Drew University College of Liberal Arts

# Evaluating Vitamin D Supplementation Compliance and Socioeconomic Status in a Pediatric Fracture Population:

Clinical Evaluation of Bone Mineral Density

A Thesis in Biology

Written by:

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## Abstract

The following thesis analyzes the impact of socioeconomic status on bone health in a pediatric fracture population and aims to validate the role of supplementation in preventing risk of fracture and poor bone mineralization. Poor bone health and vitamin D deficiencies in pediatric populations is a serious, yet understudied problem. The current literature comparing BMD directly to serum 25(OH)D levels draws conflicting reports as to the effect of vitamin D supplementation on bone mineral density accrual in healthy children; most importantly, there are limited prospective, longitudinal studies showing what happens to vitamin D deficient patients who have already proven to be vulnerable to at least one bone break after a year of proper supplementation. Additionally, there are a limited number of studies on the impact of social class or socioeconomic status on pediatric bone health. The current longitudinal interventional study presented addresses how to improve compliance to a vitamin D supplementation protocol following a fracture. The study follows patients with dual x-ray absorptiometry (DXA) scans at three time points during the healing processes in addition to vitamin D levels. Preliminary data indicate that patients following the protocol show significant improvements in bone mineral density (BMD) relative to expected changes in bone mineral content (BMC).

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# I. Introduction

Bone is a living tissue that is continually modeling and remodeling throughout our lifespan. To function correctly, bone tissue is modeled and remodeled by specialized cells known as osteoclasts and osteoblasts (Pollock 2015). Bone resorption is facilitated by osteoclasts which break down and remove old bone tissue, while bone formation is facilitated by osteoblasts which are responsible for bone formation. The modeling of bone refers to changes in the shape and size of the bone while remodeling of bone refers to bone turnover that does not change the shape and size of the bone. Starting during early childhood and continuing to early adulthood, modeling is the process in which bones become larger, heavier, and increase in density (Pollock 2015). This modeling process is essential for children to accrue the maximum level of bone mass during normal growth. This maximum level of bone mass is referred to as peak bone mass (PBM). The concept of PBM is different when directed towards an individual versus a population. At the individual level, PBM is the greatest degree of bone strength one can attain; rather at the population level, PBM is attained when age-related changes in bone outcome have reached a plateau or maximum value (Weaver et al. 2016). Used as one of the critical factors in determining the bone mass and fracture risk, PBM is attained by early adulthood for most skeletal sites (Lanham-New et al. 2013); thus, optimizing bone accrual during growth is extremely influential in preventing current and future fractures. Given that bone mass, bone density, and structural strength are all related to fractures in children and adults, failure to accrue PBM throughout growth not only increases fracture risk but can threaten subsequent bone health (Figure 1). The term bone health or bone

quality is often found difficult to measure with one variable; rather it should be understood as the combination of all the factors that control how well the skeleton resists fractures (Rizzoli 2014). Consequently, the changes in bone mass, shape, and size that occur throughout childhood and adolescence are what make assessing bone health in children more difficult than adults.

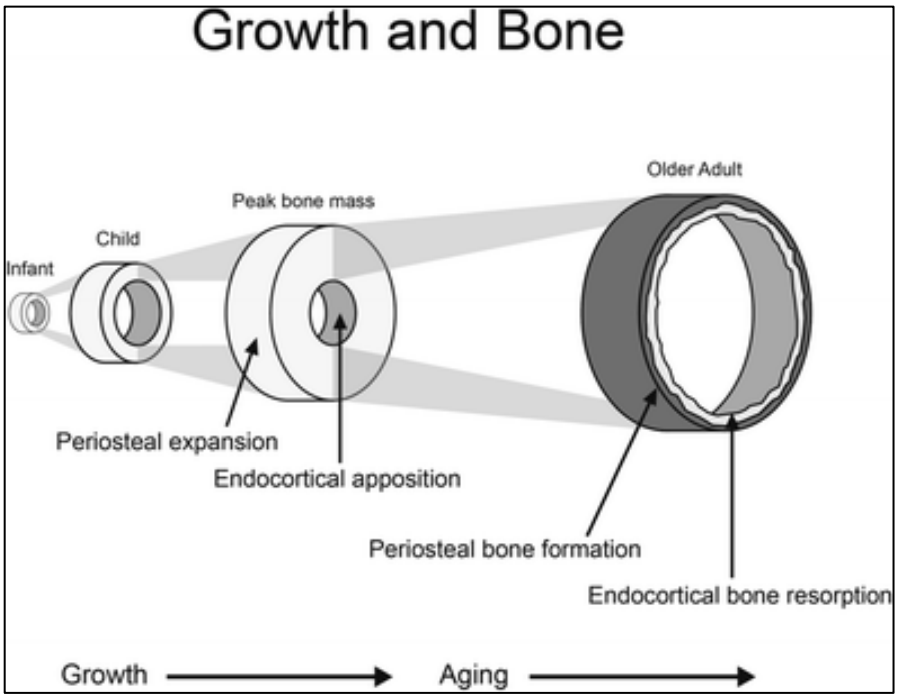


**Figure 1.** ***Changes in structural composition of bone throughout the lifespan*** (derived from (Weaver et al. 2016))

Poor bone health and vitamin D deficiencies in pediatric populations is a serious, yet understudied problem. The current literature comparing BMD directly to serum 25(OH)D levels draws conflicting reports as to the effect of vitamin D supplementation on bone mineral density accrual in healthy children; most importantly, there are limited prospective, longitudinal studies showing what happens to vitamin D deficient patients who have already proven to be vulnerable to at least one bone break after a year of proper

supplementation. Additionally, there are a limited number of studies on the impact of social class or socioeconomic status on pediatric bone health. While there is a significant amount of literature looking at the relationship between diet and nutrition and bone health, disease and bone health, and physical activity and bone health, literature on the relationship between all of these variables on pediatric bone health and vitamin D deficiencies is lacking. As a result, this thesis proposes to fill the gaps in the extant knowledge about vitamin D supplementation and bone health in pediatric populations.

In recent years, high rates of childhood fractures have generated concerns regarding pediatric bone health; resulting in an increased interest in bone densitometry for pediatric patients. Bone densitometry is a valuable tool in assessing bone health. Generally used to diagnose and monitor osteoporosis in older adults, the increased interest in pediatric bone health has made dual-energy x-ray absorptiometry (DXA) the preferred method of bone densitometry in children. DXA scanning is a fast, accurate, and easily accessible method of measuring bone mineral content (BMC) and determining bone mineral density (BMD) in children. The exposure to radiation from a DXA scan is comparable to the amount of radiation received on a round-trip plane flight (Bachrach 2005) making it a safer option for pediatric populations compared to other common imaging methods such as X-rays or computed tomography (CT) scans.

DXA scans provide information about BMC and the estimated area of bone. BMC is the measurement of bone mineral found in a specific area. BMC and expected area of bone provided by DXA scanning are used to calculate a patient's BMD. BMD is the amount of mineral matter contained in a specific volume of bone, BMD is not a true

density, but a ratio of BMC/area of bone used to determine bone strength and fracture risk (Bachrach and Gordon 2016). Favored skeletal sites for DXA measurements in pediatric patients are the lumbar spine (L1-L4) and total body less head (TBLH). Data obtained from DXA scans are areal BMD (aBMD) measurements, meaning that it cannot measure the depth of bone. This two-dimensional measurement is affected by a subject's size, allowing estimates to be inaccurate in shorter patients with smaller bones and taller patients with larger bones (Wasserman et al. 2017).

Commonly used to adjust for differences in age, height, and sex, a z-score is the difference between the patient's BMD and the mean age-matched value of the reference population, divided by the reference standard deviation. Specifically adjusting for sex, height, body composition, age, and ethnic group, reference values of healthy pediatric populations of similar age, sex, height, and ethnicity are used to calculate a more precise z-score. Since pediatric populations are continually growing, changes in BMD can be expected; thus, the Z-score can be used to account for these changes (Figure 2) (Bachrach and Gordon 2016). Additionally, adjusting for height can be done as DXA scans measure the degree of change using percent body fat, lean body mass, total body composition (TBC), BMC, BMD, and z-score. With the ability to account for the changes of a growing child, DXA scans have given physicians a way to monitor bone health to patients of all ages.

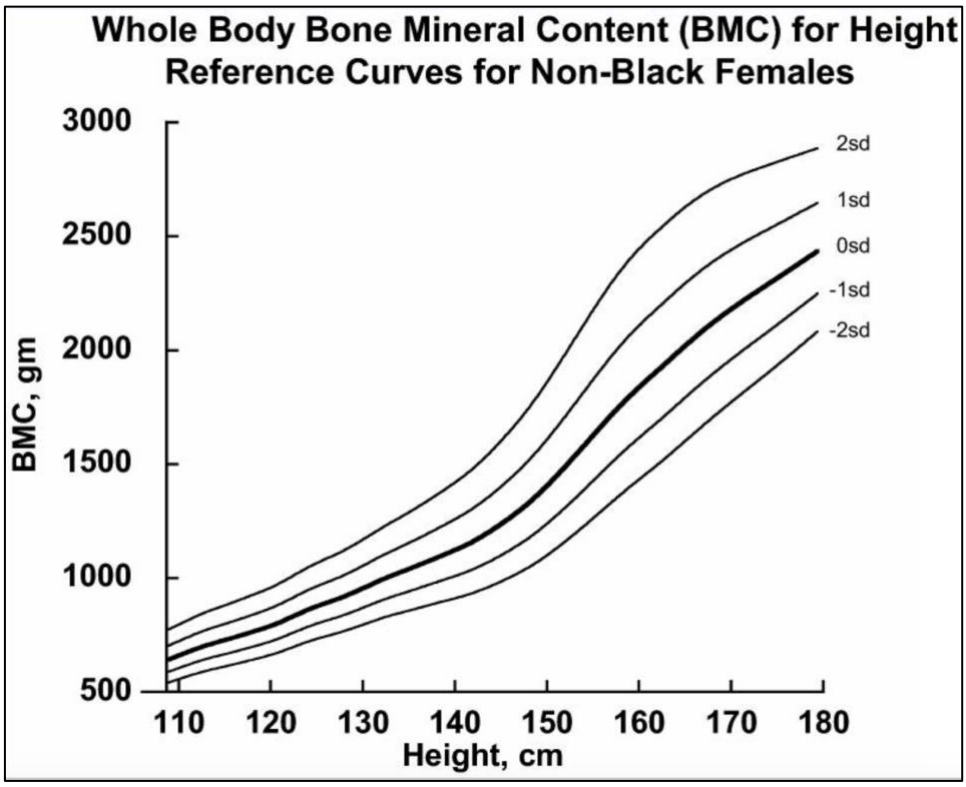


**Figure 2.** ***Example of BMC for height reference curves for generating*** $BMC_{height}$ ***Z-scores*** (derived from (Zemel et al. 2010))

DXA scanning has become more popular over the past ten years due to the increase in childhood fractures. According to the American Academy of Pediatrics, over the last four decades, there has been a 30% increase in childhood fractures, causing increased interest in then impact childhood lifestyle has on achieving proper bone health (Bachrach and Gordon 2016). Impacting bone health is multifactorial. Predetermined factors like genetics have a significant impact on bone mass variance; but, the variability in genetic factors is not necessarily the reason for the 30% increase in pediatric fractures. This being said, environmental conditions, diet, nutrition, and physical activity are all factors that directly influence bone health throughout childhood. To assess the impact certain lifestyle factors, have on bone health in children and adolescents, diseases

impacting bone health, vitamin D deficiencies, and socioeconomic status will be discussed later in this paper. Evaluation of these components will provide the context necessary for future discussion regarding the importance of vitamin D supplementation and monitoring bone health to increase compliance in pediatric fracture populations.

### *A. Disorders Associated with Poor Bone Health*

There are many chronic, genetic, and skeletal disorders that can compromise bone health throughout a person's life. Well-known disorders associated with low bone mass and increased fracture risk are rickets, osteomalacia, osteoporosis, celiac disease, and anorexia nervosa. Most commonly associated with reduced bone health in adults, osteoporosis is a metabolic condition in which bone resorption exceeds formation, resulting in decreased bone mass and deterioration of bone microarchitecture, with resulting reduced bone strength and increased susceptibility to fracture (Cashman 2007). The large holes and spaces found in osteoporotic bones, otherwise translated to “porous” bones, lead to a significant decrease in bone density, thus causing the overall strength of the bone to weaken. Often coupled with white, postmenopausal women, and white males over the age of 50, the hallmark of osteoporosis are the fragility fractures, most commonly found in the spine, hip, and distal forearm. The incidence of spine and hip fractures increase significantly with age which increases concern for the overall health of the elderly population suffering from osteoporosis. More often than not, fragility fractures resulting from osteoporosis are debilitating to an individual's health status; in fact, hip fracture patients have an overall mortality rate of 15-30%, most occurring within

the first six months after initial injury (Cashman, 2007). Along with increased mortality rates, osteoporosis also leads to increased difficulty performing one or more activities essential to daily living. According to the literature, one year after obtaining a hip fracture caused by osteoporosis, 40% of patients are unable to walk independently, 60% of patients cannot perform at least one essential daily living activity, and 80% of patients are restricted from activities such as driving or grocery store shopping (Cooper 1997).

It is uncommon for children and adolescents to be diagnosed with osteoporosis; however, they can manifest typical osteoporosis symptoms such as low bone mineral density (BMD) thus resulting in increased fracture risk (Minkowitz et al. 2017). One hypothesis is that low BMD early in life may contribute to osteoporosis later in life (Ma and Gordon 2012). A majority of an individual's bone mineral density is developed during their adolescence, making factors such as physical health and activity significantly impact the development of quality bone health in children and adolescents. Most adolescents are now being predisposed to osteoporosis because their present lifestyles are producing lower peak bone mass; the lower the peak bone mass, the higher the risk of osteoporosis later in life (Hightower 2000). This being said, an advantage of establishing good bone health early on during childhood is reaching optimal PBM, which has been shown to decreased fracture risk and osteoporosis risk later on in life.

Although children can exhibit symptoms similar to osteoporosis, according to the American Academy of Pediatrics, osteopenia and osteoporosis should not be used in diagnosing children; instead, BMC or BMD Z-score that fall more than two standard deviations below the expected for a particular age should be identified as "low for age"

(Bachrach and Gordon 2016). In adults, osteoporosis is clearly defined and can be diagnosed on basis of densitometric criteria. However, when considering children, osteoporosis does not have a widely recognized definition in pediatrics. Consequently, the International Society for Clinical Densitometry (ISCD) reviewed literature describing the relationship between bone densitometric studies and fractures in apparently healthy children and adolescents, in order to provide a better definition of osteoporosis in children and adolescents (Bishop et al. 2014). Following review, the ISCD determined that the diagnosis of osteoporosis requires the presence of both a clinically significant fracture history and low BMC or BMD. This being said, the ISCD's position still remains to be that osteoporosis should not be determined based off of densitometry results alone; rather low BMC or BMD and significant fracture history must be presented to diagnose pediatric patients with osteoporosis (Bishop et al. 2014).

Granted osteoporosis is not an appropriate diagnosis for children presenting osteoporotic symptoms; there must be other diseases or disorders impacting PBM accrual and their overall bone health. Many studies have identified disorders found in children that affect bone health. While illnesses such as cancer, cerebral palsy, celiac disease, etc. all influence bone health, studies have shown the most common chronic disorders causing poor bone health in children are obesity, anorexia nervosa, and athletic amenorrhea (Weaver et al. 2016).

Childhood obesity is a significant public health concern throughout the United States. Since BMC and BMD are influenced by overall body size and weight, body composition is likely to have an impact on bone strength. According to the literature,

obesity and low body weight have been found to increase fracture risk in pediatric patients (McDevitt and Ahmed 2010). Pediatric obesity increases the risk for many metabolic conditions like type 2 diabetes, hypertension, and cardiovascular disease. The likelihood of pediatric obesity causing such factors has been known for some time; however, with the increasing childhood fracture rates in children with high body mass indices (BMI), concerns associated to the relationship between being overweight and accruing suboptimal bone strength have increased (Çizmecioğlu et al. 2008). Body mass index (BMI) is the suggested process for classifying individuals as overweight or obese. When measuring BMI in children and adolescents, reference curves are both age and sex-specific, where BMI results in the 85th percentile are considered to be overweight, and BMI results in the 95th percentile are considered obese (Pollock 2015).

The inverse association between higher body fat and lower vitamin D levels is commonly seen in obese and overweight adolescents. This relationship has been accredited to the confiscation of vitamin D within adipose tissue. For this reason, obesity due to sedentary lifestyle increases the risk of vitamin D deficiency dramatically (Çizmecioğlu et al. 2008). In an observational study done by Reddy et al. results confirmed that vitamin D deficiency or insufficiency is greater in overweight and obese adolescents; in this study 35% of patients were overweight adolescents and 65% were obese adolescents, where 66.7% of these patients were vitamin D deficient and 13.3% were vitamin D insufficient respectively (Prashanth Reddy et al. 2017). This inverse relationship further supports the negative impact obesity has on vitamin D level. Reasons for this relationship can be attributed to the inability to recover vitamin D or the lack of

weight-bearing forces applied to the skeleton. Retrieving stored vitamin D is extremely difficult in obese and overweight individuals because the bioavailability of the fat-soluble vitamin decreases when imbedded deeper in adipose tissue stores. Furthermore, sedentary lifestyle has an increased need for vitamin D since weight-bearing exercise is not being used to maintain BMD (Prashanth Reddy et al. 2017).

In the past, excess adiposity was associated with increased bone size, specifically larger bones in males and denser bones in females during puberty. Although body fat is a determinant of bone mass and bone strength, greater skeletal growth and bone size does not result in reduced fracture risk. Taylor et al. suggested that fracture risk is higher in obese children due to excess fat tissue impairing bone strength development, greater forces generated while falling, or compromising lifestyles (Taylor et al. 2006). In a fracture study using females between the ages of 4-15 with forearm fractures, it was reported that women who suffered from a fracture were more overweight and had smaller radial cross-sectional area than their counterparts who did not sustain fractures (Skaggs et al. 2001). A certain amount of body fat is necessary for skeletal maturation to begin; therefore, when children and adolescents are considered underweight for their height and age their overall bone health is impacted too.

Moreover, children and adolescents who suffer from eating disorders are at risk for poor bone health and skeletal maturation. Anorexia nervosa, an eating disorder generally seen in adolescents, specifically females, is a chronic illness that negatively impacts skeletal integrity. Extreme malnutrition can impair bone mass gain and increase fracture risk specifically. In most women suffering from anorexia nervosa, reduced BMD

is seen in some different skeletal sites (Ducher et al. 2011). Anorexia nervosa or any disordered eating in females not only impairs bone mass gain can cause hypothalamic dysfunction and delayed or disturbed menstrual function. Excessive exercise can also cause menstrual dysfunction. Commonly referred to as athletic amenorrhea, delayed menarche often leaves young females at risk for low bone mass and increased fracture risk. Athletic amenorrhea is frequently found in young female athletes but usually goes undiagnosed(Matzkin et al. 2015). Young women who participate in extreme exercise are more often than not suffering from what is known as the female athlete triad.

Physical activity and exercise directly correlate with poor overall bone health. Physical activity is an important determinant of bone mass accrual; there is a positive relationship between weight-bearing exercise and bone mass where high-impact exercise increases bone density. Evidence has shown that adequate levels of calcium and vitamin D are necessary to acquire bone mass and enhance bone growth during exercise (Vlachopoulos et al. 2015). In a study investigating the relationship between physical activity and vitamin D concentration in 100 adolescents, results revealed that active adolescents had increased total BMC when vitamin D levels were sufficient (Valtuena et al. 2012). According to Wolf's law, when the load on a bone increases, the bone can remodel itself over time and become stronger, so it can resist the load. In other words, high intensity and weight-bearing physical activity can increase bone mass because the skeleton can adjust to the loads placed on it (Vlachopoulos et al. 2015). While physical activity is beneficial to bone health and bone mass accrual, too much exercise and inadequate nutrition can impact bone health negatively.

Originally defined as the interrelatedness of disordered eating, amenorrhea, and osteoporosis, the criteria of Female Athlete Triad were found to be too strict. Clinicians and researchers hypothesized that there was a large number of athletes with less severe conditions who were not recognized as having the triad but were suffering from its symptoms such as recurrent stress fractures and irregular menses (Taraneh Gharib and Kathryn 2012). As such, broader definitions were developed to help identify the triad in female athletes. What was labeled as disordered eating, amenorrhea, and osteoporosis are now defined as energy availability, menstrual dysfunction, and bone mineral density. These new measures allow more athletes to be recognized as having components of the triad and assist in fixing poor bone health in adolescent females that present normal vitamin D and BMI levels (Thein-Nissenbaum and Carr 2011).

The newly-defined components of the triad are now used as a spectrum when diagnosing patients with the female athlete triad. This spectrum allows physicians to diagnose patients with the triad without all three factors being present. Females do not need to have low energy availability, menstrual dysfunction, and low BMD to be considered for the triad. The average age range for women diagnosed with the triad is 13-21 years old. While the triad is most common during this age period, it does not mean younger, or older women are not suffering from the triad as well. Despite the use of a spectrum to diagnose the triad, early diagnosis and treatment remain a significant challenge in this disorder. Athletes who display low energy availability, accompanied by irregular or absent menses may not seek medical support until a more obvious symptom such as a stress fracture is sustained. Additionally, not all athletes seek medical assistance

in response to the absence of menstruation for three or more months because many believe that long-term amenorrhea is not harmful to one's health (Ducher et al. 2011).

Obesity, anorexia nervosa (disordered eating), and menstrual dysfunction are all familiar issues found in children and adolescents. A common variable in all three of these disorders: nutrition. Nutrition is a critical component in achieving and maintaining good bone health throughout childhood. Nutritional intake can, directly and indirectly, influence bone metabolism, bone structure, and modify bone turnover. The addition of vitamin D and calcium to everyday diets has been shown to have a significant impact on fracture prevention throughout childhood and later on in life (Figure 3).Calcium is one of the most abundant minerals in the human body, where 99% of it is found bones which contributes to the strength of bone (Pu et al. 2016). Dietary calcium is essential for bone formation in children as it directly influences skeletal development during growth. Not only does calcium play a role in the formation process of new bone, but also is a crucial nutrient responsible for the maintenance of existing bone. Therefore, sufficient levels of calcium are needed in children's diets to reach PBM. However, for calcium to effectively support bone quality, our bodies need Vitamin D.

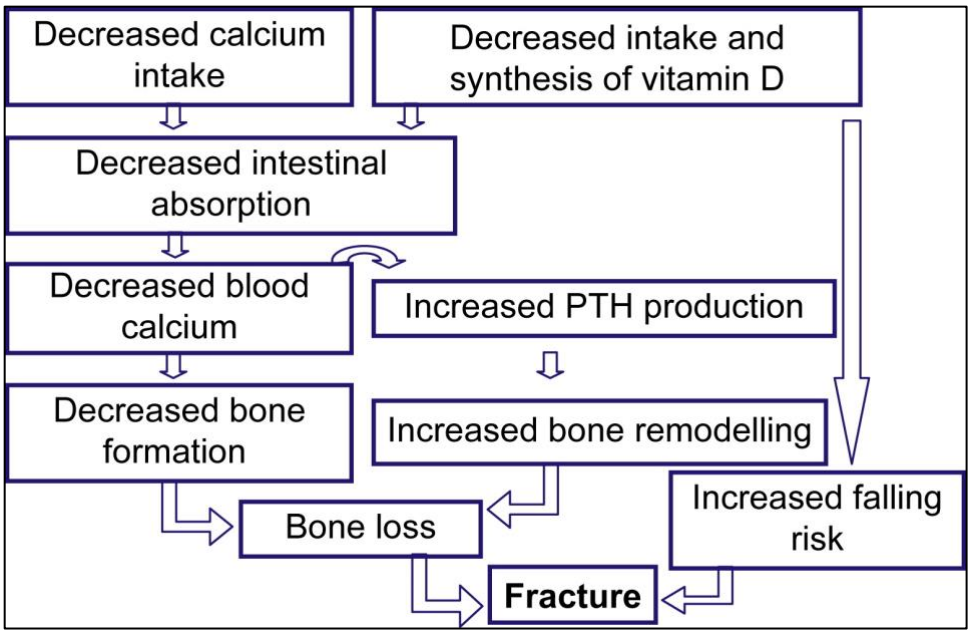


**Figure 3.** ***Mechanisms increasing fracture risk through calcium and vitamin D deficiency*** (derived from (Rizzoli 2008))

*B. Impact of Socioeconomic Status on Bone Health*

In the United States and across the globe, socioeconomic status (SES), also referred to as social class, is considered to be the most reliable and consistent predictor of a person's health and life expectancy throughout their lifetime. Social class is defined as a category or group of people who have approximately the same amount of wealth, status, and power in a society (Cockerham 2015). In most social science or social epidemiology research, SES is treated as a multidimensional construct, comprised of a number of socioeconomic factors (income, race, age, education, etc). The concept of SES is derived from ideas concerning social stratification. Used to determine class standing, SES consists of three variables: income, occupational prestige, and level of education. While these variables are all interrelated, each display different aspects of a person's position in

the class structure of society (Cockerham 2015). In health and illness studies, income indicates spending power, housing, diet, and medical care; occupation measures status, responsibility at work, physical activity, and health risks associated with a person's job; and education is suggestive of one's skills for obtaining social, psychological, and economic resources such as good jobs, health insurance, access to quality care, and knowledge about healthy lifestyles (Cockerham 2015). However, it is important to understand that different socioeconomic factors can affect health status at different phases of the life course and function at different levels (individual, household, neighborhood, etc.) Socioeconomic factors are complex in that they can all interact with other socioeconomic factors or social characteristics at any point in an individual's life span. Another study done by Langlois et al. found that youth sports participation is associated with family income level and parental physical activity participation and encouragement (Langlois et al. 2017).

When considering the impact of SES on bone health, SES has been shown to affect bone mass, bone mineral density, and vitamin D status in pediatric and adult populations. A wealth of social epidemiological research spanning seven decades has examined the effects SES on morbidity and mortality rates; hence, it is not surprising that there is increasing evidence regarding socioeconomic status as a risk factor for poor musculoskeletal health, specifically low bone mineral density (BMD). Previous studies have shown that lower SES populations more commonly have lifestyle behaviors that do not prevent the onset low BMD (Hamerman 2005). Adolescence is not only marked as a time of peak bone development but it also the time when most children establish health

and lifestyle behaviors. Associations between SES and diet, SES and physical activity, and SES fracture risk in pediatric populations are all considerable aspects that should be considered when evaluating pediatric bone health. The influence SES has on diet, physical activity, and bone health in young children all relate to one another making SES in children and their families an influential component of bone development in kids.

In a study conducted by Crandall et al. statistical analysis found that greater childhood socioeconomic advantage and higher adult education level were associated with higher lumbar spine BMD (Crandall et al. 2012). The positive association between childhood socioeconomic advantage and adult education is not surprising given that education impacts current finances, thus impacting aspects of socioeconomic status. Additionally, adult education is impacted by social conditions throughout childhood, as an adolescent, and impacts socioeconomic status as an adult. This information suggests that socioeconomic conditions over a life span are more relevant when considering adult bone health and childhood socioeconomic advantage is especially relevant to adult bone mass given that bone mass acquisition takes place during childhood.

In addition, Brennan et al.'s systematic review of socioeconomic status and BMD identified limited evidence between BMD and education. However, consistent results from three cross-sectional studies provided evidence of a positive relationship existing between level of education and BMD in females, where greater education provided a greater protective effect on BMD (Brennan et al. 2011). Income has also been shown to act as a preventative measure against low BMD. In a study done on Spanish men and women, those with higher level income were more likely to have greater BMD compared

to those with lower level income (Brennan et al. 2011). In a literature review conducted by Hanson and Chen on SES and its impact on health behaviors in adolescents, the majority of results found in previous studies found that low SES adolescents had poorer diets compared to high SES adolescents. Additionally, this review also revealed that high SES was associated with greater physical activity in teens compared to their lower SES counterparts (Hanson and Chen 2007).

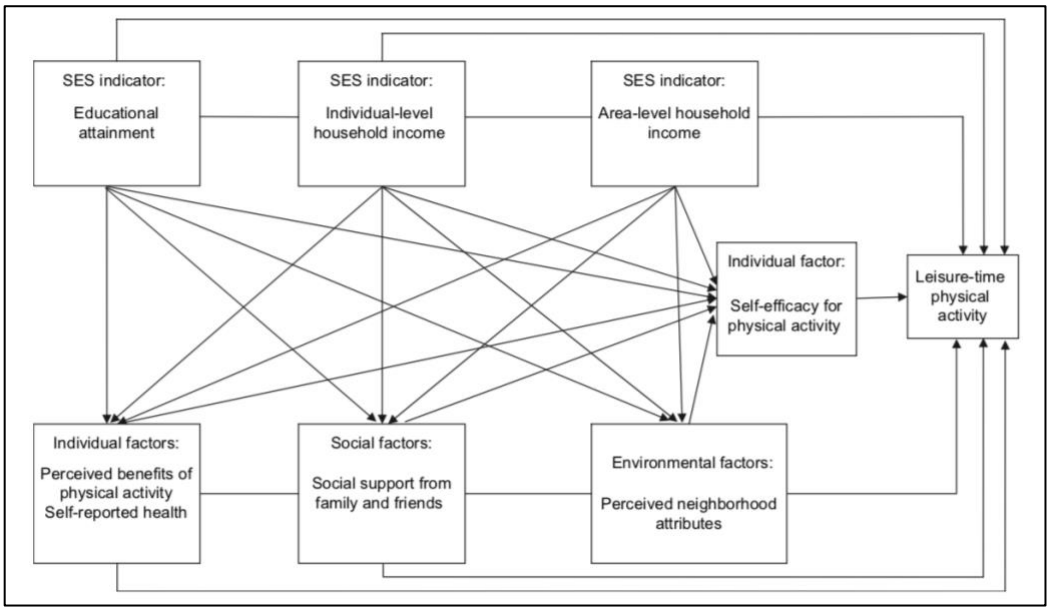


**Figure 4.** ***Socioeconomic Status and Leisure-Time Physical Activity*** Hypothetical model of differences in leisure-time physical activity among SES groups (derived from (Cerin and Leslie 2008))

While low BMD is a strong predictor of fracture risk in children and adults, socioeconomic status has also been found to be a predictor of fracture risk in adult populations. Socioeconomic status has strong relationships with obesity and chronic disease which influence fracture risk independently of BMD. Most studies looking at income and education links to fracture risk in the United States have been done on older individuals looking at hip fracture incidence. Yet, younger people who experience

fractures are more likely to fracture at sites other than the hip. Low lumbar spine BMD is a better predictor for fracture risk than femoral neck BMD for these sites and is also a stronger predictor for fractures in younger individuals than femoral neck BMD is (Crandall et al. 2014). Given that lumbar spine BMD has a stronger link to socioeconomic status than femoral neck BMD does, it is possible that association between socioeconomic status and fracture risk are greater in younger individuals.

### *C. Vitamin D Deficiency in Pediatric Populations*

Vitamin D plays an essential role in calcium absorption from the skeleton. Having a pivotal role in calcium homeostasis, vitamin D is crucial for bone mineralization (Vlachopoulos et al. 2015). Often referred to as "the sunshine vitamin" vitamin D is a fat-soluble molecule. Provided by skin synthesis, diet, or supplementation, vitamin D must undergo hydroxylation to reach its metabolically active form, 1,25-dihydroxyvitamin D. After absorption, vitamin D enters the bloodstream and first travels to the liver to form 25-hydroxyvitamin D (25(OH)D) and then kidney to form 1,25-dihydroxyvitamin D. Vitamin D levels are measured using 25-hydroxyvitamin D (25(OH)D) because it is the primary circulating form of vitamin D found in the blood. The metabolically active form of vitamin D, 1,25-dihydroxyvitamin D, is responsible for calcium homeostasis and sustaining bone health (Holick 2013). Figure 4 illustrates how vitamin D is metabolized in the body and the vital roles it plays in maintaining overall health.

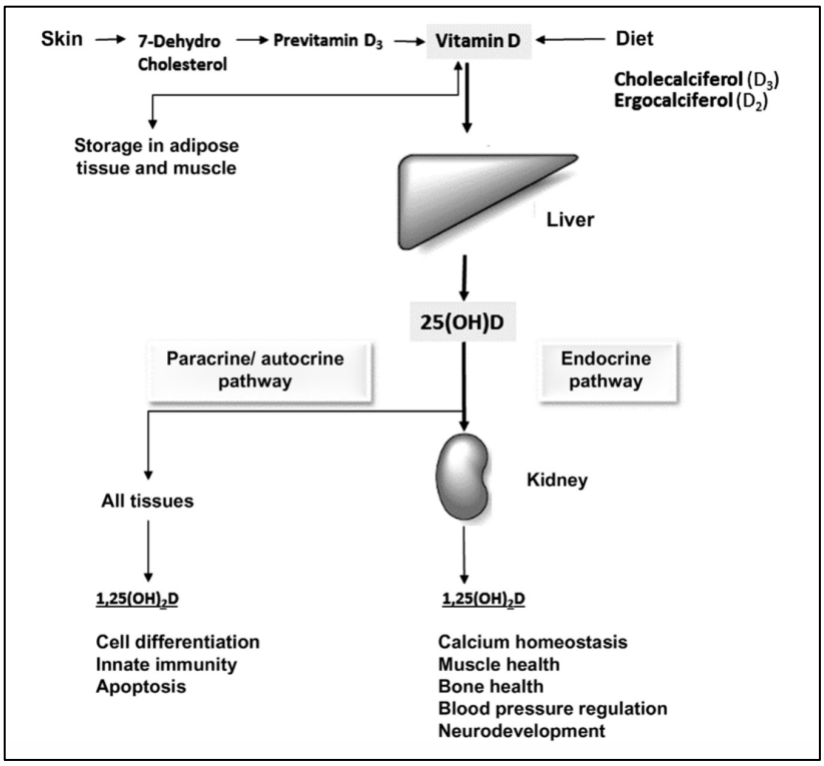


**Figure 4.** ***Vitamin D Metabolism*** (derived from (Whiting et al. 2017))

i. Sources of Vitamin D

Accounting for 95% of vitamin D found in the body, the most common source of vitamin D is provided via sun exposure. When exposed to sunlight, provitamin $D_3$ (7-dehydrocholesterol) found in epidermis and dermis absorbs ultraviolet B photons to form previtamin $D_3$. Previtamin $D_3$ is thermodynamically unstable. Thus, once created it immediately undergoes isomerization to form cholecalciferol, more commonly known as vitamin $D_3$ (Holick 2013). Once vitamin $D_3$ is synthesized, it is released from the epidermis into the bloodstream to bind to a vitamin D binding protein.

The remaining 5% of vitamin D found in the body relies solely on dietary intake. Vitamin D found in food and supplements for vitamin $D_3$ (cholecalciferol), or vitamin $D_2$ (ergocalciferol) are generally measured in international units (IU). Naturally found in fish and fish products, food sources of vitamin D can also be found in products that are fortified with vitamin D such as milk, other dairy products, and orange juice (Whiting et al. 2017). Additionally, vitamin D supplements are the final source of intake. Vitamin D supplements for vitamin $D_3$ (cholecalciferol) or vitamin $D_2$ (ergocalciferol) are frequently offered in the form of multivitamins ranging from 40 – 1000 IU per tablet.

A major misconception regarding Vitamin D deficiency is that it is more common in older adults, putting the elderly population at higher risk. However, children and teens and young adults are equally at risk for vitamin D deficiency (Holick et al. 2011). According to The Endocrine Society Clinical Guidelines, vitamin D deficiency, insufficiency, and sufficiency is defined as a serum 25(OH)D of < 12 to 20, 21 to 39, and >40ng/mL, respectively (Minkowitz et al. 2017). The American Academy of Pediatrics (AAP) only encourages "at-risk" children (usually those with 25VitD absorption or production issues) to have 25VitD levels checked. As a result, a large percentage of pediatric patients in the healthy population never have 25(OH)D levels tested throughout their childhood (Schor 2005). The concern for pediatric patients with deficient and insufficient 25(OH)D levels arises because deficient and insufficient 25(OH)D levels increase fracture risk in patients of all ages.

ii. Factors Impacting Vitamin D Deficiency

Vitamin D found naturally or fortified in food is limited, making sun exposure the primary source of vitamin D for children and adolescents. Being the primary source of vitamin D, inadequate exposure to sunlight is the most common cause of vitamin D deficiency. Many individuals believe that supplementation to maintain Vitamin D levels is only necessary if they are not exposed to sunlight; however, this common assumption is false. When looking at light-skinned individuals and their corresponding Vitamin D levels throughout the year, the change in 25(OH)D can demonstrate how sun-exposure affects this population during the winter seasons. People with naturally dark skin tones have natural protection from the sun and require three to five more hours of sun exposure to produce the same amount of vitamin D as individuals with white skin tone.

Even though people are still exposed to the sun in the winter seasons, the amount of sun-exposure is significantly less, resulting in dropping Vitamin D levels. In a study conducted in Boston, MA researchers found that sun exposure during the day was considerably shorter in October compared to the amount of sun exposure in July. As a result of the limited sun exposure in October, the percent of pre-vitamin $D_3$ formed decreased dramatically (Holick 1995). Issues surrounding the damaging effects of long-term exposure deter individuals from getting

enough exposure to the sun, even in the summer months. The detrimental effects of sun exposure such as skin cancer and sunburn can be prevented with the use of sunscreen. However, using sunscreen with a sun protection factor of 30 or greater reduces dermal vitamin D synthesis by 95% (Holick et al. 2011).

Additionally, the inverse relationship seen between BMI >30 $kg/m^2$ and 25(OH)D serum levels indicates vitamin D deficiency is also associated with obesity. Individuals with a BMI greater than 30 $kg/m^2$ that is considered obese tend to 50% less bioavailability of vitamin $D_3$ due to the deposition of vitamin $D_3$ in body fat compartments (Fuquay et al. 2011). Other causes of vitamin D deficiency can be caused by fat-malabsorption syndromes, primary hyperthyroidism, Crohn's disease, and other chronic diseases impacting the absorption of vitamin D.

iii. Consequences of Vitamin D Deficiency

Vitamin D deficiencies have been found to play a significant role in increased fracture risk and poor bone health in all types of physically active and inactive children. Two forms of bone disease most commonly associated with vitamin D deficiency are rickets and osteoporosis. Rickets, most commonly seen in early childhood is the result of chronic severe vitamin D deficiency starting at a young age. Rickets is a disabling disease that usually results in inward or outward bowed legs once children can stand and begin to walk (Whiting et al.

2017). Deficient levels of Vitamin D are associated with malabsorption of calcium and phosphate commonly result in secondary hyperparathyroidism. Secondary hyperparathyroidism caused by vitamin D deficiency heightens mobilization of calcium from the skeleton. The increase in calcium mobilization from the skeleton has been known to cause increased bone loss(Cheng et al. 2005).

Vitamin D deficiency also may be associated with other chronic diseases such as cancer, autoimmune diseases, cardiovascular disease, and neuromuscular diseases. Studies have shown that inadequate vitamin D levels were significantly associated with higher risk of colon cancer or cancer of the rectum. Research regarding the relationship between autoimmune diseases and vitamin D deficiency has found that insufficient vitamin D status may predispose individuals to type 1 or type 2 diabetes. Another relationship that has been demonstrated through several epidemiological studies is the prevalence of high vitamin D levels leading to lower risk of multiple sclerosis (Whiting et al. 2017). While there are a number of relationships between vitamin D deficiency and chronic diseases there are still some unanswered questions regarding the mechanism of action and the prevention properties of vitamin D. The broad range of illnesses connected to vitamin D highlights the significant beneficial effects maintaining vitamin D sufficiency can have on an

individual's health (Fuquay et al. 2011). While further investigation will undoubtedly lead to a better understanding of the relationship of vitamin D to the diseases mentioned above, these findings provide a great deal of evidence supporting the importance of vitamin D status in individuals of all ages.

From a public health perspective, children should be the population of interest in preventing vitamin D deficiency. Maximizing bone health is essential acutely, to fracture healing (Weaver et al. 2016). It has been shown that in children, specifically females, that having higher vitamin D serum levels results in greater BMC for the whole body compared to those with lower vitamin D serum levels (Foo et al. 2009). Children with adequate vitamin D levels tend to have significantly higher bone mass and muscle strength than those with inadequate vitamin D levels, which may be a result of lower bone remodeling rates in children with adequate vitamin D levels (Mølgaard et al. 2010). For this reason, vitamin D supplementation should be considered more often in children to promote good bone health throughout their lifetime.

*D. Vitamin D Supplementation in Pediatric Populations*

Worldwide, vitamin D deficiency is common in the general pediatric population. Past literature comparing BMD directly to serum 25(OH)D levels draws conflicting reports as to the effect of vitamin D supplementation on BMD accrual in healthy children. However, with rising interest in vitamin D supplementation in pediatric patients to promote good bone health during childhood, new evidence provides better insight into

the benefits of vitamin D supplementation. Benefits of establishing good bone health in children include decreases in fracture risk and optimization of peak bone mass which may lead to better bone health later on in life.

Current literature regarding vitamin D supplementation in children and adolescents looks mostly at populations outside of the United States. However, the results presented in these reports highlights the extreme need for pediatric vitamin D research in the United States. A cross-sectional study investigating the influence of low vitamin D status on bone mass and muscle strength in 301 healthy Chinese adolescent girls found that adolescent girls with sufficient vitamin D status (>50ng/mL) had higher (size-adjusted) whole body BMC than girls with poorer vitamin D status (Foo et al. 2009). Similarly, Du et al. revealed that the addition of 200-320 IU of vitamin $D_3$ to calcium-fortified milk increased whole body BMC over two years compared to calcium-fortified milk without added vitamin $D_3$ (Du et al. 2004).

Many meta-analysis studies looking at the impact of vitamin D supplementation on bone density in children without fractures have found that vitamin D supplementation is clinically effective in improving bone density only in children with low vitamin D serum concentrations. (Winzenberg et al. 2011). These studies investigating the effects of vitamin D supplementation on bone density in 'healthy" patients without fractures have yet to find significant results. Results regarding the overall use of vitamin D supplementation in pediatric patients to increase BMC or BMD were not statistically significant. Thus, the lack of significant results warrants further research on this topic to be done; especially since vitamin D (Serum 25(OH)D)

is essential for healthy bone development during adolescence.

Serum 25(OH)D level is a factor related to bone acquisition and peak bone mass through its role in facilitating calcium absorption. Children and adolescents with deficient and insufficient 25(OH)D levels can present symptoms comparable to osteoporosis. Adolescence is a critical period for peak bone mass development, with 80-90% of bone mass acquired by age 20, making it essential to establish good bone health during childhood. Maximizing peak bone mass is essential acutely, so the child does not have repeat fractures and refractures. Optimizing peak bone mass is essential throughout growth, so pediatric cases of low BMD do not enter into the fracture threshold prematurely (Figure 5). Hence, to curb the premature fracture risk, diagnosis of hypovitaminosis D (deficient or insufficient) and treatment should start in childhood.

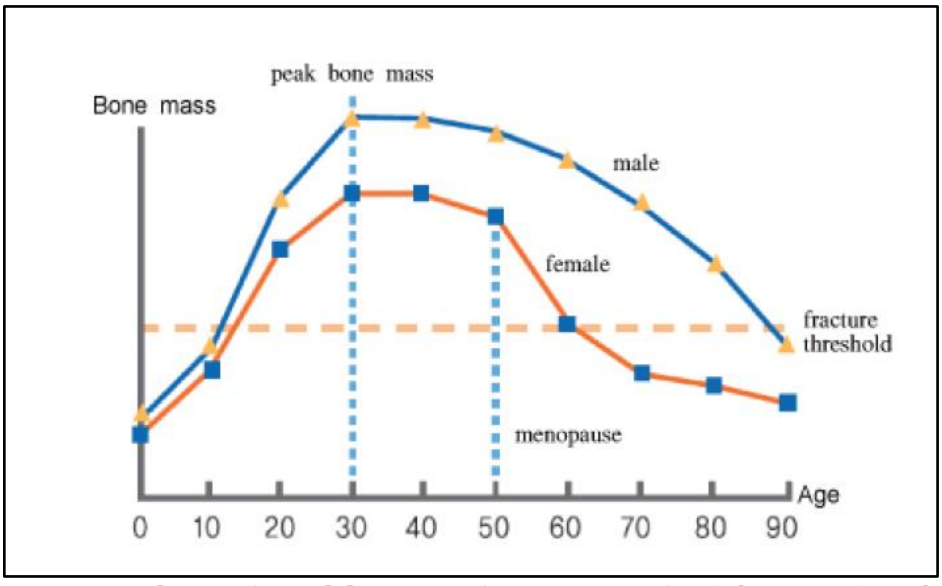


**Figure 5.** ***Changes in peak bone mass in respect to aging*** (Chung 2008) Peak bone mass is maximized between ages 20-30, and it starts to decline after that. Adolescents and postmenopausal women present comparable levels of fracture risk past the fracture threshold. Males present with consistently higher BMDs on average throughout their lifetimes.

In a previous study, Minkowitz et al. noted that fractures do not occur as a direct result of the 25VitD level, yet the severity of the fracture is related to the severity of the 25(OH)D deficiency (Minkowitz et al. 2017). The trend observed in Minkowitz et al.'s retrospective study was a motivating factor for the compliance study completed on the DXA study patient population. The objective of the compliance study was to identify compliance patterns in healthy children with fractures who were recommended to get a serum 25VitD level checked and counseled to start taking Vitamin D3 supplementation with calcium. The clinical goal of supplementation counseling in this study was to maintain compliance at least during the period of fracture healing. Specifically, the following three questions asked were, 1) is there a difference in compliance between patients with a known 25VitD level versus patients who did not know their 25VitD level; 2) is there an association between supplementation compliance and BMI, age, sex; and 3) is there an association between supplementation compliance and fracture severity or initial 25VitD level. Compliance was defined as "sustaining a pattern of compliance" meaning that patients started or were already taking and continued to take vitamin D supplements after initial encounter. Compliance was reported as parent's or patient's self-reported adherence to recommended supplementation protocol. This study found Vitamin D supplementation compliance to be significantly related to obtaining a serum 25VitD level, fracture severity, and patient age in this pediatric population. Supplementation compliance was not associated with BMI or sex and was not associated with initial 25VitD level. Patients with a known 25VitD level were more compliant than those without an initial evaluation of serum 25VitD who simply took a supplement based on

the concept of low 25VitD. Compliance was found to improve with patient age indicating that it may be valuable to empower older patients to take responsibility for themselves. Additionally, those with more severe fractures were more likely to comply with vitamin D supplementation (83% (n=104/125) of patients with AIS 3 (surgical) fractures were compliant; 49% (n= 628/1,292) of patients with AIS 1, 2 fractures were compliant ($p<0.001$).

Studies investigating BMD and supplementation in 'healthy" patients without fractures have yet to find significant results and there are no studies published that have investigated BMD and supplementation in "healthy" patients with fractures. However, this study is unique because unlike previous research, this study looks at "healthy" patients with fractures, allowing the impact of growth hormones released post fracture to also be considered. Given that other studies looking at healthy patients without fractures have not shown differences in DXA results over time, our study population may have changes in DXA as a result of overgrowth during fracture healing; thus, justifying further research on this topic.

The purpose of the current study is to determine the relationship between pediatric vitamin D levels (25(OH)D) and bone mineral density (BMD) acquired from dual-energy x-ray absorptiometry (DXA) scans in children with fractures. From a public health perspective, children should be the population of interest in curbing the incidence of osteoporosis. Adolescence is a critical period for peak bone mass development which when optimized, decreases future risk of osteoporosis. It has been shown that in children, specifically females, that having higher vitamin D

serum levels results in greater BMC for the whole body compared to those with lower vitamin D serum levels (Foo et al. 2009). Children with adequate vitamin D levels tend to have significantly higher bone mass and muscle strength than those with inadequate vitamin D levels, which may be a result of lower bone remodeling rates in children with adequate vitamin D levels (Mølgaard et al. 2010). The long-term goals of this research are to better understand pediatric bone health and the effects of supplementation on bone quality. This will guide recommendations to optimize peak bone mass, to better advocate for appropriate Vitamin D supplementation in children, and to guide the timing of safe return to sports based on DXA data with the goal of avoiding re-injury. Additionally, this study may provide insight into the effect of optimizing bone mineralization during childhood as an early method of osteoporosis prevention (Viljakainen et al. 2009).

While the primary expected outcome of this study is a better understanding of the relationships between 25(OH)D levels, bone fragility, fracture risk, BMD, bone mineral content (BMC), and muscle mass in the pediatric population, the hypothesis for this thesis is that there will be a positive relationship between vitamin D supplementation and increased BMD & BMC over 12 months. The secondary expected outcome of this study is a greater acceptance and legitimacy for the role of vitamin D supplementation in the pediatric population based on pediatric DXA data. Pediatricians and orthopedists can use information from this study to help direct patient care by identifying susceptible children and young adults to prevent or mitigate the long-term consequences of repetitive fractures and maximize peak bone mass.

Some pediatricians are unconvinced of the importance of regularly checking vitamin D levels due to the lack of formal studies addressing the consequences of vitamin D deficiency. Studies investigating BMD and supplementation in 'healthy" patients without fractures have yet to find significant results. Additionally, one of the goals of this study is to have DXA data-driven timelines developed for patients who undergo supplementation to correct low Vitamin D levels and poor bone health. This data can be used for counseling to make definitive recommendations regarding safe return to sports after injury based on changes seen on DXA. This will also help better define appropriate 25(OH) vitamin D levels in children, which has been a controversial topic between the Institute of Medicine and Endocrine Society as current guidelines from these societies are not in agreement. Altogether, this research will attempt to quantify the relationship between vitamin D levels, BMD, and BMC as they relate to bone health in healthy children and adolescents with a history of fracture, before and after supplementation.

This thesis will analyze results from both the pediatric compliance study and the ongoing DXA study and consider the implications socioeconomic status may have on the ability to comply with vitamin D supplementation and BMD status both before and during vitamin D supplementation. Overall, this paper and its research aims to:

- Correlate the relationship between 25(OH) Vitamin D level in pediatric fracture patients and bone quality seen on DXA scan (BMD and BMC).
- Test the hypothesis that vitamin D supplementation over 6 and 12 months will result in an increase in BMD and BMC in pediatric fracture patients.

- Challenge the current apathy towards pediatric vitamin D supplementation and regular monitoring of serum levels based on longitudinal data from DXA scans.
- Clarify pediatric patient guidelines for appropriate 25(OH)Vitamin D levels
- Confirm that race and ethnicity impact bone health in pediatric populations

## II. Methods

The study design is a prospective longitudinal interventional study under the leadership of Dr. Barbara Minkowitz, MD and Jennifer Ristic, PA at Atlantic Health System's Department of Sports Medicine. The duration of the study is approximately three years, where patients will only be asked to participate for one year following the time of fracture. As part of this study protocol, a DXA scan will be performed, 25(OH)D level will be drawn, and a grip hand strength test will be conducted within the first nine weeks of the time of fracture, six months after the fracture, and again one year after the fracture while patients are maintained on Vitamin D and calcium supplementation.

Vitamin D plays a significant role in bone health by assisting in absorption of calcium in the body. Calcium is essential for bone health, so it is realistic to infer that low vitamin D levels could contribute to poor BMD and BMC. It is also realistic to infer that supplementation with Vitamin D and calcium will affect bone health analyzed with DXA scan which may vary based on Vitamin D level and patient age. The following bone health protocol (Figure 6) developed by Dr. Barbara Minkowitz will be used for vitamin D supplementation regimens.

**Bone Health Protocol- Pediatric Orthopedics**
**Barbara Minkowitz, MD, Atlantic Health Systems, Morristown, NJ**
*Vitamin D and Calcium are daily supplements needed by everyone, everyday*

**Daily Vitamin D guidelines to obtain serum levels 40-60ng/ml in children without liver/ kidney dysfunction (predicated on serum 25-OHVitamin level).**
0-1 **year:** 400U Vitamin D, **1-8 years:** 600-1000U Vitamin D, **9-13 years:** 1000-2000U Vitamin D, **13-18 years:** 1500-2000U Vitamin D, **Adult:** 2000U Vitamin D
**20-50 lbs:** 500-1000U Vitamin D, **50-90 lbs:** 1500U Vitamin D,**90+ lbs:** 2000U Vitamin D
•Vitamin D taken always with calcium; 1000 mg daily except 1-4 years old= 700mg daily

**No Fracture present: Supplementation guidelines based on serum 25-OH VitD level used when fractures are not present. Labs must be repeated 2-6 months after supplementation, depending on amount given and to validate amount being used for maintenance. Some people are non-absorbers, and will require higher maintenance levels.**

| | |
|---|---|
| 30-40ng/ml: considered normal by lab, however by Endocrine Society, 40-60ng/ml is optimal. | Add 500U VitD + calcium |
| 20-30 ng/ml: considered VitD insufficient | Add 1000U VitD + calcium |
| 12-20 ng/ml: considered VitD deficient | Add 2000U VitD + calcium |
| <12 ng/ml: considered VitD deficient | 7000U VitD + calcium (If < 10 may give 14,000 VitD) and then repeat labs at 2 months, decrease if value in range, or send to endocrine This is equivalent to 50,000U or 100,000U per wk |

**Fracture present: With a fracture present, higher initial supplement can be used (dictated by serum level and not age or weight), for 2-3 months then repeat labs and decrease VitD or send to endocrinologist if not responsive. This is most important for bone fractures that can take a long time to heal, even in the best situation.**

| | |
|---|---|
| 30-40 ng/ml: considered normal by lab, however by Endocrine Society, 40-60ng/ml is optimal | Add 500-1000 units VitD + calcium |
| 20-30 ng/ml: insufficient for healing | Add 4000 units VitD + calcium |
| 16-20 ng/ml: insufficient for healing | Add 4000 units VitD + calcium |
| <15 ng/ml: deficient | 7000U VitD + calcium (If < 10 may give 14,000 VitD) and then repeat labs at 2 months, decrease if value in range, or send to endocrine This is equivalent to 50,000U or 100,000U per wk |

**Fracture present but no serum25-OH VitD is available: Patients are empirically started on supplementation while awaiting serum level. VitD is then adjusted up or down as dictated by lab values:**
≤12 years of age or < 90 lbs: 2000 units VitD + calcium
≥12 years of age or > 90 lbs: 4000 units VitD + calcium
**Plan B: The Noncompliant Child: A weeks worth of VitD can be given once a week and calcium given daily.**

•Some children have absorption problems that are recognized and some are not. They may need up to 7000 units of VitD daily for maintenance (equivalent 50,000 units per week). Serial 25-OH VitD levels help discovery and allow customization of supplements.

**Figure 6.** ***Minkowitz Bone Health Protocol*** (Minkowitz et al. 2017) recommendations for daily vitamin D and calcium supplementation based on age, weight, and presence of fracture to obtain serum levels 40-60 ng/ml

*A. Patient Population:*

We will screen 600 fracture patients with the goal to enroll 250 between the ages of 4-18 years of age who are interested in participating in the study and can lay still for the DXA scan. Repeat DXA will be checked after six months and one-year post fracture. We will monitor patients with phone reminders to encourage compliance with their supplementation and that their serum vitamin D levels are within the 40-60 ng/ml recommended range. The source of study participants is from the Children's Orthopedic and Sports Medicine group in Atlantic Health System. The pediatric patients will already have been treated for fractures and will be able to have the DXA performed within the first nine weeks after fracture. In the summer months, only subjects with a low vitamin D level can be included to avoid false high levels often seen during the summer time. During every other month, subjects with a vitamin D level greater than 20 will be included. Patients with 25(OH)D levels under 20 will not be accepted into the study. Children with the following conditions will be excluded from the study:

> *amyloidosis, ankylosing spondylitis, collagen vascular diseases, congenital porphyria, epidermolysis bullosa, prior gastrecTaray, hemochromatosis, hemophilia, homocystinuria, idiopathic juvenile osteoporosis, idiopathic scoliosis, inflammatory bowel disease, insulin-dependent diabetes, leukemia, lymphoma, bone cancer, malabsorption, nutritional/eating disorders, organ failure/transplantation, osteogenesis imperfecta, parenteral nutrition, severe liver disease, thalassemia, thyrotoxicosis, acromegaly, adrenal atrophy, Cushing's*

*syndrome, gonadal insufficiency, hyperthyroidism, hyperparathyroidism, and hypophosphatasia.*

Only participants who give assent and whose parents give consent can be included in the study. The materials being used to recruit patients are the consent form, assent form, an informative brochure about DXA scanning, and a $25 stipend in the form of a gift card (given at each DXA, $75 per participant).

*B. Consent and Assent:*

Those obtaining consent will use respectful and child-friendly language to the prospective participants or their legally authorized representatives. We will give participants and their parent(s) sufficient opportunities to ask questions throughout the entirety of the study. We will tell participants and their parent(s) that they can withdraw from the study at any time for any reason with no consequences. Research investigators will refrain from any language or body language that may be perceived as coercive to either the patient or their guardian(s). We will document the consent (assent) of the participant in writing in language suitable for their age group. We will also document the consent from the parent(s) in writing.

*C. Standard Practice:*

*i. DXA Study Questionnaire:*

Upon consent and assent, the patient's parent or guardian will be asked to complete an initial DXA questionnaire. Patients will also be asked to complete a follow-up questionnaire at both their second and third DXA scan. The initial DXA study questionnaire includes questions regarding patient demographics,

family history, supplementation regimen, previous fracture history, physical activity, and diet. Follow-up questionnaires will ask for updated information supplementation usage after being provided with supplementation recommendations.

*ii. X-ray and Bloodwork:*

The patient to have an x-ray performed as part of the orthopedic care, blood drawn early on in fracture care, and a DEXA at the time of his/her fracture or within nine weeks. Further blood draws may be required to address vitamin D serum levels. The blood draws will be done at an appropriate lab, with approximately 15 ml (about ½ an ounce) obtained. A series of tests initially drawn will include complete blood count (CBC), comprehensive metabolic panel (CMP), phosphorus, parathyroid hormone (PTH), calcium, and 25 hydroxyvitamin D. 25 hydroxyvitamin D draws will be repeated at 6-month intervals depending on serum 25(OH)D level and response to supplementation. The goal is to optimize the serum 25(OH)D level to 40-60 ng/ml. Serum 25(OH)D levels under 40 ng/ml are considered insufficient or deficient, and those over 60 ng/ml are considered hypovitaminosis (high levels of a vitamin to the point of toxicity).

*iii. Tanner Stages Questionnaire:*

Pubertal stage is strictly correlated to skeletal maturation in pediatric patients. The timing of puberty and pubertal stage align with changes in structure and composition of bone, thereby impacting bone health. Pubertal stage was self-reported or reported by the patient's parent(s) or legal guardian(s). Tanner Stage forms were provided to all patients at each DXA scan. Being the standard clinical system for describing pubertal status, subjects were classified as Tanner stage I, II, III, IV, or V. The Tanner system included assessment of the pattern of development of breasts in females, genitals in males, and pubic hair in both girls and boys (Figure 7).

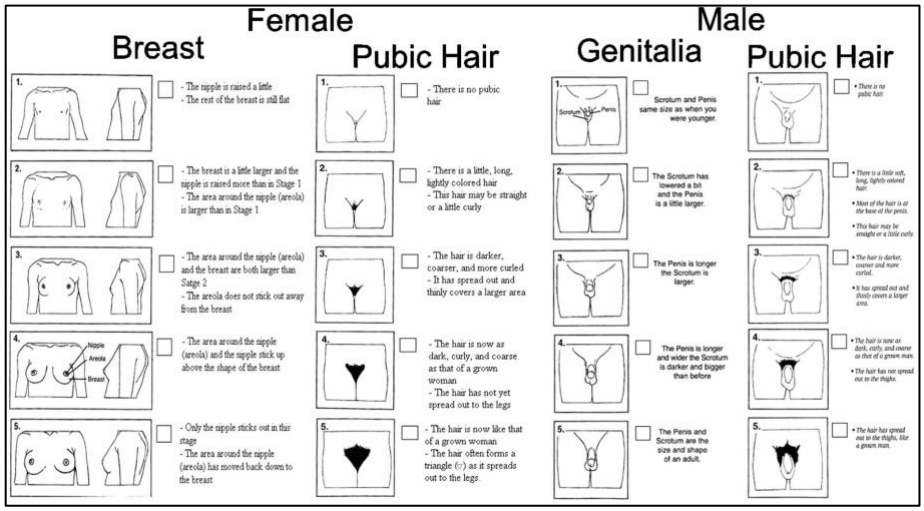


**Figure 7.** ***Tanner Stages*** Gender-specific self-assessment questionnaire for children, containing both illustrations and explanatory text (Chavarro et al. 2017)

*iv. Hand Grip Strength:*

We will test patient grip strength using a Jamar Dynomometer gripper. The gripper uses the mechanical effort needed to move the bar

within the hand pump as an indicator for musculoskeletal capacity. We will perform the test three times to get an average strength score (presented with a standard deviation).

*v. DXA Scanning:*

The radiologist will perform the DXA at 111 Madison Avenue, Morristown, NJ on a Lunar DXA machine. The first DXA scan must be performed within the first 9 weeks after the fracture. The vertebral DXA takes 1 minute, and a whole body DXA (less head) takes 5-6 minutes. Although callus formation, bone remineralization, and muscle atrophy may be problematic for the vertebral scan, they will not affect the whole body DXA (less head). There cannot be a cast in place at the time of DXA scanning. A second DXA will be required six months later and a third one year later. The radiologist will analyze these DXA results using z-scores, since pediatric body mass will likely change over time.

*vi. DXA Analysis:*

A radiologist with Atlantic Health Systems analyzed the results from the DXA scans. The DXA provided bone mineral content (BMC), bone mineral density (BMD), and z-score for the lumbar spine (L/S) region, the total body less head "TBLH" region, and the left and right femoral heads. For the purposes of analysis, the results were separated into two racial categories: African American/Black and White/Asian because of skin tone-based differences in UVB absorbance.

*vii. Follow-up and Compliance:*

The patients will be able to get information about supplementation and reminders for visits and lab draws from Dr. Minkowitz and her staff. We will remind the patients by phone (as frequently as needed to get a response when it is time to schedule a follow up) that they are part of this study and advised to continue taking vitamin D and calcium supplementation as per the Minkowitz protocol (Figure 6). We will tell patients if any further blood testing is required and when to return for follow-up DXA.

Patients may decide not to continue in the research study at any time without it being held against them. If patients decide to leave the research study, they must contact the investigator so that the investigator can ensure that the removal process is complete. The person in charge of the research study or the sponsor can remove them from the research study without approval from the coordinator of research data. Possible reasons for removal include inability to sit still for a DXA scan or blood draw.

## III. Results

At this time the study has had a total of 42 patients enrolled; nine patients have completed the study and nineteen patients withdrew from the study or were lost to follow-up during their participation. Currently, there are 14 active patients participating. The average patient age is 12.564 (±2.963) and the ethnic distribution is 33.33% Hispanic or Latino and 66.57% not Hispanic or Latino. Racial distribution is 7.69% Asian, 2.56%

Native Hawaiian or Other Pacific Islander, 17.94% Black or African American, 66.67% White, 2.56% more than one race, and 2.56% unknown or not reported. Average initial Vitamin D level is 18.52 (ranging from 6-46.2).

DXA baseline data that has been collected is described in Table 1. Multi-variate analysis will include sports activity (weight-bearing versus non-weight bearing) in different seasons, amount of active time per week, and any differences in DXA data between sedentary and active children. Multi-variate analysis will also include fracture severity, mechanism of injury, amount of sun exposure, sunscreen use, and dietary preferences (calcium intake, caffeine intake, etc). Initial and follow-up strength analysis with grip-strength testing will be reported with multi-variate analysis and change over time will be reported. The study's current DXA data for 23 patients with two completed DXA scans is reported below (Table 2). Please note that TBLH Z score is not available for African American patients because there is not a reference population for African American children in the software for the GE Lunar Prodigy DXA machine at our facility. Our statistician is working with GE and we anticipate being able to correct for this problem using a programmable database with reference data for this population from a previously published study.

Data presented in Table 2 is an analysis of 23 patients, including patients who are Black or African American. The data shown highlights that there is a statistically significant increase in BMC between visit 1 and 2 (Table 2). Additionally, the data for BMD is trending towards statistically significant results; future data analysis with a larger number of patients may confirm this trend.

**Table 1. Patient Demographics**

| | | |
|---|---|---|
| **Age** | mean±SD | 12.564 ± 2.963 |
| | median (min-max) | 13 (7-18) |
| **Gender** | Female | n=15 (38.46%) |
| | Male | n=24 (61.53%) |
| **Ethnicity** | NOT Hispanic or Latino | n=26 (66.57%) |
| | Hispanic or Latino | n=13 (33.33%) |
| **Race** | White | n=26 (66.57%) |
| | Black or African American | n=7 (17.94%) |
| | Asian | n=3 (7.69%) |
| | Other | n=3 (7.69%) |
| **Vitamin D Level** | mean±SD | 18.5 ± 8.6122 |
| | median (min-max) | 17.9 (6-46.2) |

**Table 2. DXA Results Comparison between Scan 1 and Scan 2**

| | **Scan 1** | **Scan 2** | **p-value*** |
|---|---|---|---|
| ***L/S BMD*** | 1.071 (0.146) | 1.085 (0.145) | 0.18 |
| ***L/S Z Score*** | 0.1 (1.332) | -0.067 (1.375) | 0.01 |
| ***L/S BMC*** | 50.42 (10.421) | 52.66 (10.678) | 0.035 |
| ***TBLH BMD*** | 0.996 (0.107) | 1.023 (0.122) | 0.038 |
| ***TBLH Z Score*** | 0.33 (1.515) | 0.4 (1.671) | 0.497 |
| ***TBLH BMC*** | 1857 (383) | 1944 (413) | 0.01 |
| ***Right Femoral Neck BMD*** | 1.022 (0.160) | 1.03 (0.179) | 0.486 |
| ***Right Femoral Neck Z score*** | 0.14 (1.372) | 0.08 (1.504) | 0.492 |
| ***Right Femoral Neck Z BMC*** | 4.671 (1.025) | 4.849 (1.049) | 0.224 |
| ***Left Femoral Neck BMD*** | 1.045 (0.161) | 1.045 (0.165) | 0.997 |
| ***Left Femoral Neck Z score*** | 0.34 (1.468) | 0.2 (1.495) | 0.296 |
| ***Left Femoral Neck Z BMC*** | 4.627 (0.910) | 4.754 (0.970) | 0.37 |
| ***Grip strength (average of test trials)*** | 51.667 (14.883) | 57.82 (15.684) | 0.036 |
| ***Standard deviation*** | 4.653 (4.496) | 4.747 (2.315) | 0.932 |
| ***Coefficient of variation*** | 0.073 (0.134) | 0.033 (0.047) | 0.189 |
| **p-values calculated using paired t-test* | | | |

Completed DXA data (patients with 3 DXA scans) is available for 9 of the 23 patients who have under gone two DXA scans. The current demographics of the completed study data are not representative of the total population. Of the 9 patients who have completed the DXA study, six are male and three are female (Table 3). All follow-up patients were compliant to the vitamin D and calcium supplementation six months and one year after the initial fracture and DXA scan. Only twenty-two percent of the follow-up patients showed statistically significant increases in BMD after six months of supplementation and healing; however, the seventy-eight percent of patients who did not have significant increases in BMD after six months of supplementation did have significant increases in BMD after one year of supplementation (Table 3). Furthermore, seven out of the nine patients showed general increases in BMD in the L1-L4 regions and eight out of the nine patients showed general increases in BMD at TBLH regions after six months (Table 5a) and after one year of vitamin D supplementation (Table 5b). Patient 7 was the only child to have a decrease in BMD at the L1-L4 regions and no changed in BMD at the TBLH regions. Data were not available for the DXA measurements on the left and right femoral heads because there was not complete data on all the follow-up patients for analysis.

Eighty-eight percent of the follow-up patients were within a normal range of BMD for their age, weight, height, and sex (Table 5a & 5b). Table 6 shows the percent change in BMD relative to the percent change in BMC, which is relevant for growing populations (all patients were in an age range where normal body growth would be

expected). All of the patients' BMCs increased at a greater rate than their BMDs for both the L1-L4 and the TBLH regions.

**Table 3. Completed DXA Study Data:** Patient Characteristics

| Patient | Race | Sex | Age | BMI | Tanner Stage | Compliant | Significant increase at 6-month follow up? | Significant increase at 12-month follow up? |
|---|---|---|---|---|---|---|---|---|
| **1** | White | M | 9 | 13.90 | II (Boy) | Yes | No | No |
| **2** | White | M | 7 | 13.75 | I (Boy) | Yes | No | No |
| **3** | Black | M | 14 | 35.14 | V (Boy) | Yes | Yes | Yes |
| **4** | Hispanic | M | 11 | 22.42 | II (Boy) | Yes | No | Yes |
| **5** | White | M | 14 | 27.02 | IV (Boy) | Yes | No | Yes |
| **6** | Black | F | 11 | 25.29 | IV (Girl) | Yes | Yes | No |
| **7** | Hispanic | F | 8 | 21.73 | III (Girl) | Yes | No | Yes |
| **8** | White | M | 14 | 18.13 | III (Boy) | Yes | No | Yes |
| **9** | White | F | 13 | 23.62 | IV (Girl) | Yes | No | Yes |

**Table 4. Patient Diet and Weight Demographics**

| Patient | Weight Status | Physical Activity | Soda (1 can) | Ice Cream (1.5 cups) | Healthy Eater |
|---|---|---|---|---|---|
| **1** | underweight | >10 HRS | 2 | | Yes |
| **2** | healthy weight | 6-10 HRS | 1 | | Yes |
| **3** | obese | >10 HRS | 2 | 2 | Yes |
| **4** | overweight | 6-10 HRS | 2 | 1.5 | Yes |
| **5** | obese | 6-10 HRS | 3 | | Yes |
| **6** | obese | 6-10 HRS | 2 | 1.5 | No |
| **7** | obese | 3-5 HRS | 0 | 1 | Yes |
| **8** | healthy weight | >10 HRS | 5 | | Yes |
| **9** | overweight | >10 HRS | 0 | | Yes |

**Table 5a. Completed DXA Study Data:** BMD & BMC results after 6 months of supplementation

| Patient | L/S BMD ($gm/cm^2$) | L/S BMD increase (gm/cm2) | L/S Z-score | L/S BMC (g) | TBLH BMD ($gm/cm^2$) | TBLH BMD increase (gm/cm2) | TBLH Z-score | TBLH BMC (g) | BMD Impression |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.663 | -0.018<br>-2.64% | -0.8 | 24.64 | 0.718 | 0.008<br>1.10% | -0.7 | 804 | normal |
| **2** | 0.588 | 0.016<br>2.90% | -1.2 | 15.75 | 0.609 | 0.023<br>3.90% | -1.7 | 501 | low |
| **3** | 1.078 | 0.002<br>0.20% | 0 | 49.01 | 1.145 | 0.064<br>5.80% | * | 2902.7 | normal |
| **4** | 0.733 | 0.079<br>12% | -0.5 | 22.2 | 0.85 | 0.02<br>2.50% | 0.4 | 1236.2 | normal |
| **5** | 1.228 | 0.051<br>4.50% | 1.3 | 68.1 | 1.179 | 0.103<br>9.50% | 2.3 | 2736.2 | normal |
| **6** | 1.083 | 0.029<br>2.82% | 0.9 | 49.35 | 1.036 | 0.035<br>3.81% | * | 2667.8 | normal |
| **7** | 0.804 | -0.02<br>-3.07% | 0.3 | 32.94 | 0.865 | 0.00<br>0.00% | 1.0 | 1218.9 | normal |
| **8** | 1.248 | 0.055<br>4.78% | 1.4 | 69.31 | 1.171 | 0.027<br>5.19% | 2.2 | 2352 | normal |
| **9** | 1.224 | 0.023<br>1.95% | 1.5 | 58.17 | 1.097 | 0.01<br>1.00% | 2.1 | 2073.3 | normal |

*TBLH Z score is not available for African American patients because there is not a reference population for African American children in the software for the GE Lunar Prodigy DXA machine at the AHS facility.

**Table 5b. Completed DXA Study Data:** BMD & BMC results after 1 year of supplementation

| Patient | L/S BMD gm/cm$^2$ | L/S BMD increase (gm/cm$^2$) | L/S Z-score | L/S BMC (g) | TBLH BMD (gm/cm$^2$) | TBLH BMD increase (gm/cm$^2$) | TBLH Z-score | TBLH BMC (g) | BMD Impression |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.682 | 0.01<br>1.47% | -0.6 | 23.42 | 0.705 | 0.008<br>1.15% | -0.8 | 812.3 | normal |
| **2** | 0.564 | 0.016<br>2.92% | -1.4 | 16.01 | 0.611 | 0.023<br>3.91% | -1.6 | 493.4 | low |
| **3** | 1.05 | 0.002<br>0.19% | 0.1 | 45.79 | 1.177 | 0.064<br>5.75% | * | 2497.9 | normal |
| **4** | 0.739 | 0.079<br>11.97% | -0.4 | 21.11 | 0.833 | 0.02<br>2.46% | 0.4 | 1131.1 | normal |
| **5** | 1.192 | 0.051<br>4.47% | 1.3 | 67.19 | 1.191 | 0.103<br>9.47% | 2.6 | 2689.2 | normal |
| **6** | 1.057 | 0.029<br>2.82% | 1 | 47.26 | 0.953 | 0.035<br>3.81% | * | 2083.1 | normal |
| **7** | 0.759 | -0.024<br>-3.07% | 0.3 | 28.61 | 0.809 | 0<br>0% | 0.9 | 1095.9 | normal |
| **8** | 1.205 | 0.055<br>4.78% | 1.4 | 66.08 | 1.11 | 0.027<br>2.49% | 1.9 | 2214.2 | normal |
| **9** | 1.205 | 0.023<br>1.95% | 1.7 | 55.83 | 1.068 | 0.01<br>0.95% | 1.9 | 1941 | normal |

*TBLH Z score is not available for African American patients because there is not a reference population for African American children in the software for the GE Lunar Prodigy DXA machine at the AHS facility.

**Table 6: Percent BMC change relative to percent BMD change**

| Patient | Initial L/S BMC (g) | 1 Year L/S BMC (g) | % BMC Change | %BMD Change | Initial TBLH BMC (g) | 1 Year BMC (g) | % BMC Change | %BMD Change |
|---|---|---|---|---|---|---|---|---|
| **1** | 23 | 24.64 | 7.13% | 1.47% | 719 | 804 | 11.82% | 1.15% |
| **2** | 14.56 | 15.75 | 8.17% | 2.92% | 426.5 | 501.5 | 17.58% | 3.91% |
| **3** | 44.13 | 49.01 | 11.06% | 0.19% | 2401.2 | 2902.7 | 20.89% | 5.75% |
| **4** | 19.13 | 22.2 | 16.05% | 11.97% | 934.2 | 1236.2 | 32.33% | 2.46% |
| **5** | 59.41 | 68.1 | 14.63% | 4.47% | 2440 | 2736.2 | 12.14% | 9.47% |
| **6** | 43.27 | 49.35 | 14.05% | 2.82% | 1793.1 | 2667.8 | 48.78% | 3.81% |
| **7** | 29.45 | 32.94 | 11.85% | -3.07% | 1018.5 | 1218.9 | 19.68% | 0% |
| **8** | 61.25 | 69.31 | 13.16% | 4.78% | 2084 | 2352 | 12.86% | 2.49% |
| **9** | 52.89 | 58.17 | 9.98% | 1.95% | 1987.9 | 2073.3 | 4.30% | 0.95% |

Figures 8 and 9 display DXA imaging data from patient 3's one-year DXA scan. Figure 8 contains the BMD report for the L1-L4 region. Patient 3 has a 1.05 BMD measurement for the L1-L4 region, which is broken down into the four vertebrae, and then compared to the original BMD measurement from 6 months prior, showing an overall increase in BMD at the L/S region. Figure 9 contains the BMD report for the TBLH region. Patient 3 has a 1.177 BMD measurement for the TBLH region, which is compared to the original BMD measurement from 6 months prior, showing an overall increase in BMD at the TBLH region.

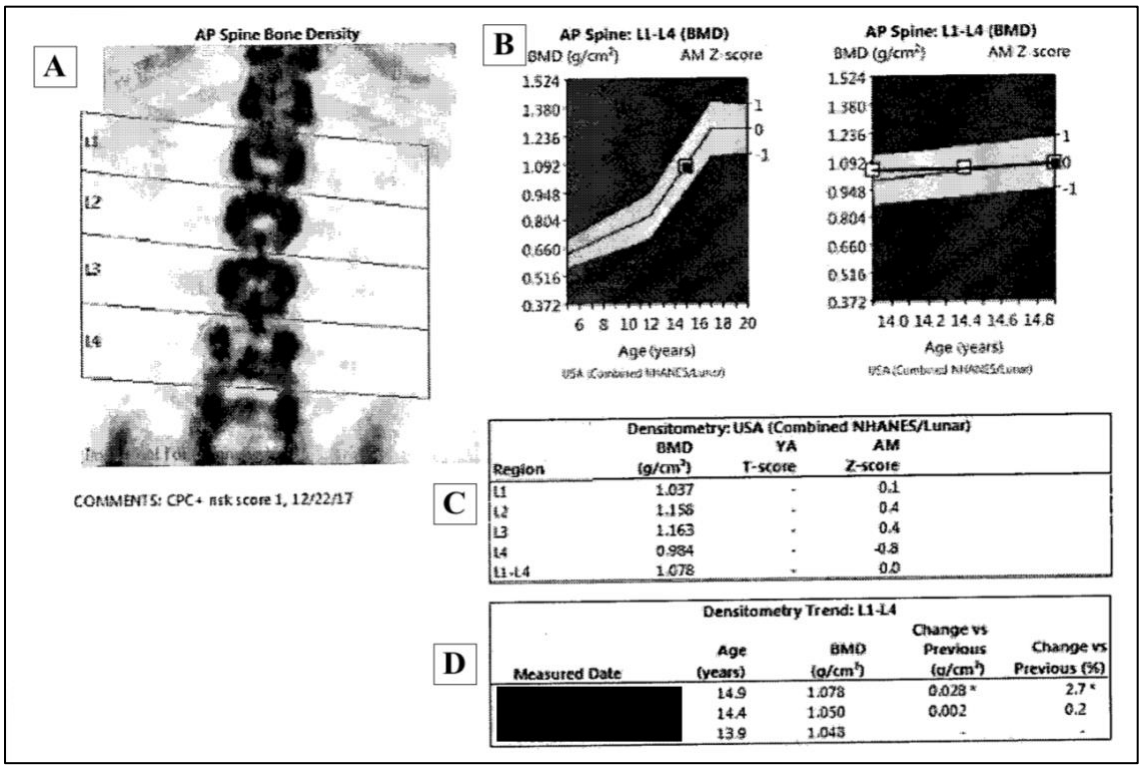


**Figure 8: DXA L1-L4 1 Year Scan Patient 3:** image obtained from EPIC Hyperspace (Epic Systems Corporation) close-up density scan of the L1-L4 vertebrae (a), BMD Z-score charts for patient 3's demographic (b), the BMD break down for each of the four vertebral BMD score that make up the composite L1-L4 score (c), and the progress made in BMD over a 12-month time period (d).

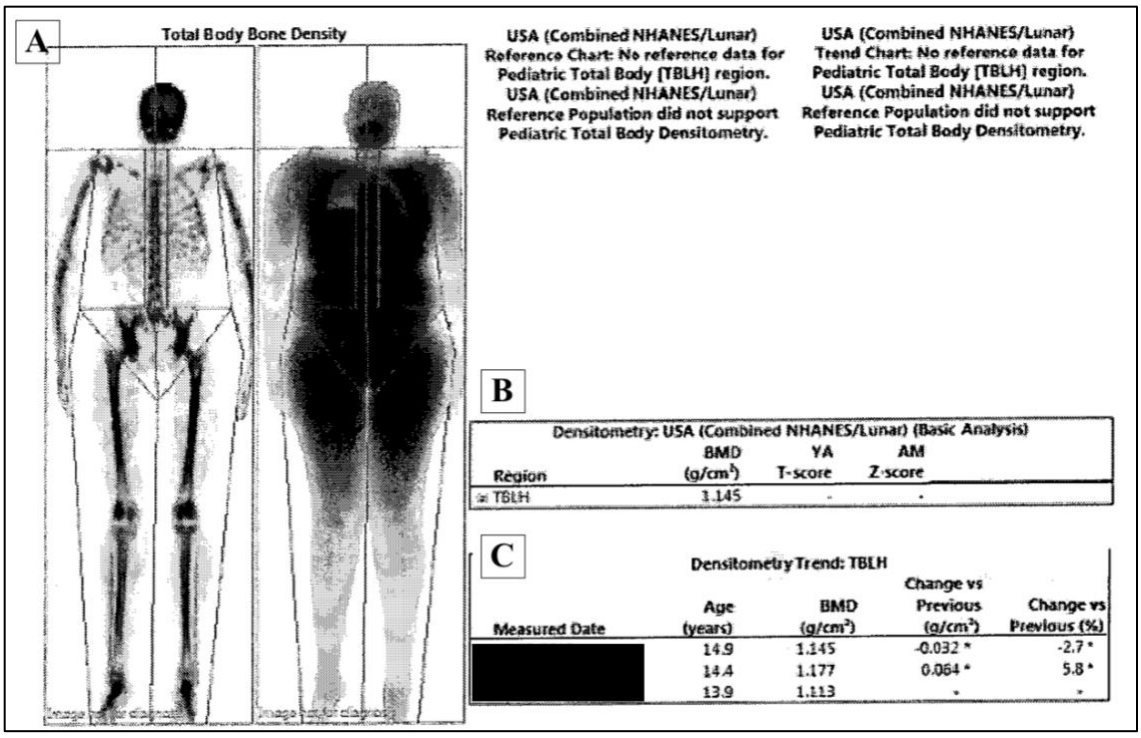


**Figure 9: TBLH DXA 1 Year Scan Patient 3:** image obtained from EPIC Hyperspace (Epic Systems Corporations) density scan of the total body less head (TBLH) region (a), the BMD break down for TBLH region (b), and the progress made in BMD over a 12-month time period (c). *Please note that patient 3 is Black or African American so TBLH BMD Z-score charts for this patient's demographic is not available.*

## IV. Discussion

Observations can be drawn from the nine patients who have completed the DXA study. While there are more males and females who have completed all three DXA scans, there is diversity amongst race and ethnicity (Table 3). As the remaining fourteen patients continue to participate in the study and complete all three DXA scans, increases in ethnic/racial diversity and gender diversity can be expected. Of the twenty-three patients who have either completed the DXA study or are currently active participants, the mean baseline vitamin D level of 18.52$\pm$8.61 with a range of 6.0-46.2; more specifically, the

mean baseline vitamin D level of the nine patients who have completed the study was 23.07±11.70. Both average vitamin D levels indicate that a majority of the patients are presenting with a vitamin D deficiency at the time of fracture. The data indicates that there may be a heightened association between fracture risk and vitamin D deficiency.

The completed DXA data indicates racial differences in BMC, BMD, and z-scores in the L/S and TBLH regions (Table 5a&5b). Typically, African-Americans tend to have the greatest aBMD, followed by Caucasian, Hispanic and Native Americans. Asian Americans, on average, have the lowest aBMD. Within the lumbar spine region, African American or Black participants present with higher BMC and BMD on average with a lower median z-score 0.2 (0-1.0) compared to 0.3 (-1.4-1.7) for the White or Hispanic participants. The results are mirrored with the TBLH region, since the BMC and BMD are higher for the African American or Black participants. The observation that the White or Hispanic demographic is presenting with poorer BMD and BMC outcomes at the baseline indicates evidence contrary to what is currently known about the relationship between skin pigmentation and vitamin D absorption. The data presented in Tables 5a & 5b illustrate how the African American and Black participants are presenting with higher baseline BMC, BMD, and z-scores in the regions studied.

The completed DXA data reports approximately 1 years' worth of growth, however the data are still preliminary, due to the small number of patients with three completed DXA scans. Even though the data are preliminary, there is a basic trend showing that patients are showing a higher than average increase in BMD and BMC

relative to their expected increase in BMC. As the study continues to collect patient data and increased the number of completed study participants, additional data analysis can be conducted to draw stronger conclusions.

Of the completed DXA data reports, four of the nine participants were consistently physically active for more than 10 hours per week; the remaining five participants did not consistently participate in physical activity every week, as time spent physically active ranged from 0-6 hours per week. Physical activity is known to play a key role in the accrual of bone mass during childhood and adolescence. Physical activity and exercise directly correlate with overall bone health as there is a positive relationship between weight-bearing exercise and bone mass where high-impact exercise increases bone density. Evidence presented from literature on this topic shows that there is a higher prevalence of physical inactivity seen in racial/ethnic minority groups with lower SES. Differences in physical activity are extremely influenced by SES and its role in individual, social, and environmental factors (Cerin and Leslie 2008).

Previous research has shown that the most prominent factor found to impact children and adolescent physical activity is area-level income. In a screening of patient's family income, about 50% of the study's patient population fell within the low-income range. This being said, areas with higher household income (and thereby higher SES) are known to have greater access to sports facilities and youth sports organizations (Cerin and Leslie 2008). Given that physical activity and exercise have a prominent role in healthy bone development during childhood and adolescence, access and participation in sports and exercise is necessary. Additionally, lower SES has been associated with

increased prevalence with overweight and obese children. Whether this likelihood is due to lack of access to fitness facilities or just the overall lack of physical activity in daily lifestyle has yet to be determined. Thus, when taking into account the role and importance of SES in children and teen participation in physical activity, future studies should also look at the impact of socioeconomic factors such as area-level income on physical activity as it can be an underlying factor influencing bone health.

Another lifestyle characteristic often influenced by SES that can impact the overall bone health of a child is nutrition and diet. In the data presented six of the nine patients to complete the DXA study were overweight or obese. Lower SES has been associated with increased pediatric obesity incidence; while it could be caused by a lack of physical activity, it may also be the result of a poor diet. Seven of the nine patients were considered a "healthy eater" despite the child's considerable consumption of soda and/or ice cream every day and low level of physical activity (Table 4). When looked at independently, diet/nutrition is a critical component in achieving and maintaining good bone health throughout childhood. Nutritional intake can, directly and indirectly, influence bone metabolism, bone structure, and modify bone turnover. However, when diet is not measured independently, socioeconomic factors can impact the ability to have and/or maintain a healthy diet. A child's access to health foods such as fresh fruits and vegetables help to determine if a child or adolescent is maintaining a "healthy diet." It is common for parents and children with lower SES to eat fast food products more often than higher SES families due to the low price of fast-food meals (Babar et al. 2010). Although these children are provided food, they may not be consuming enough nutritious

foods to assist their growing bones. Families with low SES tend to have poorer diets than high SES families because they cannot afford to buy fresh produce or food beneficial to one's diet. In order to provide children and their parents with necessary information to keep children healthy, recommendations to educate families on the importance of nutrition and a healthy diet for a growing child can be made.

The impact SES has on a child's diet does not only exist in the ability to afford nutritious food but also relates to parental education. Maternal education and household wealth have a significant impact on diet/nutrition in children. Literate mothers are more likely to influence the health of their children, specifically their diet (Babar et al. 2010). Poverty, parental education/occupation, access to food and healthcare services all play a part in childhood nutritional status, where their nutritional status influences the physical health of growing pediatric populations. Without proper nutritional intake, the ability for pediatric patient during childhood and adolescence have a greater chance of impairing bone mass accrual, heightening fracture risk throughout life.

In order to better identify and understand the factors that influence pediatric bone health, relationships between other components of lifestyle and SES should be accounted for. One could suggest that patients do not participate in youth sports or physical activity because their parents cannot afford it, or the child does not have access to a gym/recreational area. When collecting information on a patient to help improve bone health, it is not only important to collect their medical and family history, but also the family's average income, parent's education status, household situation, participation in physical activity, and even the child's daily diet.

When considering the number of participants who were withdrew or were lost to follow-up in the study, population and socioeconomic status are factors to highlight. Being that all participants in the DXA study are between the ages of 2 and 18 years old, parents and/or legal guardians are the primary decision-makers for the patients. Seeing as participants are asked but not required to participate in this study, the ability to recruit and retain participation often falls under the responsibility of the parent. According to the literature on pediatric participation in clinical studies, researchers found that nonparticipating parents has a higher SES, more social support, and were less motivated to contribute to medical research. Moreover, factors such as free gifts, were shown to positively influence patients with lower SES (Rothmier et al. 2003). As discussed earlier in this paper, SES is likely to have a large influence on pediatric bone health; not only does SES impact pediatric bone health, but also impacts participation in pediatric clinical studies such as the one being presented.

The limitations of the current study are a result of the small sample size of completed study participation, the loss to follow-up or withdraw of patient participation, and the inability to determine if patients are completely compliant throughout the duration of the study. As the study continues to collect patients and complete current participation, the study's sample size will increase; thus, correcting its current small sample size issue. Although patients are also reminded to comply at each visit and by mail, compliance cannot be completely contributed to the changes in the DXA scans. Additionally, as the research progresses this portion of the study might have more data to look at impact of obesity. However, in order to gain a better understanding of how SES

impacts pediatric bone health future studies should determine a scale to look at obesity, nutrition, physical activity, and family income. By adding social determinants of health to future research, physicians may find that there are many outside sources influencing the overall health of both children and adults.

## V. Conclusion

Patients presented in this thesis completed the study in its entirety by getting three DXA scans in addition to follow-up vitamin D blood draws. Instead of quantifying the risk of poor bone mineralization (a possible consequence of vitamin D deficiency at the time of fracture), the DXA scans provide the actual level of bone mineralization; providing visibility of consequences to both the physician and the patients. DXA scans provide a risk indicator of future fracture, refracture, and unsatisfactory healing. The supplementation and overall bone health data will be provided in the full version of the observational DXA study. The data presented indicate that vitamin D supplementation improves the bone mineralization in all patients following the protocol. While the data pool of completed DXA scans and supplementation is small, the compliance rate from all nine patients was 100%, a rate not presented in any of the literature previously mentioned in this study.

Present evidence from follow-up DXA and Vitamin D results after one year of supplementation support the Minkowitz Protocol. There is a positive trend between indicators of bone mineralization (BMD and BMC) and vitamin D. Furthermore, it would be wise for the AAP to not only show their support for the supplementation protocol, but

to recommend regular vitamin D monitoring in pediatric patients at risk for poor bone mineralization that could lead to a lifetime of fractures and increased osteoporosis risk.

An unaddressed observation inferred from this study was the patients' (in addition to the parents') desire to return back to sports and physical activity. Premature return to sports can impede proper healing of fractures and can often lead to subsequent fractures or refractures. The implication of this longing to return back to sports prematurely has not been explored. By including vitamin D levels in athletic annual physicals, physicians and coaches may be able to include vitamin D and bone strength in the conversation of sports injuries. Outcomes of reinforcing the importance of vitamin D in general pediatric health may include less impatience to prematurely return to sports and less disregard for the importance of vitamin D supplementation post fracture or even to prevent a fracture from initially occurring.

Future studies should address limitations from this analysis as well as other variables such as SES and other social determinants of health. Studies could incorporate broader racial, ethnic, and geographic diversity in the study sample. Upbringing, diet, and environment play significant roles in vitamin D deficiency risk, fracture risk, and the likelihood to comply with treatment regimens. These studies could also be duplicated in different age, income, education, household status, lifestyle, and neighborhood demographics, examining behavioral habits, and its preventive role of in fracture risk alongside vitamin D supplementation.

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