of Nursing Care, Medical/Surgical Nursing (I, II, III), Nursing Care of the Older Adult, Obstetrics/Gynecology and the Childbearing Family, Pediatric Nursing, Community and Family Health Nursing, Mental Health Nursing

Clinical Capstone

Mount Nittany Medical Center Emergency Department, State College, PA

Provided total care for patients under the supervision of a Registered Nurse

RESEARCH EXPERIENCE

2013-2016

Undergraduate Thesis | Cytokines, Depression, and Bone Health: Exploring the Associations in Healthy Adolescent Females

The Pennsylvania State University, University Park, PA

Advisor: Lorah D. Dorn, Ph.D., CPNP

- Researched and analyzed the physiological relationships among systemic inflammation via cytokines, depressive symptoms, and bone health in healthy adolescent females
- Cleaned and managed data from parent dataset, created and carried out plan for data analysis, interpreted results, developed abstract

April 2015

Shaping the Future Summit

The Pennsylvania State University, University Park, PA

Supervisor: Darlene Clark, M.S., R.N.

- Studied the interaction of ethical, legal, and genetic issues as they apply to health care organizations
- Facilitated a discussion on global issues and leadership scenarios, particularly related to “The Power of Money” in organizations that influence health care practice

GRANTS

- 2015 Student Summer Research Grant, Funded by: Schreyer Honors College
- 2014 Student International Travel Service Grant, Funded by: Schreyer Honors College

PROFESSIONAL MEMBERSHIPS

- 2015-Present Member, Sigma Theta Tau International Honor Society of Nursing
- 2012-Present Member, Student Nurse Association of Pennsylvania

CERTIFICATIONS

- 2013-Present Basic Life Support (BLS) Provider from American Heart Association

COMMUNITY SERVICE INVOLVEMENT

- 2015-2016 OPPerations Captain, Penn State IFC/Panhellenic Dance Marathon
- 2014-2015 OPPerations Lieutenant, Penn State IFC/Panhellenic Dance Marathon
- March 2014 Global Medical/Public Health Brigade, Nicaragua
- 2013-2014 OPPerations Committee Member, Penn State IFC/Panhellenic Dance Marathon
- March 2013 Team Leader, Schreyer Honors College Day of Service

2012-2013

Rules & Regulations Committee Member, Penn State IFC/Panhellenic
Dance Marathon