Don’t Break a Leg: The Effects of Vitamin D Intervention and Lactose Intolerance on the Bone Health of Pediatric Patients

A Thesis in Biology

by

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Abstract

This study is a longitudinal study that aims to determine whether providing supplemental vitamin D will increase total BMC and BMD in pediatric patients that have experienced a fracture, taking into account how lactose intolerance may impact pediatric bone health. Pediatric fracture patients that had low vitamin D serum levels were given vitamin D supplements to increase their serum levels. Patients got a DXA scan 12 weeks, 6 months, and 12 months post-fracture where lumbar spine (LS) and total body less head (TBLH) bone mineral content (BMC) and bone mineral density (BMD) were measured. Height-adjusted z-scores (HAZs) for the data at each time point were calculated. A total of 17 patients out of 55 enrolled completed all three scans. There was no significant difference in HAZs from the first scan to the third scan. The average HAZs at the LS had a decrease from the first to third scan, whereas TBLH measurements had an increase in average HAZs. Tanner stage was shown to have a large impact on the data with R^2 values above 0.25 for all measurements. No conclusions could be drawn about the impact of lactose intolerance on bone health due to a small sample size (n=2).

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Introduction

Bones are the basic component of the human skeleton and are important living tissues that are constantly changing throughout a person's lifespan. The health of bones is important because it can have large impacts on quality of life. In particular, osteoporosis is a common condition affecting millions of people annually where bones are weakened and break more easily. Vitamin D deficiency has a negative impact on bone health and is a widespread condition, yet there is no consensus as to the beneficial effects of vitamin D supplementation. This study focuses on the effectiveness of vitamin D supplementation in pediatric fracture patients, its effects on bone density, and the potential impacts of lactose intolerance on bone density. Potentially, this will also lead to reframing the recommendations for vitamin D supplementation and interventions in children in order to decrease rates of osteoporosis for these individuals later in life.

Understanding the process of how bones form can help determine how to best improve bone development and health. Bones first undergo the process modeling as they form. This involves changes in the bone that affect bone shape; later in life, remodeling becomes the major process of bones. Remodeling is the turnover of bone that does not have an effect on the shape of the bone (Pollock 2015). Since bones are an essential part of the human body, having healthy bones is crucial to having good health. Bone health is generally measured by the strength of bones and their resistance to fractures, and modeling and remodeling of bones determines their strength.

While bones are initially formed by the body during the modeling process, they are also constantly being remodeled with fluctuating build-up and break-down.

Remodeling is the main process after peak bone mass has been reached, which typically is achieved around puberty. Remodeling is central to damage repair and control of calcium and phosphorus levels in the body (Office of the Surgeon General 2004). Osteoclasts and osteoblasts are responsible for this process. Osteoclasts are cells that are formed from differentiated monocytes, and these cells decrease bone mass by activating resorption (Schwartz 2007). Resorption occurs when the osteoclast, assisted by transporter proteins, transports hydrogen ions to the bone to degrade the calcium complexes (Office of the Surgeon General 2004). This leads to the creation of a tunnel or depression in the bone (Schwartz 2007). Osteoblasts are responsible for the build-up of bone, and often follow the path of osteoclasts, forming fresh bone tissue in the newly open space (Schwartz 2007). Once the osteoblasts are laid down and become part of the bone matrix, they are referred to as osteocytes. These osteocytes form a network and help bones respond to injury, which is crucial to lifelong skeletal health (Office of the Surgeon General 2004). This study aims to contribute to skeletal health by using an intervention of vitamin D supplementation to make the network of bone stronger by increasing bone mineral density and therefore reducing the impacts of injury.

Osteoblastogenesis is stimulated by various transcription factors including Runx1, osterix, and MSx2, as well as the Wnt/B-Catenin pathway (Pollock 2015). Osteoblasts are partially responsible for osteoclastogenesis as osteoblasts express the gene RANKL which then triggers osteoclast differentiation and maturation (Pollock 2015). After menopause, women have increased expression of RANKL, which also increases inflammatory cytokines, leading to more osteoclast activity and higher levels of

osteoporosis in women than in men (Pollock 2015). The activity of these two cell types is largely responsible for the shaping of bone and determining its strength throughout a person's life.

In order to evaluate how different factors impact bone health, it is important to understand how bone strength is measured. Two important measurements to take into consideration when evaluating bone strength are bone mineral content (BMC) and bone mineral density (BMD). Bone mineral content is the total bone mass in the body (g), and bone mineral density is a calculation that considers the total bone mass in terms of the total area of the bones (g/cm^3) (Bachrach and Gordon 2016; Wasserman et al. 2017). As BMD is not a direct measurement, there is a higher chance of error in this value, though it is important as it factors in the size of bones. Therefore, while BMC and BMD are often closely correlated, they are not always correlated because a high BMC in someone with a large body and large bones may result in low BMD and vice versa. Lower BMC and BMD have been correlated with increased risk of fractures (Clark et al. 2006; Bachrach and Gordon 2016). These measures are most commonly obtained through the use of a dual energy x-ray absorptiometry (DXA) scan, which is used in our study in order to determine if the BMC and BMD are increasing over time with increased vitamin D.

DXA scans are the most widely accepted measure of bone health currently in use. They are a non-invasive measure akin to a regular x-ray that measures BMC and total bone area, with which the DXA machinery can calculate BMD (Wasserman et al. 2017). This scan emits much less radiation than an x-ray and it has no known associated health risks; there is ten times more radiation exposure on a transatlantic flight than in a DXA

scan, but experts recommend that DXA scans occur no more than once every 6-12 months (Bachrach and Gordon 2016; Wasserman et al. 2017). Precision errors that occur with DXA are less than 1%, and the entire scan takes less than 30 minutes to complete, so it is an accurate and quick diagnostic measure (Wasserman et al. 2017). The most common measurements taken with DXA, especially in children, include the total body less head (TBLH) and the lumbar spine (LS). TBLH is a useful measurement as the skull is a large part of the skeleton but is not impacted by environmental factors such as exercise, and skull fractures are not due to osteoporosis, so including it in a measure of BMD is not representative of overall bone health. To try and determine the change in bone density due to increasing vitamin D, our study includes the measurement of TBLH as the skull is not an area that would be affected by osteoporosis or by many interventions and therefore would not be relevant to the study.

One of the most important measures of bone health is an individual's peak bone mass (PBM), which is the maximum bone content an individual will achieve in their lifetime. Most of the PBM is accumulated during pre-puberty, with up to 50% of a person's total bone mass being obtained during childhood and adolescence (Vlachopoulos et al. 2015). In women, the majority of PBM is achieved around menarche, and for men it is achieved around the end of puberty; in both cases, it is established by the end of the second decade of life (Matkovic et al. 1994; Bonjour and Rizzoli 2001; Rizzoli et al. 2010; Zhu and Prince 2012). The highest rates of bone mass accrual occurs in females from ages 12-15, and in males from ages 14-16 (Vlachopoulos et al. 2015). After PBM is reached, there is very little subsequent increase, and both bone

density and bone mass decrease throughout the rest of the person's lifespan, as shown in Figure 1 (Matkovic et al. 1994; Bonjour and Rizzoli 2001). This is why intervention at the time when PBM is being established is the most effective to increase lifelong health.

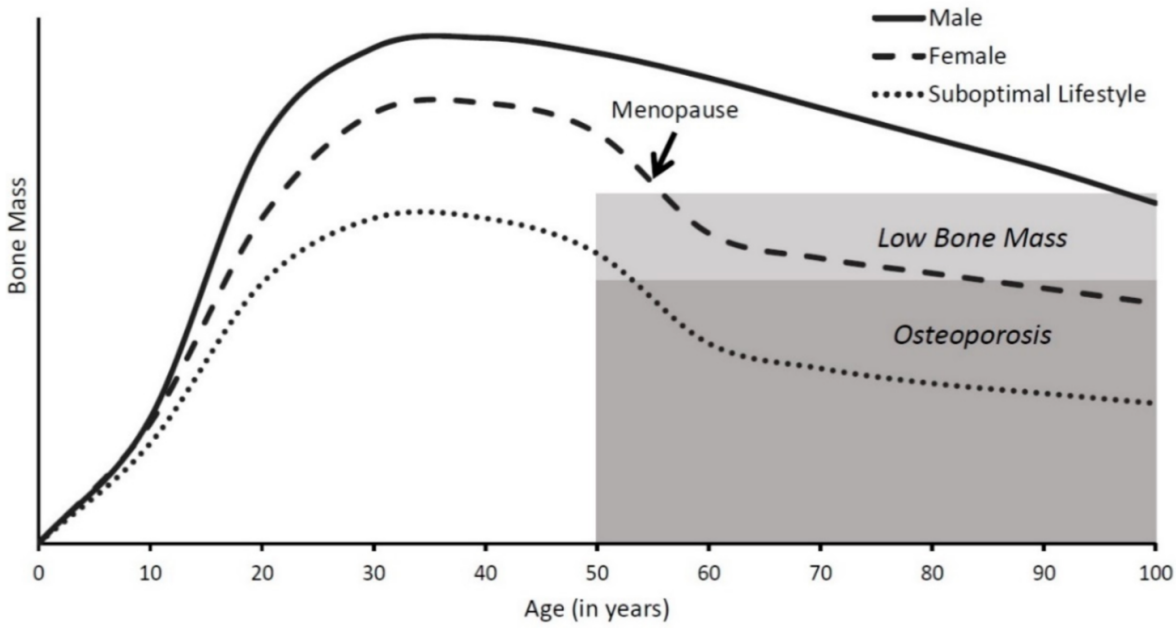

Figure 1. Bone mass over time. Figure from Hodges et al. 2019.

The period of pre-puberty is significant in establishing life-long bone health because it is the time period when PBM is established, and it has the highest amount of bone remodeling (Rizzoli et al. 2010). Children are an important population to examine as it is in childhood when interventions to increase bone strength may have the most impact, which is why our study is looking at pediatric populations. This is the time period where we may have the most effect on bone health later in life. Measuring the changes in bone in children has its challenges as children are still growing, and it may be difficult to

ascertain whether changes in BMC are due to normal growth or solely due to the benefits of any given intervention or activity.

People who have lower BMD and BMC (and therefore PBM) have higher risks of fracture, and for every standard deviation that an individual's PBM is lower than the mean, their risk of fracture doubles (Clark et al. 2006; Rizzoli et al. 2010). Therefore, taking steps to increase peak bone density during the critical pre-pubertal period may have the largest impact on life-long bone health including reducing the rates of osteoporosis in adults. While there is some controversy over whether or not PBM has a significant impact on bone health later in life, it is largely agreed that PBM is correlated to lifelong bone health. This research will assume that increasing peak bone density does increase lifelong bone health. However, even if this intervention has no long-term impact, it may still decrease the risk of fracture during the time the intervention is taking place.

Gafni and Baron argue that, in rabbits, decreasing early bone mass acquisition with treatment with glucocorticoid is not permanent. After the treatment was stopped, the bone mass went back up to normal (2006). They argue that any disruption to skeletal growth, whether positive or negative, will be corrected over time. This suggests that any change that was the result of an intervention may last for a few years, but not decades. However, many experts disagree and believe that improving PBM is vital to lifelong bone health. While the role of PBM may not be completely elucidated, our goal remains to increase PBM in order to decrease fractures and increase long-term bone health.

Another major factor that determines bone strength is the development of different diseases affecting bones; one of the most well-known being osteoporosis.

Osteoporosis is a disease that leads to weakened bones due to over reabsorption of bones from overactive osteoclasts. This leads to an overall loss in total bone mass which makes bones more prone to breaking. Osteoporosis is defined as an individual having BMD 2.5 standard deviations lower than the mean (Boyle et al. 2003; Rizzoli et al. 2010; Liberato et al. 2013). Osteoporosis is a large issue especially in developed countries and in older female populations; osteoporotic fractures were the number one cause of hospitalizations for women over 55 from 2000 to 2011, and it is estimated the costs of osteoporosis in US health care will reach 45 billion US dollars by 2020 (Pollock 2015; Singer et al. 2015). Approximately half of all women and one third of all men over the age of 50 will have an osteoporotic related fracture in their life (Pollock 2015). It should be noted here that a majority of the studies presented in this thesis will have more emphasis on female subjects as weak bones and osteoporosis are more common in females than males, leading to a disparity in the number of studies conducted involving women versus men.

The majority of osteoporotic bone fractures occur at the hip, humerus, wrist, and spine, so interventions that can increase density in these areas may be particularly important to reduce the number of fractures (Chen et al. 2014). Osteoporosis and weak bones are of growing concern as people live longer and develop more of these chronic diseases. A study conducted using osteoporosis measurements from the National Health and Nutrition Examination Survey estimated that by 2020 the rate of osteoporosis in adults over 50 would increase by 19% compared to 2010, and increase another 11% by 2030 (Wright et al. 2014). However, the rates of this disease can be potentially decreased

by increasing the peak bone mass (PBM) in adolescents. This study is focused on increasing the bone mass of pediatric patients in order to achieve this goal.

Bone health is not just important in older people but is also a prevalent issue at younger ages as well. Fractures are often seen as a childhood ailment, as kids are often the most active and participate in activities that are likely to cause fractures. Up to half of all children from ages 5 to 18 experience a fracture, and the rate of fractures has been increasing from past decades (Khosla et al. 2003; Rizzoli et al. 2010). When kids are growing the most, they are also the most likely to incur a fracture (Bonjour and Rizzoli 2001). In particular, children tend to fracture the forearm most often of any bone in the body (Lyons et al. 1999; Clark et al. 2006). However, if patients have recurring fractures, particularly from falling at standing height or less, it may be an indication of bone fragility, which is a lifelong issue (Bachrach and Gordon 2016). Bone mass and strength during childhood and adolescence may have a direct correlation with the risk of developing osteoporosis later in life because this is the period where bone mass is established. By increasing the bone health beginning in these pediatric populations, fracture risk could decrease and public health overall would improve.

Factors influencing bone health

There are many factors that influence bone health and have an impact on PBM. One factor that has a considerable impact on bone health is genetics, with 60-80% of bone health being determined by one's genes (Bonjour and Rizzoli 2001). Genetics impacts bone mineral density by changing both the initial amount of bone accrual during

childhood and puberty as well as determining the flux of bone remodeling later in life. While some portion of bone density is determined by height, there are genes independent of height that control bone density (Mitchell et al. 2016). The genes that control bone remodeling in adults also have been shown to have an impact on initial bone growth during childhood (Mitchell et al. 2016)

To determine the degree to which genetics actually control overall bone density, a study on fraternal and identical twins found that bone density was strongly correlated to heritability in every region of the body examined except for the distal forearm, showing that genetics do strongly influence skeletal strength, with ranges in heritability from 42-92% for different regions (Pocock et al. 1987). Another study conducted on twins found the heritability to be in a narrower range, between 60 and 80% (Seeman et al. 1986). As can be seen in Figure 1, though a majority of bone density is determined by genetics, the 20-40% determined by other factors can have significant impacts on whether or not osteoporosis is developed. Even with the high heritability of bone density, there is still a significant portion determined by environmental factors, including physical activity and diet. This is important as these factors are able to be controlled by the individual.

Physical activity is an important environmental factor that exerts many different forces on the bones including the force of muscle contraction and gravitational loading which work to stimulate the activity of osteocytes. This causes them to release molecular signals that increase the growth of osteoblasts, increasing the levels of bone formation above rates of resorption, which leads to increased bone mass and density (Weaver et al. 2016). Children that are active have been shown to have higher bone mineral density than

those that are not by up to 17% (Bailey et al. 1999). Weight-bearing exercise is found to be the most important in increase bone health, and though the best forms of exercise to help bone density have not yet been determined, high-impact sports have been shown to lead to the largest increases in BMC and BMD where sports like swimming and cycling actually resulted in lower BMC values (Vlachopoulos et al. 2015). In order to have a significant impact, the activity needs to increase the load borne by the bone by more than normal strain for the individual (Weaver et al. 2016). Low strain does not lead to further development of bones as it desensitizes the osteocytes that are essential in stimulating the process of bone formation (Weaver et al. 2016). During pre-puberty and early puberty, interventions involving physical activity are the most effective; the increase in total BMC in these stages ranges from 1-5.5%, but during puberty the benefits were only up to 2% increase in total BMC, once again showing the importance of the prepubertal period in establishing bone health (Vlachopoulos et al. 2015). The effects of these physical activity interventions, while lessened, were still seen up to three years later, showing that it is useful for increasing gains in BMC and BMD for long-term increases in bone strength (Vlachopoulos et al. 2015).

Increasing activity is a change that individuals can make in their lives in order to improve their bone health which would then decrease their risk of fracture in the future. However, increasing activity may also temporarily slightly increase risk of fractures because when people are more active there are more opportunities for fractures to occur. To address the risk of increased fractures with increased physical activity, one study had 121 kids participate in a physical activity intervention. The experimental group

demonstrated only a slightly higher risk of fracture than the control group in a ratio of 1.11:1 (Lofgren et al. 2012). This study also found that BMC was increased in these prepubertal and early pubertal kids over the four years of this intervention, with the most gain in BMC taking place at the lumbar spine with 7% increase in the girls and a 3.3% increase in the boys compared to their respective control groups (Lofgren et al. 2012). The study only found an increase in total body BMC in girls, but did not report a measurement for total body less head, which would have been useful because the cranium makes up a significant portion of the total BMC and it is not impacted by interventions of physical activity (Lofgren et al. 2012). The increased risk of fractures does not increase to a level that would outweigh the benefits of the exercise (2012).

Dietary nutrients can also have important impacts on bone health. Calcium supplementation is another well studied way to increase bone density and health, and calcium one of the most important nutrients to consider in the discussion of bone health as it plays a significant role in determining BMD. When there is not enough calcium supplied to the body, bones are broken down to increase the amount of calcium available in the bloodstream, which can weaken the bone over time (Surgeon General 2004). 99% of the calcium in the body is stored in bones (Zhu and Prince 2012; Vlachopoulos et al. 2015). Three different hormones regulate calcium: parathyroid hormone, calcitriol, which is a derivative of vitamin D, and calcitonin (Surgeon General 2004). Many thorough studies have concluded that increased calcium intake does have a positive effect on bone mass and density. An article by Rizzoli et al. 2010 demonstrates this as groups that were

eating calcium-rich food had significantly more bone mass compared to the control groups.

Even when not directly in the diet, but in the form of a supplement, calcium has been shown to increase gains in bone density and mass (Rizzoli et at. 2010). However, there have been some cardiac as well as kidney stone risks associated with supplementation, though these risks are not present when the calcium is consumed as part of the diet (Rizzoli et al. 2008). Calcium intake can account for up to 5% of PBM and total BMC variance in individuals (Vlachopoulos et al. 2015). Not only do these increased calcium intake interventions have immediate impacts on the bone density, the results last after the intervention has ceased with effects on PBM lasting up to 3 years afterwards (Rizzoli et al. 2010). The amount of calcium consumed in the diet can influence how much calcium is retained in the skeleton during growth, making it an important factor in one's PBM. People whose diets contain 750mg of calcium or less per day obtain up to 77g less of bone than people who have sufficient calcium in their diets (Zhu and Prince 2012). Calcium intake has also successfully been used as a predictor of future total body BMC (Zhu and Prince 2012). The recommended amount of calcium is 1000-1200 mg daily, a quantity necessary for good bone health and sufficient to decrease bone loss (Rizzoli et al. 2008). Increasing calcium intake has been shown to decrease the risk of fracture by up to 30% in some studies, as long as the level of compliance was 80% or higher (Zhu and Prince 2012).

Lactose intolerance

Lactose intolerance may be another health condition that has an impact on bone health due to its effect on calcium in the diet. To understand this impact, lactose intolerance needs to be examined. Lactose is a disaccharide that is generally only found in mammalian milk (Szilagya and Ishayek 2018). Lactose intolerance is the inability to break lactose into glucose and galactose due to the lack of the lactase phlorizin hydrolase (LPH or lactase) enzyme (Obermayer-Pietsch et al. 2004). More specifically, lactose maldigestion refers to the inability to digest lactose whereas lactose intolerance signifies the symptoms and condition that accompanies not being able to digest lactose. For the purposes of this paper, these terms are interchangeable. When the lactase enzyme is missing, the prokaryotes in the individual's microbiome are left to digest this sugar, which then produce metabolic byproducts that cause the symptoms of lactose maldigestion and intolerance (Szilagya and Ishayek 2018). The symptoms of lactose intolerance are most commonly gastro-intestinal discomfort after ingesting products containing high amounts of lactose (Szilagya and Ishayek 2018). There are four ways that lactose intolerance may occur: a genetic condition from birth where even as an infant an individual cannot digest lactose, premature infants that cannot digest lactose because they have not yet developed lactase, adult-onset hypolactasia, or an injury to or removal of the small intestine (Szilagya and Ishayek 2018). This paper will focus on adult-onset hypolactasia as the main form of lactose intolerance and examine the role it has in determining the BMC and BMD of pediatric patients with low vitamin D.

Lactose intolerance is an autosomal recessive condition due to a mutation in the *LCT* gene on chromosome 2q21 with this condition affecting up to 75% of all people (Obermayer-Pietsch et al. 2004; Szilagya and Ishayek 2018). The mutation from thymine to cytosine in the DNA sequence causes methylation of the DNA to occur. The methylated DNA has more limited transcription and translation and therefore the amount of lactase produced is reduced, so people with the cytosine mutation have lactose intolerance. If there is a thymine in that position, less methylation is present and transcription can still occur, so lactase is produced and an individual is lactose tolerant.

There are three main tests to measure lactose intolerance. One is the lactose breath hydrogen test, where following a dose of 20-50 mg of lactose, the breath is measured for hydrogen and methane content. If this content is higher than the standard, it shows that the person is unable to digest lactose. Testing the blood after ingesting lactose is another method of determining lactose intolerance. If the level of glucose in the blood does not rise significantly, then the lactose is not being broken down into glucose and galactose, signifying intolerance. The third method is genotyping to determine if the individual has the mutation in *LCT* that leads to lactose tolerance. People can present with three classic genotypes: CC - lactose intolerance, CT - potentially able to digest some lactose, or TT - lactose tolerant (Szilagya and Ishayek 2018). In cultures where dairy consumption is not common and most of the population is lactose intolerant, the onset of intolerance may occur as early as ages 2-5. In cultures where there is a more even mixture of lactose tolerant and intolerant people, onset of intolerance may occur as late as the teen years (Szilagya and Ishayek 2018).

The indigestion that accompanies lactose intolerance may have an impact on bone health as it decreases the intake of foods rich in calcium and vitamin D, which would also decrease bone density and increase fracture risk (Szilagya and Ishayek 2018; Hodges et al. 2019). A correlation has been shown between a country's rate of osteoporosis and the number of lactose digesters; as the rate of non-lactose digesters increases, so does the number of cases of osteoporosis (Szilagya and Ishayek 2018). This is exemplified by the UK and Japan. In the UK it is estimated that 8% of the population is lactose intolerant, and the rate of osteoporosis in females is 27% while 73% of Japan's population is lactose intolerant, and 38% of females have osteoporosis. In general, as the rates of lactose intolerance in a country increase, so does the rate of osteoporosis as demonstrated in Figure 2 on page 16. Another study found that there was an association between lactose intolerance and lower BMC in the spine even when the subjects had similar calcium intakes (Lee et al. 2018). This evidence points to a unique factor with lactose intolerance that has a negative impact on bone health besides avoiding dairy. However, not consuming dairy has been correlated to poorer bone health, and avoidance of milk has been shown to increase a person's fracture risk by 1.7 times compared to a person who consumes milk (Zhu and Prince 2012). These statistics may be due to the fact that the presence of lactose helps increase calcium absorption; when lactose was present in people naturally producing the lactase enzyme, the absorption of calcium rose by 14%, but in lactose intolerant people, lactose decreased the absorption of calcium by 5% (Zhu and Prince 2012). Thus, not only are lactose intolerant people consuming less calcium, but the calcium they do consume from dairy is not processed by the body as well as in people

who can digest lactose, which could be the reason for decreased BMC between lactose tolerant and intolerant individuals with the same ingestion of calcium. However, another study found that the presence of lactose does not influence calcium absorption (Hodges et al. 2019). The role of lactose in calcium absorption is unclear, and so the role of lactose intolerance also needs further investigation. This study is looking into lactose intolerance as another variable in determining bone health because of this potential interaction between calcium intake and lactose tolerance.

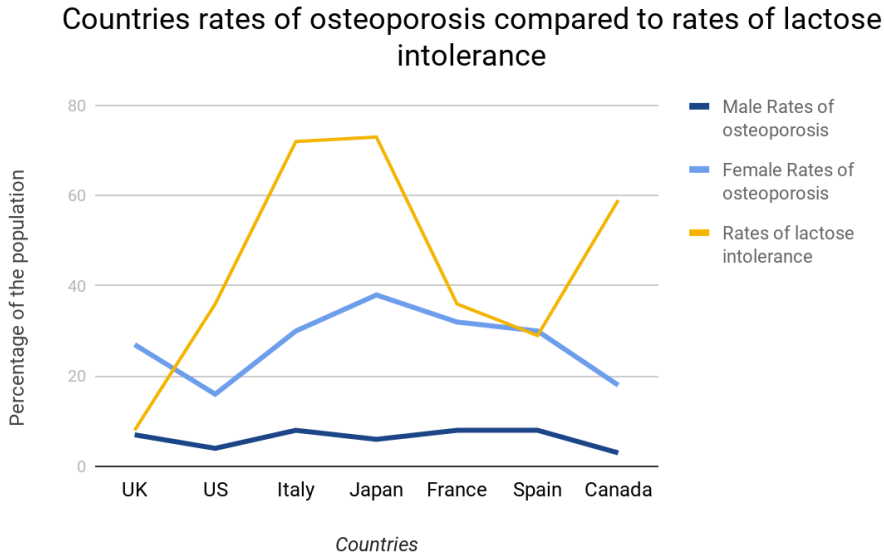

Figure 2. Countries rates of osteoporosis and prevalence of lactose intolerance based on data reported in Storhaug et al. 2017

Lactose intolerance may also affect the levels of vitamin D in the body. A study that used genotyping to determine lactose intolerance found that people who had the CC intolerant genotype had significantly lower levels of vitamin D than both the CT genotype and the TT genotype individuals (Alharbi and El-Sohemy 2017). Vitamin D

deficiency in this study was measured as less than 30 ng/mL, and in CT genotypes the risk of deficiency was increased by 50% over the TT genotype. Additionally, individuals with the CC genotype were 100% more likely to have vitamin D deficiency than TT individuals (Alharbi and El-Sohemy 2017). However, a separate study found that there were slightly higher levels of vitamin D in lactose intolerant individuals. People with the CC genotype were found to have a vitamin D increase of nearly 8 ng/mL above the TT genotype (Obermayer-Pietsch et al. 2004)

There are also various ways to self-treat the symptoms of lactose intolerance and still consume dairy. One way to do this is to ingest a dose of the lactase enzyme when consuming dairy products (Szilagya and Ishayek 2018). Taking this enzyme generally does not cause any side effects and the enzyme is widely available in most pharmacies in a variety of brands. To avoid lactose, some people also switch to non-dairy substitutes such as almond or soy for traditional animal dairy products, or purchase animal-product based dairy products that have had the lactose removed (Szilagya and Ishayek 2018). People who continue to consume dairy products may still ingest the same amount of calcium as people eating the correlating products, but these people may still have lower BMC. The role of lactose intolerance in bone health is still uncertain, and more research needs to be done to elucidate this. For the purposes of this study, it is hypothesized that lactose intolerance may increase the risk of having low levels of vitamin D as well as decreasing the BMC and BMD of subjects that are lactose intolerant.

Vitamin D

Vitamin D also plays an important role in supporting bone density by controlling levels of parathyroid hormone (PTH) in the body and increasing the absorption of calcium (Rizzoli et al. 2008; Rizzoli et al. 2010). Vitamin D inhibits the synthesis of PTH, so low levels of vitamin D reduce the inhibition of PTH synthesis, leading to increased levels of PTH. PTH decreases the activity of osteoblasts and increases the activity of osteoclasts, leading to a decrease in bone formation (Pollock 2015). Therefore, vitamin D is needed to inhibit PTH to continue bone development. Additionally, vitamin D is necessary because it increases the ability of the intestines to absorb calcium and phosphorus and increases the levels of proteins that result in osteoblast formation (Rizzoli et al. 2010). Most of the vitamin D in our bodies is made from a reaction with sunlight, but 5% of our total vitamin D is from dietary intake (Vlachopoulos et al. 2015).

In the body after UVB from sunlight is absorbed, vitamin D is made in the skin in the form of vitamin D^2 or D^3. This is converted to 25-hydroxyvitamin D (25(OH)D) in the liver or 1,25-dihydroxyvitamin D in the kidney, also known as calcitriol. This process is shown in Figure 3 (Rizzoli et al. 2010; Vlachopoulos et al. 2015; Farrar et al. 2016). There is no agreement over how much time is needed in the sun to obtain the necessary amount of vitamin D. This is because there are many factors involved in regulating the amount of vitamin D a person is able to make from sunlight including age, skin tone, latitude, time of year, and amount of skin exposed (Holick 2002). Estimates of the necessary time in the sun range from having one's extremities exposed for 15 minutes a

day to being in the sun for 25% of the amount of time it would take to get a sunburn (Holick 2002; Holick 2008).

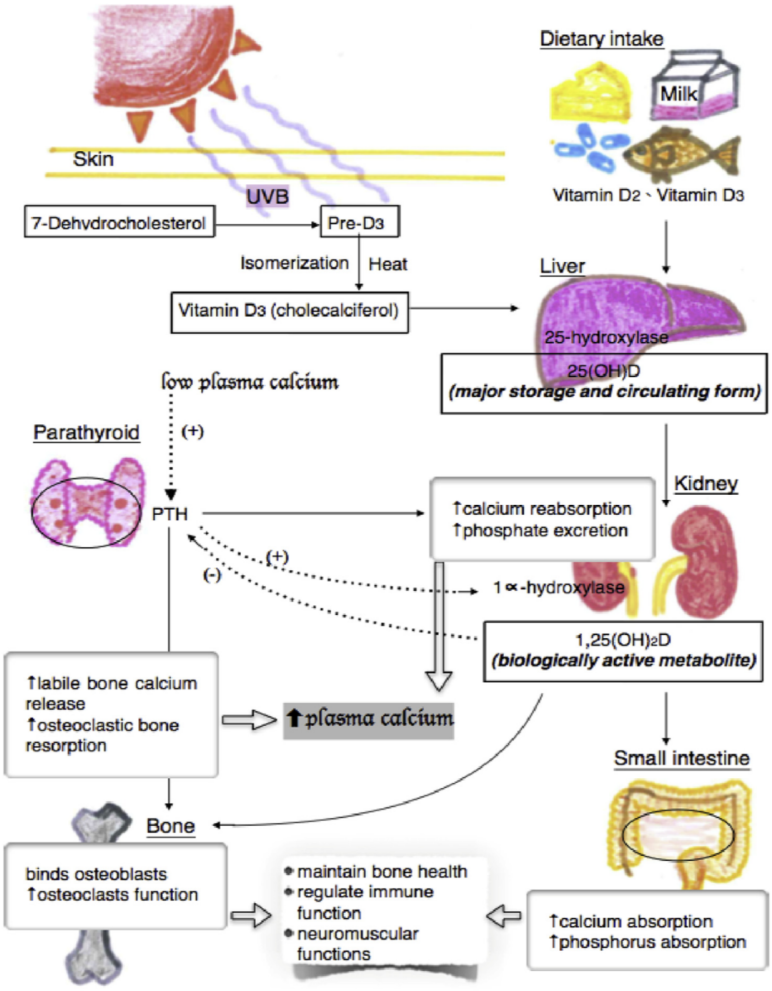

Figure 3. The absorption of processes of vitamin D in the body. Figure from Chen et al. 2014

To determine levels of vitamin D present in the body, serum 25(OH)D is measured. This molecule has a half-life of 30 days compared to the active metabolite $1,25(OH)D_3$ which has a half-life of up to 6 hours (Rizzoli et al. 2010). When levels of 25(OH)D in the body are low, it was shown that a protein responsible for bone turnover,

CTX, was present at higher levels, showing again that vitamin D is necessary to reduce bone loss (Lehtonen et al. 2002).

Lack of vitamin D can cause rickets and osteomalacia, whereas large amounts of vitamin D can also cause problems with bone strength as it may increase the rate of bone resorption, causing bones to be porous (Schwartz 2007). It has generally been agreed upon that 20 ng/mL of 25(OH)D is needed to suppress PTH enough to promote bone growth. At levels higher than 20 ng/mL, there was no further impact on PTH (Rizzoli et al. 2008). The US Institute of Medicine has defined vitamin D deficiency as less than 12 ng/mL, insufficiency as less than 20 ng/mL, and sufficiency as 20 ng/mL or more (Pettifor et al. 2017). However, other studies have determined that the optimal range for good bone health is between 36-48 ng/mL, and the Endocrine Society in 2011 stated that 40-60 ng/mL is the optimal range of vitamin D serum levels for bone health (Bischoff-Ferrari 2008; Holick et al. 2011). Vitamin D is also important in the discussion of osteoporosis because as a person ages, their skin synthesizes less vitamin D, and people spend less time outside which further decreases their level of vitamin D and can increase fracture risk (Rizzoli et al. 2008).

There are many factors affecting the level of vitamin D in the body. As stated previously, most of the vitamin D in the body comes from exposure to sunlight, which means that vitamin D deficiency is more widespread in the winter as people are outside less and the UV rays from the sun are not as strong. People who always wear sunscreen are also more likely to have a vitamin D deficiency even when they spend time outside because sunscreen blocks UVB rays which are needed to make vitamin D in the skin

(Holick 2008). One study done on adolescents in the UK found that 28% of the sample had vitamin D deficiency or insufficiency defined by less than 20 ng/mL in one season, and 78% had deficiency or insufficiency in at least one season (Farrar et al. 2016). It was also determined that going on vacation was a factor that affected the amount of serum 25(OH)D a person had, with up to 17% of the variance in peak levels being due to vacation (Farrar et al. 2016). This is also shown in the trends in vitamin D levels, as the peak of serum 25(OH)D levels occurs in October and the trough is in April (Ning et al. 2015). However, up to 5% comes from the diet. Foods naturally rich in vitamin D include salmon, cod liver oil, and shiitake mushrooms, but there are also various foods that have been fortified with vitamin D such as milk and orange juice (Chen et al. 2014).

The prevalence of vitamin D deficiency is a common and widespread condition. In a study of vitamin D deficiency in China, of the 5531 subjects that participated, 96.1% had some type of vitamin D insufficiency as defined by less than 30 ng/mL (Ning et al. 2015). Another study found that the subjects that had vitamin D insufficiency or deficiency (less than 20 ng/mL) had a significantly lower BMD in the femoral neck (Farrar et al. 2016). In a survey of vitamin D deficiency in the US, 70% of the pediatric population was found to be either deficient or insufficient (less than 30 ng/mL) (Melamed and Kumar 2010). In Canada, a survey of mothers and infants in Manitoba found that 75% of mothers included in this study had levels of vitamin D below normal, as did 43% of children (Lebrun et al. 1993). Additionally, people of color are more at risk for developing vitamin D deficiency because the melanin in their skin acts similarly to sunscreen and blocks the UVB rays that are necessary for making vitamin D (Holick

2008; Melamed and Kumar 2010). Vitamin D deficiency is a widespread issue in public health that needs to be addressed.

Vitamin D deficiency is important to consider because it can lead to rickets. Rickets can be caused not only by genetics, but also by vitamin D deficiency, which will be the focus here (Itoh et al.2017; Pettifor et al. 2018). Rickets occurs most commonly in children from ages 1-3 years old, and while rates of rickets largely decreased after more foods were fortified with vitamin D, it is currently on the rise again in many places including Japan, the United States, and the United Kingdom (Pettifor et al. 2017; Itoh et al. 2017). The symptoms of rickets include a delay in motor milestones in young children, hypotonia, and deformities in the bones resulting in a bowing shape (Pettifor et al. 2017). This can typically be corrected by giving the child vitamin D, and both D_2 and D_3 forms have been shown to be effective. Depending on the severity of the case, abnormalities can be corrected within a few weeks to a few months (Pettifor et al. 2017). A variety of doses are given based on the severity and the regularity of the treatment. The regularity and dose are determined by how likely patients are to comply - patients who are less likely to take their vitamins daily are given larger doses less frequently (Pettifor et al. 2017). Decreasing the prevalence of vitamin D deficiency will increase bone health overall, which is why this study aims to correct vitamin D deficiency to increase bone density.

There is very little risk associated with vitamin D supplementation. Vitamin D excess can occur when serum levels are higher than 100 ng/mL but this requires extremely high doses of vitamin D over a prolonged period of time (Minkowitz et al.

2018). Levels over 100 ng/mL are defined as vitamin D toxicity, with symptoms including vomiting, confusion, and abdominal pain (Minkowitz et al. 2018; Macinowska-Suchowierska et al. 2018). However, vitamin D toxicity is a very rare condition and not a major consideration in supplementation interventions.

While vitamin D does impact the process of building bones, there is conflicting evidence on whether or not supplementation of vitamin D may be beneficial to bone health. Low vitamin D levels resulting in vitamin D insufficiency or deficiency have been positively correlated with forearm fracture risk in African American children (Ryan et al. 2010). However, this study had a very small sample size of 17 participants and showed only an association, as it was not a clinical trial. This study implies that insufficient vitamin D is a factor in decreased bone density. Another study completed in Australia showed that of pediatric patients with fractures, 52% were vitamin D deficient as defined by the study with less than 11.6 ng/mL of 25(OH)D serum (Kwon et al. 2016). The large number of fracture patients with vitamin D deficiency lends credence to the supposition that vitamin D is a necessary component for good bone health. However, a study conducted in California found that there was no correlation between serum 25(OH)D and BMC or BMD (Kremer et al. 2009). Even with the participants living in a sunny place, 59% of subjects were vitamin D insufficient with a serum level of less than 12 ng/mL (Kremer et al. 2009).

Randomized control studies have also shown mixed results, with many finding little to no impact of vitamin D on BMC or BMD. One randomized control trial found that a lower dose of vitamin D at 35 ug/week lead to more increases in BMD than a

higher dose of 350 ug/week, though both experimental groups had a BMD that was higher than that of the control (Al-Shaar et al. 2013). However, this was a post-hoc analysis, and the age range of the population was variable, so this is not the best study to analyze the effects of vitamin D on bone density specifically in pre-pubertal adolescents. Another study, conducted on Lebanese adolescents given either a placebo, 200 IU/d (international units per day) of vitamin D, or 2000 IU/d, did not find significant changes in overall BMC or BMD. It did find slight insignificant changes in both the experimental groups compared to the control group in premenarchal girls; boys and postmenarchal girls showed no difference (El-Hajj Fuleihan et al. 2006). There were significant changes in the serum level of the high-dose group compared to the placebo as well as some significant increases in total hip BMC (El-Hajj Fuleihan et al. 2006). As predicted by the investigators, the largest impact was seen in the girls with the lowest baseline vitamin D serum level that were given the high-dose treatment. The lack of change in BMD and BMC could be due to the fact that the dose of vitamin D was given once a week rather than daily, though the serum vitamin D levels of the high dose group did increase (El-Hajj Fuleihan et al. 2006). Cheng and colleagues did not find any differences in the BMC or BMD of girls based on their levels of vitamin D via DXA, though some differences were detected via pQCT, which is another method of determining the density of bones (2003). Another study conducted in postmenarchal girls did not find any significant difference in those that received vitamin D supplementation compared to those who did not, but their method of supplementation was 150,000 IU every three months, which was likely less effective than smaller, daily doses as the half-life of vitamin D in the body is

rather short with a maximum of five weeks (Rizzoli et al. 2010; Ward et al. 2010). Additionally, their population was postmenarchal girls, when it has been previously established that the most effective time for interventions to have an effect on bone health is in the prepubertal period. Lehtonen and colleagues also did not find a correlation between dietary intake of vitamin D and bone health; however, when looking at the serum level of 25(OH)D in the blood, the girls with high 25(OH)D as defined by the study had 27% higher BMD in the lumbar spine than girls with low 25(OH)D (2002). This difference was only found in the girls in the study who were more progressed in their Tanner stages, and girls at stage I or II did not have any difference in the BMD of the lumbar spine (Lehtonen et al. 2002). The Tanner scale is a 5-point scale to determine the stage of puberty a person is in by looking at breast development in girls, genital development in boys, and pubic hair development in both as shown in Figures 4 and 5 on pages 36 and 37. The scale classifies the person as Tanner stage I, II, III, IV, or V with I being the least developed and V being fully developed. As with other interventions, it was found that the last premenarchal years are the most important for levels of vitamin D to have an impact on the overall BMD of a person (Lehtonen et al. 2002). However, another study looking at the effects of vitamin D with calcium found that the baseline levels of vitamin D and calcium were not predictors of fracture risk in menopausal women, and only the intake of these nutrients was a factor (Jackson et al. 2006).

There are some studies that have found a positive impact of vitamin D on bone health and density. In postmenopausal women with normal BMD, the supplementation of 1000 IU of vitamin D did increase the serum level of study participants by 12.5 ng/mL

whereas the serum 25(OH)D levels of subjects receiving a placebo dropped by 2.9 ng/mL (Nahas-Neto et al. 2018). This study also found that the levels of vitamin D corresponded to the levels of PTH, with the supplemental group's level of PTH decreasing by 12 pg/mL, and in the placebo group the level of PTH increased by 5 pg/mL (Nahas-Neto et al. 2018). This shows that supplementation does increase the level of serum 25(OH)D, the widely accepted biomarker. This study does not comment on the changes in BMD as associated with vitamin D; however, the lower levels of PTH in the experimental subjects allows for the hypothesis that those women would also have less bone loss than their control counterparts that had elevated levels of PTH in comparison. A meta-analysis looking at six randomized-control double-blind placebo trials involving vitamin D and bone density concluded that there were no significant effects of vitamin D across the studies, though there was a small increase in BMD in the lumbar spine as the only region of the body where there was marginal impact (Wizenberg et al. 2011). A study examining adolescent Finnish girls found that with vitamin D supplementation of 5 ug daily, the BMC of the femur increased by 14.3% over the placebo group, and the group receiving 10 ug had an increase of 17.2% over the placebo group after 1 year of intervention (Viljakainen et al. 2006). This study adjusted for growth by taking into account the total bone area, weight, and Tanner stage of the subjects in the ANCOVA analysis that was performed (Viljakainen et al. 2006). They also found that there was significantly higher BMC in the lumbar spine of girls whose compliance was over 80%, with the group taking 10 ug having BMC 12.5% higher than the placebo (Viljakainen et al. 2006).

A few studies have examined the interactions between vitamin D and calcium. One study found that with calcium alone, the risk of fracture decreased by 10%, but providing calcium and vitamin D in conjunction lowered the risk of fracture another 3% for a total of 13% (Zhu and Prince 2012). Other studies have also found that providing calcium and vitamin D together is more successful than either alone. When giving the two supplements in conjunction, hip fractures were reduced by 25% more than when vitamin D was given alone (Rizzoli et al. 2008). In another study looking at the effects of combined calcium and vitamin D, postmenopausal women taking 1000 mg of calcium and 400 IU of vitamin D daily were found to have a 12% lower risk of hip fracture than the women taking a placebo (Jackson et al. 2006). In terms of compliance, participants that followed the protocol 80% of the time or more had a higher reduction in hip fractures, 29% less, than the control group (Jackson et al. 2016).

Vitamin D is a very important nutrient in the body that is needed to strengthen bones. Vitamin D deficiency is a very common problem that affects a large percentage of the population and can lead to weaker bones. There are mixed results concerning whether or not vitamin D has significant positive effects on bone density over a long period of time. Some results show no increase at all in bone density with supplementation compared to others that show a 17% increase in bone density or up to 13% decrease in fractures.

The study we have conducted aims to specifically determine whether providing supplemental vitamin D and calcium will increase the speed of healing in pediatric patients that have experienced a fracture as well as increase total BMC and BMD. This

study also hopes to contribute to a body of knowledge on pediatric bone health, which is currently lacking as most studies focus on bone health in older populations. Children and adolescents are examined in this study as discussed earlier because this is the life-period in which the most bone accrual occurs, and so should be the focus for long-term reductions in fracture risk and related bone diseases. The goal of this thesis is to determine if supplementation with vitamin D is a sufficient intervention to increase the PBM of children and adolescents. This may also provide guidelines on the use of vitamin D supplementation in assisting the healing of fractures.

This thesis will also examine how lactose intolerance may have an impact on bone mass and therefore fracture risk. Lactose intolerance is a very common condition, and while it is well known, its effects on bone density and various factors impacting bone health are less clear. The hypotheses of this thesis are that increasing vitamin D to optimal levels for bone health will significantly increase BMC and BMD in pediatric patients after experiencing a fracture and that lactose intolerance may decrease an individual's level BMC and BMD, so increasing their vitamin D will lead to the greatest increases in BMC and BMD.

This study was conducted by studying a sample of pediatric patients that experienced a recent fracture and had a low vitamin D level. Patients were asked to take vitamin D to boost the levels in their blood and aid with the healing of their fracture as well as to undergo a DXA scan at 12 weeks, 6 months, and 12 months post fracture so that we could determine if the increase in their serum vitamin D levels had an impact on their overall bone density. With these data, the participants were asked if they had a milk

allergy or regularly consumed dairy which was then used to examine the impacts that lactose intolerance had on bone density.

Methods

This study is a longitudinal intervention that was conducted by Barbara Minkowitz M.D. and Jennifer Ristic P.A. at the Atlantic Health System's Department of Sports Medicine and Pediatric Orthopedics in Morristown, NJ. The study has run for approximately 3 years. Each patient participated for one year with participation involving DXA scans, checking of 25(OH)D levels, hand grip tests, and surveys. This study was submitted and obtained approval from Atlantic Health Systems Institutional Review Board.

Patient Population

600 fracture patients were screened with the goal of enrolling 250 patients aged 5-18 from patients presenting at the Children's Orthopedic and Sports Medicine Group as part of the Atlantic Health System. It is necessary to acquire a sufficient sample size so that reliable conclusions may be drawn. Patients presenting with a fracture were asked if they would like to enroll in this study if they had a vitamin D level below 20 ng/mL, and they must have been able to get a DXA scan in the first 12 weeks after the fracture occurred. Patients that have any of the following conditions were excluded: amyloidosis, ankylosing spondylitis, collagen vascular diseases, congenital porphyria, epidermolysis bullosa, prior gastrectomy, 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. These conditions all affect the bone formation of patients, so the exclusion is to limit these variables in the analysis of results. Only patients that have signed the assent and guardians that have signed the consent were enrolled, and patients were informed that they will receive a stipend totaling $125 for participating in the study, with $25 given as a gift card after the first DXA, and $50 gift cards given at the second and third DXA scans in order to increase patient compliance.

Standard Procedure

After being told about the study, patients and their guardians were counseled about the correct amount of vitamin D supplementation for their size and the extent of their deficiency as outlined by Dr. Minkowitz and shown in Figure 4. The protocol was developed by Dr. Minkowitz to increase patient vitamin D serum levels to between 40 and 60 ug/mL. Each patient was followed in a year-long longitudinal study with two follow-ups after the initial visit and DXA scan. The initial DXA scan was taken within 12 weeks of the fracture, followed by 2 more scans and vitamin D levels taken at 6 and 12 months. The 6-month follow up DXA was scheduled within 6 months of the first scan, and at that time another serum sample for vitamin D level was taken. The 12-month scan was taken 12 months after the fracture with another serum sample for vitamin D level

taken at that time. The study aimed to measure the increase in bone density and mass over time following supplementation with vitamin D to bring vitamin D to optimal levels for bone health. At the time of the DXA scans, patients were also asked to fill out a survey regarding their compliance in taking their vitamin D and what other vitamins they are currently taking as well as taking a brief hand-grip strength test. The hand-grip strength test measures the force with which the apparatus is grabbed in order to assess muscle strength. This test measured if increasing vitamin D and bone density also has an effect on strength and muscles. After the first scan is completed, participants received a $25 dollar gift card, with a $50 gift card following the second and third scans to encourage follow-up. At the present time, patients are not being enrolled in this study.

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Children's Orthopaedic and Sports Medicine Center
Bone Health Sheet

Vitamin D and Calcium are daily supplements needed by everyone, everyday

Barbara Minkowitz, MD
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261 James St. , Suite 3C, Morristown, NJ
333 Mt. Hope Ave., Rockaway, NJ

Guidelines for daily Vitamin D needed to obtain serum levels 40-60ng/ml for children without liver or kidney dysfunction. (must have baseline Vitamin D25 level)

0-1 year: 400 units Vitamin D
1-8 years: 600-1000 units Vitamin D
9-13 years: 1000-1500-2000 units Vitamin D
13-18 years: 1500-2000 units Vitamin D
Adult: 2000 units Vitamin D

Up to 50 lbs: 500-1000 units Vitamin D
50-90 lbs: 1500 units Vitamin D
90+ lbs: 2000 units Vitamin D

- Vitamin D must be taken with calcium; 1000 mg daily except 1-4 years old then, 700mg daily
- Children are encouraged to take a MVTs with their supplement. This can be counted in as part of the total VitD given. There are anywhere from 200 to 800 units of Vitamin D in children's MVTs. Parents are asked to read the label several times to figure out how much they are giving their child and pay close attention to serving size (daily dose can be 2-3 tablets and child may be getting only one).

No Fracture present:

Supplementation guidelines based on serum VitD level used when fractures are not present. Labs must be repeated 3-6 months after supplementation 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 500 units VitD + calcium
20-30 ng/ml: considered VitD insufficient	Add 1000 units VitD + calcium
12-20 ng/ml: considered VitD deficient	Add 2000 units VitD + calcium
<12 ng/ml: considered VitD deficient	Supplement with 4000-7000 units VitD + calcium for 2 months then repeat labs and decrease VitD if value in range, or send to endocrinologist

Fracture present:

With a fracture present, higher initial supplementation can be used, dictated by the 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 units VitD + calcium
20-30 ng/ml: insufficient for healing	Add 4000 units VitD + calcium
12-20 ng/ml: insufficient for healing	Add 4000 units VitD + calcium
<12 ng/ml: deficient	Add 7000 units VitD + calcium for 2 months then repeat labs and decrease VitD if in range, or send to endocrinologist. Equivalent to 50,000 units/week for 8 wks

Atlantic Health System
Trusted Network of Caring™

Figure 4. Minkowitz vitamin D protocol (Minkowitz 2018).

Consent and Assent

After being asked if they would like to participate in the survey, legal guardians and patients were told about the study and given a document outlining the study in child-friendly language as well as describing what their consent would entail. Researchers did not use language or body language to coerce enrollment and participation in this study. Guardians and patients were given sufficient time to ask questions both at the intake as well as any time throughout the study. They were informed that they are free to leave this study and stop participating at any time with no consequences. Assent of the participant and consent of the guardian was documented by the research associate.

DXA questionnaire

When patients agreed to participate in the study, they were given an initial survey to complete which document patient demographics, previous fractures, regular activities, family history of bone disease, diet, and current supplementation. This provided information on the patients habits which can then inform the interpretation of the DXA data that was obtained.

DXA Scans

The DXA scans were conducted at 111 Madison Avenue, Morristown, NJ on a Lunar DXA machine. DXA scans measure the bone density of the patient at various sites. This study focused on total body less head (TBLH) and lumbar spine (LS) bone mineral content (BMC) and bone mineral density (BMD) as the measurements obtained from the DXA scans. The entire DXA scan is completed in less than 10 minutes. No cast or covering was in place at the time of the scan. The first DXA must occur within 12 weeks of the injury, then 6 months after the injury, and again 12 months after the injury.

Tanner Scale

Pubertal status is an important factor in bone health, and Tanner stages are the accepted method of determining pubertal stage (Viljakainen et al. 2006). At the time of the DXA scan, technicians provided a short survey that asked patients or their guardians to self-identify their Tanner stage based on their body's development, and this form is shown in Figures 5 and 6. Patients were given the form that corresponds to their sex. Girls identified their stage of breast development, boys their stage of genital development, and both identified their pubic hair development to classify the patient as Tanner stage I, II, III, IV, or V. As pubertal status affects the development of the bones, Tanner stage is important data to look at in order to understand where the patients are in their bone development.

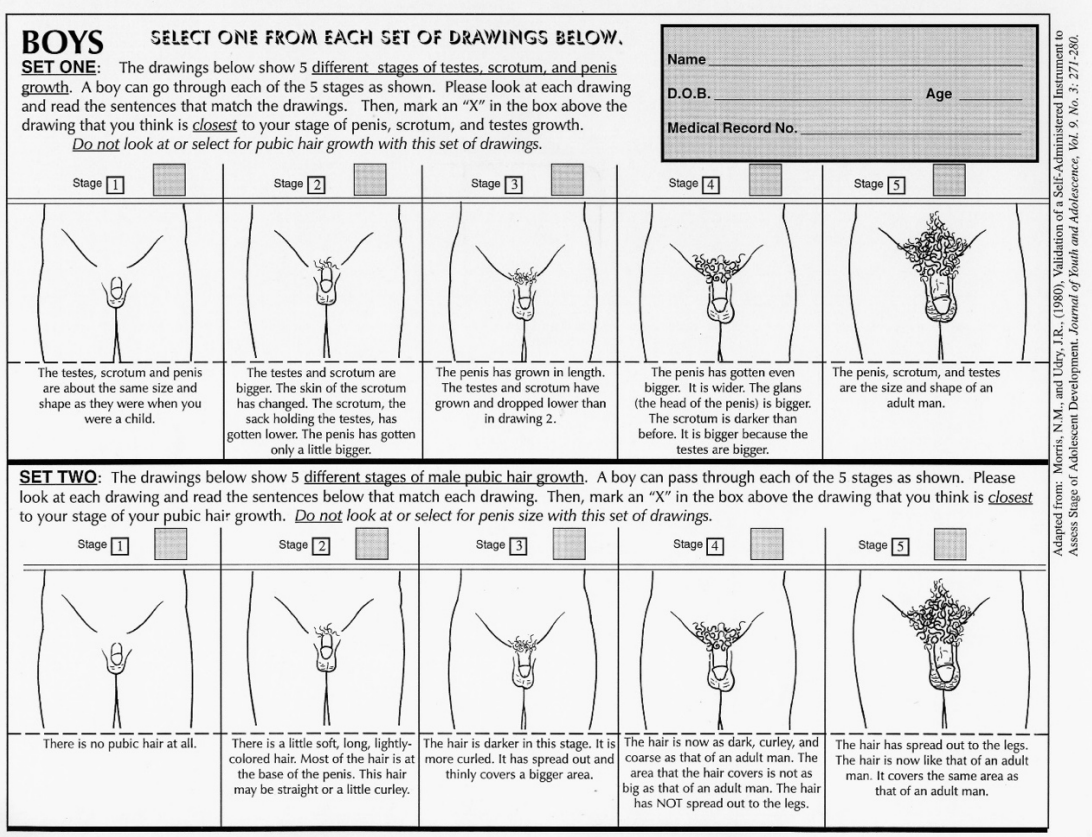

Figure 5. Tanner Stage chart for boys.

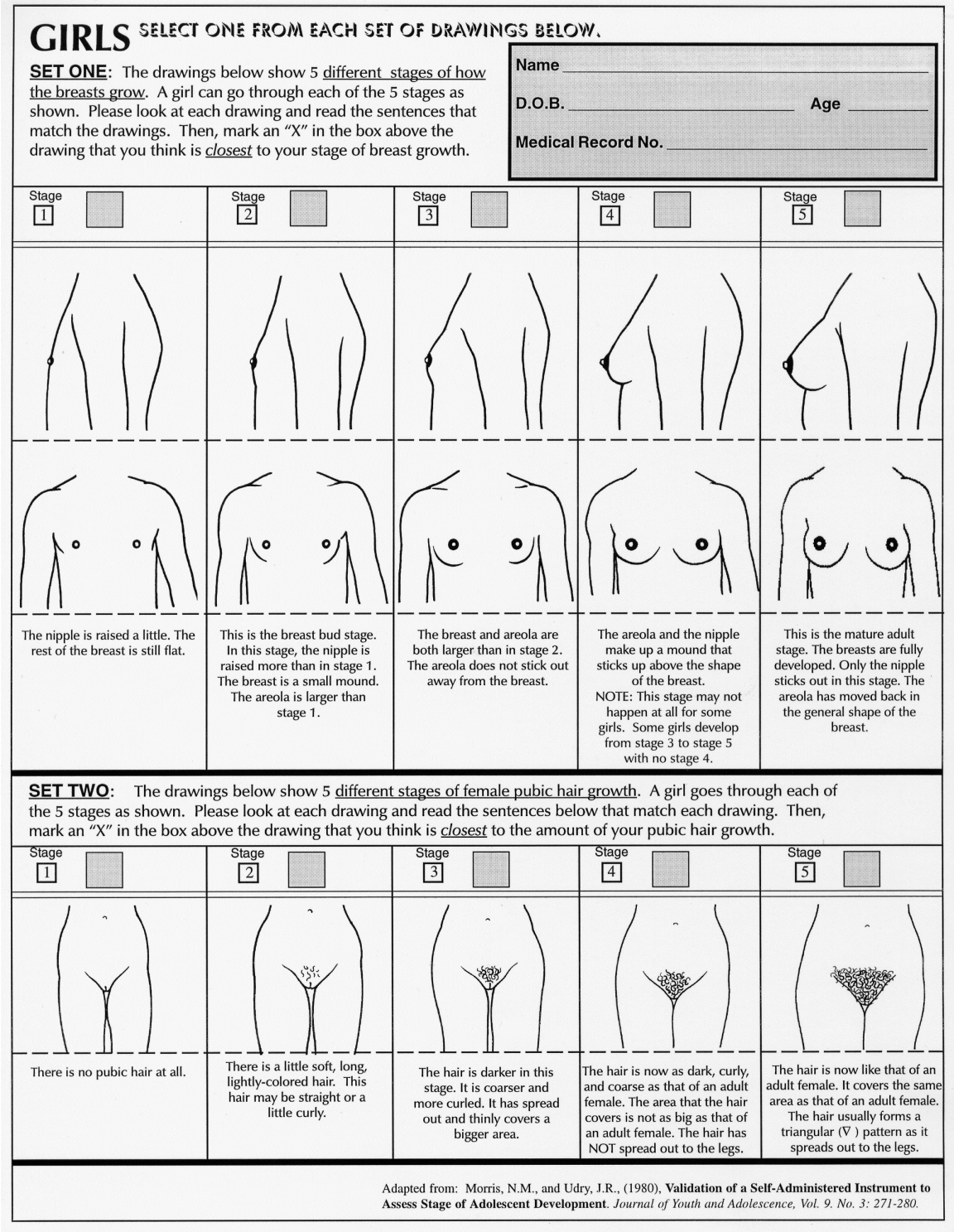

Figure 6. Tanner Stage chart for girls.

Hand grip strength

At the time of the DXA, hand strength was also measured in order to determine if vitamin D supplementation had an impact on muscle strength. This was done with a Jamar Dynamometer gripper. This machine is a handle that patients squeeze to test the strength of their grip. A dial reports the strength of the grip in pounds of pressure. The hand strength was tested three times to obtain an average, and it is presented with standard deviation.

Statistical analysis

The DXA for all non-Black participants was automatically analyzed for Z-scores using a normal set of data for white children. Raw DXA data for Black participants is not reported with a Z-score. Further analysis was conducted on the data after the scans were completed, including paired t-tests, ANOVA analysis, and height-adjustment using SPSS and a calculator developed by Zemel et al.

Follow-up and Compliance

Patients were contacted via phone reminders about scheduling DXA scans and continuing to take vitamins. We contacted patients if any additional testing or procedures

are necessary for their continued fracture care. Patients were considered compliant if at their follow-up scans they reported taking vitamin D 4 days a week or more.

Results

At the present time, there are 17 patients of the 55 who have been enrolled have completed the study. Table 1 describes the characteristics of the patients that have completed the study. Of these 17 patients, 9 are male and 8 are female. Patients 14 and 15 are the only 2 that reported having a milk allergy or lactose intolerance on their intake survey. The majority of patients are White (n=11), followed by Black (n=4). There is one Asian patient and one patient of mixed background. Patient's height and total growth was recorded in Table 2. As shown in Table 3, the mean age of participants is 12.9 years of age at the beginning of the study and the mean Tanner stage is 3.18. The average growth that a patient had during the study was 1.69 inches, with a standard deviation of 1.42 inches. Some patients were shown to have no growth.

Table 1. Patient Demographics

Patient	Sex	Age	Ethnicity	Tanner Stage	Milk allergy
1	M	14	Black	V	No
2	M	11	White	II	No
3	M	14	White	IV	No
4	F	11	Black	IV	No
5	F	9	White	III	No
6	M	14	White	III	No
7	M	13	More than one	III	No
8	M	14	White	I	No
9	F	14	White	IV	No
10	F	13	White	IV	No
11	F	14	Black	IV	Yes
12	M	10	Black	I	Yes
13	F	17	White	IV	No
14	M	16	White	V	No
15	F	15	Asian	IV	No
16	M	10	White	I	No
17	F	11	White	II	No

Table 2. Patients' recorded heights at the time of each DXA scan in inches

Patient	Height #1	Height #2	Height #3	Total growth
1	69	70	71.5	2.5
2	56	57	58	2
3	69	70	70	1
4	60.5	63	63.3	2.8
5	58	58	60.7	2.7
6	68.5	70	70	1.5
7	62	64	64	2
8	60	61.2	62.5	2.5
9	61	62.2	62	1
10	61.2	64	62	0.8
11	61	61	61	0
12	57	60	60	3
13	61.2	61.2	61.2	0
14	66	65.7	66	0
15	62	61.4	62	0
16	54.5	55	56	1.5
17	51.6	56	57	5.4

Table 3. Patient Demographic Averages

Measures	**Mean**	**Standard Deviation**
Age (years)	12.9	2.25
Tanner Stage	3.18	1.33
Height at 1st scan (inches)	61.1	4.96
Height at 2nd scan (inches)	62.3	4.65
Height at 3rd scan (inches)	62.8	4.43
Growth during study (inches)	1.69	1.42

Overall, the majority of patients had a large increase in their lumbar spine BMC, with 11 of the patients having a percent change over 10% or more in a year since beginning supplementation as seen in Table 4. Of the remaining 6 patients, two did have slight decreases in the BMC of their lumbar spine during this time period, and the other 4 had a smaller increase in BMC. The data are similar for the BMD, with 2 patients having a slight negative change in their BMD with all other patients experiencing at least a slight increase.

Table 4. Changes in lumbar spine BMC and BMD

Patient	L/S BMC #1 (g)	L/S BMC #2 (g)	L/S BMC #3 (g)	Percent Change	L/S BMD #1 (g/cm^3)	L/S BMD #2 (g/cm^3)	L/S BMD #3 (g/cm^3)	Percent Change
1	44.13	45.79	49.01	11.06	1.05	1.05	1.08	2.86
2	19.13	21.11	22.20	16.05	0.66	0.74	0.73	11.06
3	59.41	67.19	68.10	14.63	1.14	1.19	1.23	7.62
4	43.27	47.26	49.35	14.05	1.03	1.06	1.08	5.35
5	29.45	28.61	32.94	11.85	0.78	0.76	0.80	2.68
6	61.25	66.08	69.31	13.16	1.15	1.21	1.25	8.52
7	51.11	56.99	64.57	26.34	1.09	1.13	1.25	14.72
8	27.84	27.87	28.52	2.44	0.74	0.70	0.77	3.92
9	43.52	48.64	51.55	18.45	1.11	1.16	1.22	9.45
10	52.89	55.83	58.17	9.98	1.18	1.21	1.22	3.55
11	54.19	57.22	55.27	1.99	1.22	1.21	1.21	-0.49
12	33.68	34.48	37.48	11.28	0.95	0.93	0.96	1.48
13	58.89	52.66	56.66	-3.79	1.32	1.26	1.29	-2.28
14	48.89	51.49	48.81	-0.16	1.03	1.06	1.03	0.10
15	42.48	43.56	43.08	1.41	1.07	1.10	1.09	1.02
16	28.40	30.21	30.62	7.82	0.96	1.00	0.98	2.09
17	23.79	26.93	28.49	19.76	0.845	0.907	0.926	9.59

Similar changes were also observed in the TBLH measurements shown in Table 5. For TBLH BMC, 15 patients showed an increase. The largest increase was seen in Patient 4 who had an increase of 48% in their TBLH BMC. Two patients did have a slight decrease in TBLH BMC after one year of supplementation. For the BMD, 16 of the patients had a positive percent change, with only one patient having a slight negative change.

Table 5. Change in TBLH BMC and BMD

Patient	TBLH BMC #1 (g)	TBLH BMC #2 (g)	TBLH BMC #3 (g)	Percent Change	TBLH BMD #1 (g/cm^3)	TBLH BMD #2 (g/cm^3)	TBLH BMD #3 (g/cm^3)	Percent Change
1	2403.20	2497.90	2902.70	20.78	1.11	1.18	1.15	2.88
2	934.20	1131.10	1236.20	32.33	0.81	0.83	0.85	4.55
3	2440.00	2689.20	2736.20	12.14	1.09	1.19	1.18	8.36
4	1793.10	2083.10	2667.80	48.78	0.92	0.95	1.04	12.85
5	1018.50	1095.90	1218.90	19.68	0.81	0.81	0.87	6.92
6	2084.00	2214.20	2352.00	12.86	1.08	1.11	1.17	8.13
7	1592.50	1809.20	1948.00	22.32	1.00	1.07	1.11	10.46
8	929.40	975.00	1116.10	20.09	0.75	0.75	0.80	6.55
9	1495.10	1536.50	1569.20	4.96	0.93	0.93	0.96	3.66
10	1987.90	1941.00	2073.30	4.30	1.06	1.07	1.10	3.69
11	1873.90	1922.20	1900.30	1.41	1.08	1.10	1.12	2.86
12	1436.40	1485.90	1789.80	24.60	0.91	0.98	0.96	5.39
13	1931.00	1981.40	1917.00	-0.73	1.05	1.08	1.08	2.94
14	1899.90	1864.70	1863.20	-1.93	1.08	1.04	1.05	-2.23
15	1735.40	1774.90	1845.00	6.32	0.94	0.96	0.95	1.49
16	989.90	1030.50	1094.50	10.57	0.85	0.85	0.87	2.24
17	903.9	1045	1132.3	25.27	0.801	0.836	0.856	6.87

Patient 14 had a decrease in both BMC and BMD for their TBLH measurements as well as for their LS BMC. This patient began the study at Tanner stage V and had no growth during the study. The other patient with negative data, patient 13, had decreases in LS BMC and BMD as well as TBLH BMC, but did have a 3% increase in TBLH BMD. Patient 13 was at Tanner stage IV at the beginning of this study and also showed no growth. Neither of these patients reported having a milk allergy or lactose intolerance.

Results of paired t-tests run on the DXA data from patients that completed 3 scans are shown in Table 6. The p-values (Sig.) are under 0.05, demonstrating significance in the change from DXA 1 to DXA 3 for all measurements.

Table 6. Results of paired t-test for DXA data between DXA#1 and DXA#3

Measurement	**Mean**	**P-value**
LS BMC	4.22	<0.001
LS BMD	0.05	0.001
TBLH BMC	230.25	0.001
TBLH BMD	0.05	<0.001

Height adjusted z-scores (HAZ) were calculated using the Children's Hospital of Philadelphia's Pediatric Z-Score Calculator by Zemel et al. Table 7 shows that 10 out of 17 patients had a negative change in their HAZ for LS BMC with an average overall difference between HAZ 3 and HAZ 1 at -0.12. As shown in Table 8, 11 patients had a

negative change in their HAZ for LS BMD with an average difference of -0.25. For TBLH measurements, Table 9 shows 7 patients with a negative change in TBLH BMC HAZ and an average difference of 0.30, and Table 10 shows 6 patients with a negative change in TBLH BMD HAZ and an average difference of 0.14. All other patients have a negative change in these measurements.

Table 7. Height adjusted z-scores for LS BMC

Patient	LS BMC HAZ 1	LS BMC HAZ 2	LS BMC HAZ 3	Difference HAZ 3 to HAZ 1
1	-1.31	-1.21	-1.51	-0.20
2	-1.86	-1.15	-1.40	0.46
3	0.53	0.74	0.69	0.16
4	1.03	0.52	0.61	-0.42
5	0.27	0.07	0.17	-0.10
6	0.79	0.65	0.80	0.01
7	1.99	1.81	2.39	0.40
8	-0.67	-1.01	-1.42	-0.75
9	0.06	0.18	0.44	0.38
10	1.49	0.87	1.51	0.02
11	1.09	1.21	0.86	-0.23
12	1.26	0.68	1.11	-0.15
13	1.36	0.56	1.03	-0.33
14	-0.24	-0.08	-0.61	-0.37
15	-0.63	-0.38	-0.73	-0.10
16	1.04	1.28	1.10	0.06
17	0.86	0.12	0.04	-0.82
Average	0.42	0.29	0.30	-0.12

Table 8. Height adjusted z-scores for LS BMD

Patient	LS BMD HAZ 1	LS BMD HAZ 2	LS BMD HAZ 3	Difference HAZ 3 to HAZ 1
1	1.18	0.86	0.55	-0.63
2	0.66	1.49	1.12	0.46
3	2.34	2.28	2.39	0.05
4	3.44	1.73	1.74	-1.70
5	1.22	1.01	0.96	-0.26
6	2.51	2.45	2.55	0.04
7	3.77	3.46	4.26	0.49
8	0.66	-0.08	0.11	-0.55
9	2.08	2.61	2.62	0.54
10	2.90	2.45	2.87	-0.03
11	2.66	2.33	2.22	-0.44
12	4.12	3.21	3.39	-0.73
13	3.56	3.02	3.27	-0.29
14	1.30	1.41	0.95	-0.35
15	1.37	1.72	1.44	0.07
16	4.66	4.89	4.47	-0.19
17	2.90	2.38	2.24	-0.66
Average	2.43	2.19	2.19	-0.25

Table 9. Height adjusted z-scores for TBLH BMC

Patient	TBLH BMC 1	TBLH BMC 2	TBLH BMC 3	Difference HAZ 3 to HAZ 1
1	1.31	1.22	1.50	0.19
2	0.62	1.55	1.66	1.04
3	1.77	1.99	2.04	0.27
4	3.35	3.29	5.06	1.71
5	0.92	1.33	1.08	0.16
6	1.03	0.86	1.19	0.16
7	1.67	1.68	2.04	0.37
8	-3.22	-3.08	-1.01	2.21
9	0.99	0.71	0.83	-0.16
10	2.87	1.85	2.84	-0.03
11	2.20	2.17	1.98	-0.22
12	2.76	2.05	3.10	0.34
13	2.59	2.78	2.51	-0.08
14	0.85	0.65	0.46	-0.39
15	1.54	1.93	1.97	0.43
16	2.09	2.16	1.79	-0.30
17	1.74	1.10	1.20	-0.54
Average	1.48	1.43	1.78	0.30

Table 10. Height adjusted z-scores for TBLH BMD

Patient	TBLH BMD 1	TBLH BMD 2	TBLH BMD 3	Difference HAZ 3 to HAZ 1
1	1.07	1.44	0.62	-0.45
2	1.27	1.31	1.29	0.02
3	1.44	2.08	1.83	0.39
4	1.26	0.79	1.93	0.67
5	0.86	0.80	1.03	0.17
6	1.42	1.25	1.74	0.32
7	2.18	2.40	2.71	0.53
8	-2.92	-3.26	-1.09	1.83
9	0.82	0.44	0.80	-0.02
10	2.64	2.00	2.76	0.12
11	2.12	2.08	2.19	0.07
12	1.93	2.26	1.89	-0.04
13	2.09	2.40	2.40	0.31
14	1.40	0.84	0.78	-0.62
15	0.55	0.94	0.60	0.05
16	2.42	2.18	2.20	-0.22
17	2.23	1.45	1.45	-0.78
Average	1.34	1.26	1.48	0.14

The graph shown in Figure 7 demonstrates the change over time for the average HAZ score for BMC and BMD. In all cases, there is a slight decrease in the measurements from DXA 1 to DXA 2. From DXA 2 to DXA 3, all measurements increased except for LS BMD which remained constant at 2.19. From DXA 1 to DXA 3, there was an increase in the HAZs for the TBLH measurements and a decrease in the LS measurements.

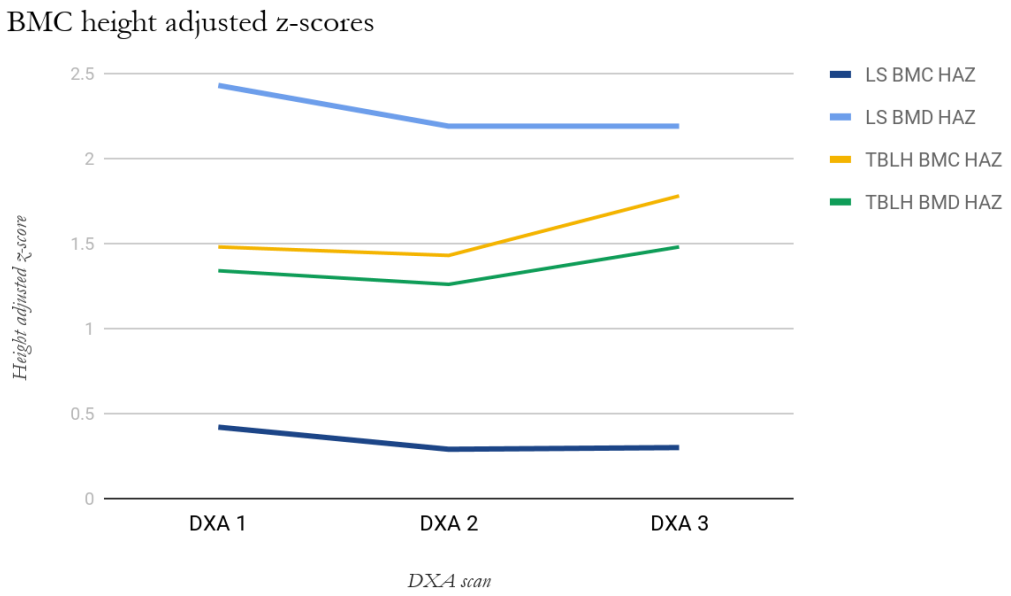

Figure 7. Height-adjusted z-scores over time for all measurements

Paired t-tests were run on the HAZs for the patients that completed 3 scans and these results shown in Table 11. The p-values (Sig.) are all above 0.05, demonstrating no significance in the change from HAZ 1 to HAZ 3 for all measurements.

Table 11. Results of paired t-test for DXA data between HAZ#1 and HAZ#3

Measurement	Mean	P-value
LS BMC HAZ	-0.12	0.178
LS BMD HAZ	-0.25	0.066
TBLH BMC HAZ	0.30	0.087
TBLH BMD HAZ	0.14	0.313

The ANOVA tests in Tables 12-15 show further analysis of the data by comparing the percent change in the various measurements taken by the Tanner stage of the patient when they were enrolled in the study. These data show that, while there was no statistical significance, the impact of Tanner stage is very high due to the high R^2 value. The mean percent change by Tanner stage was highest for stage II in each variable except TBLH BMD, where stage III had a higher mean by almost 3 % which is shown in Figure 8 on page 59.

Table 12. Results of ANOVA for Percent Change in Lumbar Spine BMC by Tanner Stage

	N	**Mean**	**Std. Deviation**	**Std. Error**
1	3	7.18	4.45	2.57
2	2	17.90	2.62	1.85
3	3	17.12	8.01	4.63
4	7	8.11	8.29	3.13
5	2	5.45	7.94	5.61
Total	17	10.37	7.96	1.93

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	364.86	4	91.22	1.684	0.218
Within Groups	649.93	11	58.46		
Total	1014.79	15			
R^2	0.360				

Table 13. Results of ANOVA for Percent Change in Lumbar Spine BMD by Tanner Stage

	N	Mean	Std. Deviation	Std. Error
1	3	2.50	1.27	0.73
2	2	10.32	1.04	0.74
3	3	8.64	6.02	3.48
4	7	3.46	4.31	1.63
5	2	1.48	1.96	1.38
Total	17	4.78	4.66	1.13

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	155.749	4	38.937	2.435	0.104
Within Groups	191.906	12	15.992		
Total	347.654	16			
R^2	0.448				

Table 14. Results of ANOVA for Percent Change in Total Body Less Head BMC by Tanner Stage

	N	Mean	Std. Deviation	Std. Error
1	3	18.42	7.17	4.14
2	2	28.80	4.99	3.52
3	3	18.29	4.88	2.82
4	7	11.02	17.13	6.48
5	2	9.43	16.06	11.36
Total	17	15.51	13.26	3.21

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	616.492	4	154.123	0.843	0.524
Within Groups	2194.910	12	182.909		
Total	2811.402	16			
R^2	0.219				

Table 15. Results of ANOVA for Percent Change in Total Body Less Head BMD by Tanner Stage

	N	Mean	Std. Deviation	Std. Error
1	3	4.73	2.23	1.29
2	2	5.71	1.64	1.16
3	3	8.50	1.80	1.04
4	7	5.12	4.03	1.52
5	2	0.32	3.61	2.55
Total	17	5.05	3.72	0.93

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81.505	4	20.376	1.886	0.178
Within Groups	129.678	12	10.806		
Total	211.183	16			
R^2	0.386				

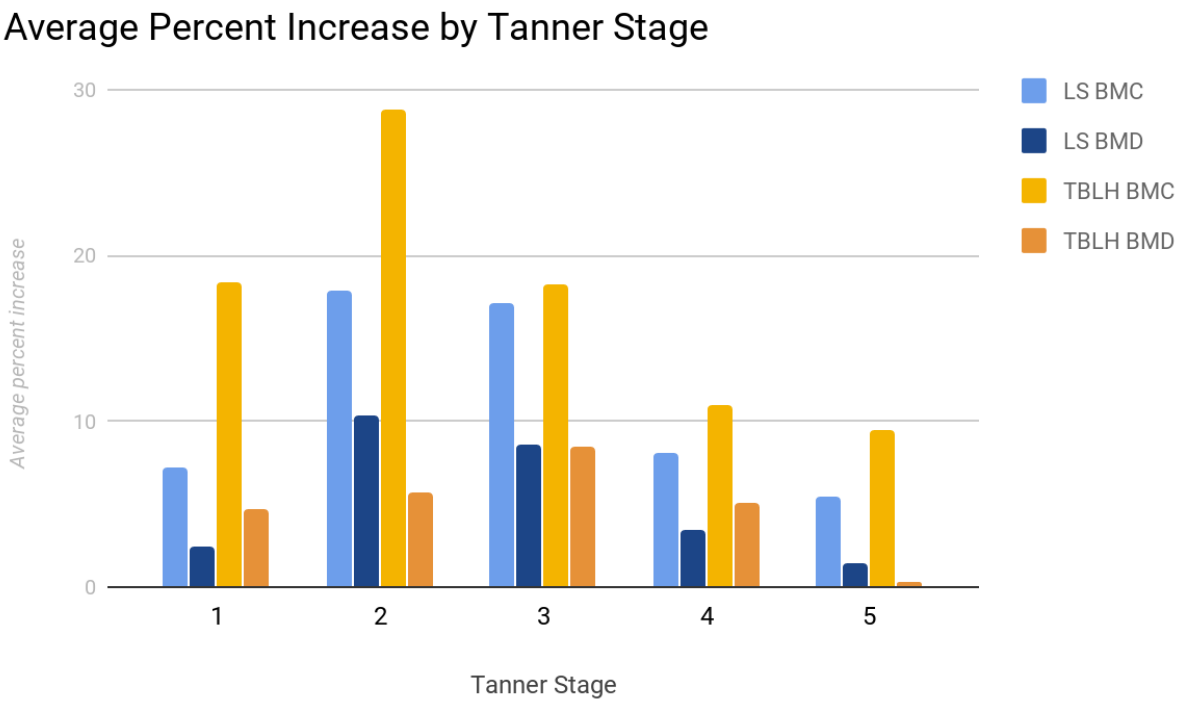

Figure 8. Average percent increase in bone measurements by Tanner Stage

Most patients did have an increase in BMC and BMD, though after the values were adjusted for height, only about half of patients had a positive increase. The average increase in HAZs for both LS measurements was negative, but the HAZs for TBLH measurements were positive. None of the values had any statistical significance. Tanner stage had a large impact on changes in BMC and BMD as the R^2 values for each measurement was above 0.25, even though the p-values were not significant. Patients with a milk allergy did not differ from patients without milk allergy in any measurements.

Discussion

This study was conducted in order to contribute to the literature on the impact of vitamin D on bone density in pediatric patients. After supplementing patients that presented with a fracture and low vitamin D, 3 DXA scans were taken in order to determine if the increased level of vitamin D would increase the bone density in the patients. We found that most patients did show an increase in both the LS and TBLH measurements; however, there were 2 patients that showed slight decreases. It was expected that patients with remaining growth would have increased bone mass and density as they grew, but our results do suggest that some patients bones showed more than the expected increase.

As there were only two patients that had a documented milk allergy, no significant statements can be made from this data on the impact of lactose intolerance or milk allergy on bone density. However, the data we have collected from patients 11 and 12 who have reported having a milk allergy did have some increase in their BMC and BMD in both the LS and TBLH as shown in Figures 1 and 2. Patient 11 had a smaller increase than the majority of participants, and had a slight negative change in their LS BMD. This patient's greatest positive percent change increase was only 2.8%, whereas even patient 12 had up to 24.6% increase in their TBLH BMC. However, patient 12 did have 3 inches of growth over the course of the study whereas patient 11 did not have any

growth, so this may account for the differences in their measurements. Patient 11 was also at Tanner stage IV at the beginning of this study compared to patient 12 who was at stage I; there is more growth left to occur at the beginning Tanner stages compared to the later stages. When looking at their HAZ, these patients both had a negative change in their LS BMC and BMD HAZs. Patient 11 had -0.22 difference in their TBLH BMC HAZ, and a 0.07 difference in TBLH BMD HAZ. Patient 12 had 0.34 HAZ difference for TBLH BMC and a -0.04 difference for TBLH BMD. The consistent negative changes for these two patients indicate that their bone health actually decreased via measures of BMC and BMD based on their height. The intervention of increasing vitamin D did not have a more positive impact on these patients with milk allergy than on any other patient, and may have been less beneficial than for other patients, contradictory to the hypothesis of this study. Our lactose intolerance patients might not be showing as significant a change in their bone health because all patients started with very low vitamin D. The percent change may still be greater than the general population that has a more normal starting point of vitamin D. It should be noted that these patients were siblings, so they would have both similar genetics and potentially similar dietary patterns.

It is unusual that there are only 2 patients with reported milk allergy when lactose intolerance is a common condition. While the sample size of the entire study is small, rates of lactose intolerance in the US are reported to be 36%, so there should be at least

one more patient reporting lactose intolerance or milk allergy (Storhaug et al. 2017) This may be due to the fact that this is a pediatric population, so lactose intolerance may still develop in many of these patients as they continue to age. However, people with lactose intolerance should naturally be selected for our study as they are much more likely to have low levels of vitamin D (Alharbi and El-Sohemy 2017). It may also be that there are patients who experience lactose intolerance symptoms but do not know what is causing these symptoms and so are unaware of their lactose intolerance.

The other patients that showed negative changes did not have a milk allergy. These patients, 13 and 14, had no growth during the duration of the study and were Tanner stage IV and V respectively. It is likely that growth and Tanner stage rather than having lactose intolerance is more indicative of whether or not vitamin D would have a significant impact on BMD changes.

The patients with the greatest growth over the study, patients 1, 4, 5, and 8, all had over 2 inches of growth during the study. These patients all did have large positive changes in their DXA measurements over the course of the study, especially in the TBLH measurement. Patient 4 had the most change with a 48.7% increase in TBLH BMC and 12.8% increase in TBLH BMD. Patient 8 had less change in their LS measurements, only increasing 2.44% BMC and 3.92% BMD, whereas the other patients that had a lot of growth had over 10% change in their LS BMC, though BMD scores were similar. Patient

8 still had a 20% increase in TBLH BMC, similar to the other patients with over 2 inches of growth. The patients that did not grow, 11, 13, 14, and 15, all had much smaller increases in all of their measurements. The average change in TBLH BMC for these patients is 1.27%. For the LS BMC and BMD, both averages were slightly negative at less than -1%. This confirms that changes in height are closely associated with increases in BMC and BMD.

Not all bone mass and density increases in the study were due to growth. Patient 15, who showed no overall growth during the study and began at Tanner stage IV, still did experience an increase in BMC and BMD. Their TBLH BMC increased by 6.3% and BMD increased by 1.5%. While this is not the same level of increase as those who did have growth during the study, this shows that there still were effects on bone density independent from growth and Tanner stage that were due to the increase in vitamin D.

The p-values in Table 6 show that the changes in the TBLH BMC and BMD as well as the LS BMC were all significant as the p-values were lower than 0.05. However, these numbers are skewed by the natural increase that occurs in these values due to growth. The average growth a patient had was 1.46 inch, which, while not an extreme change, still would have a large impact on the size of bones and therefore BMC and BMD. Additionally, the standard deviation is 1.09, so there is variation in the amount of growth experienced. Even with the differences in growth, nearly all patients showed

some increase in their LS and TBLH BMC and BMD, showing that their increased levels of vitamin D likely had an impact.

Change in height was normalized by calculating the height adjusted z-score (HAZ) for each patient, at each measurement, at each time point. The decrease from DXA 1 to DXA 2, shown in Figure 7, is very unusual. As the data reveals, most patients had an increase from DXA 1 to DXA 2, though a few did have decreases. This decrease is more consistent in the HAZs, so it is likely due to growth in the patients that is more than their gains in bone mass and density and so the HAZ decreases. The overall increase in TBLH measurements and decrease in LS measurements indicates that the LS is not benefitting from the intervention, but potentially other body sites are. Scanning more regions of the body would lead to more accurate conclusions being drawn about where in the body bone health was increasing. Overall bone health in the body is increasing, but not in the area of the lumbar spine.

Our study varied widely from what is stated in the literature. While many studies did not find an impact of vitamin D supplementation on bone mass or density, the doses of vitamin D were either much lower than our recommendations or much higher and less frequent (Cheng et al. 2003; El-Hajj Fuleihan et al. 2006; Ward et. al 2010). The supplementation in our study was tailored to each patient in order to increase their serum vitamin D to sufficient levels and was supposed to be administered daily, though there

was no way to verify that patients were taking their vitamins as prescribed. While other studies only found increases in bone density in the LS, our study showed far less positive impacts in that region and instead had increases in the TBLH measurement (Viljakainen et al. 2006; Wizenberg et al. 2011).

Tanner stage was shown to have a large impact on the change in both BMC and BMD and therefore bone health. While no statistical significance was found in any of the ANOVA tests shown in Tables 7-10, the small sample size may be the limiting factor in determining significance. A larger sample size would lead to greater significance. The coefficient of determination, R^2, did show that Tanner stage did influence the percent change in each of the four variables measured. The largest impact was shown to be on LS BMD; Table 8 shows that the R^2 value is 0.448, showing that over 44% of the increase in BMD is related to the Tanner stage. LS BMC and TBLH BMD both had R^2 values higher than 0.35, and the value is significant if it is over 0.25. The only measure that does not show this level of significance was TBLH BMC, but the R^2 value is 0.219, so still nearly 22% of the change in TBLH BMC is due to pubertal development. This confirms what was found in other studies, which is that pubertal status plays a large role in the development of bones (Bonjour and Rizzoli 2001; Lehtonen et al. 2002; Viljakainen et al. 2006; Rizzoli et al. 2010). The impact of puberty is another challenge to interventions in

pediatric populations, and these effects need to be considered when looking at pediatric data.

The percent change for each measurement separated by Tanner stage in Figure 8 shows that there was variation in the increase of the varying measurements by Tanner stage. Stage II was shown to have the greatest amount of growth, which aligns to the literature that states that pre-puberty and early puberty are the most effective times for bone health interventions.

A limitation of this study is the small sample size with only 17 patients who have completed this study. There were 55 patients enrolled in the study, but 38 were lost to follow-up over time. Increasing patient compliance in the future could improve conclusions that are able to be drawn from the study.

The size of this study also limited any conclusions that could be made about lactose intolerance and bone density. This study only had 2 patients that had reported a milk allergy, and their data was not significantly different from the other patients. A future study could use individuals who were pre-screened to have lactose intolerance in order to increase the sample of people with this condition, rather than patients that have experienced a fracture. They could then be compared to other subjects who were lactose tolerant, and the impact of vitamin D on both groups could be tracked through the DXA scans. As Tanner stage had a large impact on changes in bone density, screening and

controlling for Tanner stage would lend clarity to whether or not results were due to the intervention.

The study was also unable to regularly measure the serum 25(OH)D levels of participants. If these data were collected, more conclusions could have been reached about whether participants with higher serum levels had greater increases in BMC and BMD than participants with low serum levels. This also would have been one way to measure whether or not study participants were compliant. If this research were to be repeated, it would be useful to have another way to determine if patients were compliant with taking their vitamin D rather than relying on a survey, as patients may have written that they were compliant without taking their vitamins, leading us to make inaccurate conclusions about the data.

While this study does not conclusively confirm that taking vitamin D supplements has positive impacts on bone health, there are no side-effects or negative consequences of taking some vitamin D daily. People should continue to take vitamin D regularly to keep their serum levels in the optimal range, and more studies need to be done to further solidify the role of vitamin D in increasing bone health. Future experiments should also focus on the pediatric population, taking Tanner stage into consideration, as there is very little literature on this population. The design should include regular tests of serum vitamin D as well as a method to measure compliance in taking vitamins. Comparing

experimental data to control data where subjects did not take vitamin D would be another step to that could help to clear ambiguity about whether or not it is vitamin D having an impact on the bones of the subjects.

Conclusion

This research was conducted in order to contribute to the body of research on the impact of vitamin D on bone health, specifically in pediatric patients where the literature is lacking. Vitamin D has been shown to be an important factor in bone health. Increasing a person's bone health during pre-puberty is the most effective time in order to prevent problems later in life such as osteoporosis. By supplementing pediatric patients that had experienced a fracture with vitamin D, it was hypothesized that their bone density and mass would increase. It was found that there was a large change in both the LS and TBLH BMC and BMD for the majority of these patients. No conclusions could be drawn about the significance of lactose intolerance on bone health.

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