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TOTAL-BODY BONE MINERAL CONTENT IN ADOLESCENT GIRLS CORRELATES WITH BODY MASS, GROWTH AND PUBERTAL DEVELOPMENT, PHYSICAL FITNESS, AND PARENTAL PERCEPTIONS OF DAIRY PRODUCTS

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ABSTRACT

Maximizing bone accretion in young girls during growth is strategy that could decrease the severity of bone loss later in life. Associations between bone mineral content and genetics, growth and pubertal development, body weight, and physical activity have been observed in previous research, and evidence about the relationship between bone health and diet is inconclusive. This study aims to identify variables that correlate with bone mineral accretion between the ages of 11 and 13 years and to plan an intervention strategy for promoting bone health in girls based on the associations found. In this study, BMI at age 11 ($p<.0001$), height velocity between age 9 and 11 ($p=.0056$), starting menstruation by age 13 ($p=.0066$), maternal attitudes toward milk at girl age 11 ($p=.0216$), and physical fitness at age 11 ($p=.0015$) all significantly correlated with bone mineral content at age 13.

The findings of this study suggest that clinical recommendations for maximizing bone accumulation during the early years of adolescence should aim to encourage adequate caloric intake to promote growth and weight gain to maintain bone mass and ensure achievement of menses in a timeframe that is appropriate for the individual. Physical activity should be encouraged to improve bone strength and physical fitness, but moderation should be advised to avoid excessive activity that results in a chronic caloric deficit that could lead to delayed growth, weight loss, or amenorrhea. Future studies may benefit from categorizing physical activity to examine the difference between impact-loading activities and non-impact exercise on bone deposition. Additional research may also be required to understand the influence of habitual dietary intake throughout childhood on subsequent bone mineral content measures.

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Introduction

Almost half of all women in the US demonstrate osteopenia, or reduced bone mass. This staggering statistic results in approximately 1.3 million osteoporosis-related fractures each year in the US, resulting in approximately $13.8 million per year in treatment costs. Chronic low bone mineral content is one determinant in lifelong osteoporosis and osteopenia, especially during the postmenopausal period, because females lose bone mass rapidly after menopause (DeBar et al., 2006). Thus, taking a preventative approach to minimizing bone demineralization in older women by increasing bone mass in growing girls is a tactic that may improve bone health throughout the lifespan. Several factors significantly impact bone mineral content such as body mass index, rate of growth, and earlier onset of puberty, which can be measured by age at menses. However, research does not conclusively indicate the degree to which other modifiable factors influence bone mineral content. The purpose of this study is to reaffirm the strong role that non-modifiable factors play in influencing bone mineral content of 13-year-old girls while also examining the influence of modifiable factors including diet composition, physical activity and fitness, and parental attitudes toward dairy.

Significance of bone mineral content development in girls during childhood and adolescence

Bone mass accumulates throughout childhood and adolescence. The highest level of skeletal mass reached during the life span is known as peak bone mass. A considerable percentage of total bone mass is acquired during adolescence, with bone mass peaking between late adolescence and early adulthood (Bonjour, Theintz, Buchs, Slosman, & Rizzoli, 1991; Holick & Dawson-Hughes, 2004; Valimaki et al., 1994). Given the timing of bone accumulation, it is crucial to maximize bone accretion during childhood and adolescence to minimize the damage that could be incurred by osteopenia and osteoporosis later in life. Maximizing peak bone mass has several benefits. A 1998 study by Goulding and colleagues demonstrated that high peak bone mass was associated with greater resistance to fracture in a sample of 100 Caucasian girls ages 3-15 (Goulding et al., 1998). In addition to the protective role that high peak bone mass plays against fractures, a higher peak bone mass is also associated with lower rates of bone loss later in life (Mora & Gilsanz, 2003; Theintz et al., 1992).

Measuring bone health: Bone Mineral Content vs. Bone Mineral Density

Bone health is typically assessed by one of two measurements: bone mineral content (BMC) or bone mineral density (BMD). Each of these measurements has its own unique limitations in yielding a value that indicates bone health.

Bone Mineral Content. Bone mineral content is the preferred measure for assessment of bone status in children. BMC indicates the raw number of grams of mineral in the skeleton or in a given segment of bone. Although BMC may be skewed if body size is not taken into consideration, this assessment generally yields measurements that are reliable even during periods of growth. In single-energy measurements, BMC is measured in g/cm; a segment of bone is measured and bone mineral content is calculated per length of bone. Dual energy methods, including the dual-energy absorption (DXA) scale that is used in this study, calculates bone mineral content by determining the total grams of mineral throughout the entire body. This particular measurement may be flawed because comparing total-body BMC values without controlling for body size may skew data (Prentice, Parsons, & Cole, 1994). For the purpose of this study, BMC measurements are considered more reliable indicators of bone status than BMD measurements.

Bone Mineral Density. Bone mineral density is measured by dual energy X-ray absorptiometry (DXA), which is a non-invasive, rapid measurement device that is easy to use to assess most members of the population. Bone mineral density is the most common measure used to determine bone health in children and adults (Leonard, Propert, Zemel, Stallings, & Feldman, 1999; Prentice et al., 1994). Despite the popularity and simplicity of measuring BMD (expressed in g/cm^2), relying solely on BMD as an indicator of bone health can yield misleading results. This measurement fails to calculate true density because it does not take into account bone volume, bone length, or bone thickness (Heaney, 2003; Prentice et al., 1994). Calculating bone mineral density requires a value for bone area, which is the areal measurement of bone in square centimeters that is calculated by multiplying the length of the bone by the width of the bone (Katzman, Bachrach, Carter, & Marcus, 1991). The equation that uses bone mass and bone area to find density is $BMD = BMC\ (g)/BA(cm^2)$. However, using bone area to calculate bone density results in a flawed calculation of BMD. A true density measurement should derive from a measure of mass over volume, rather than a measure of mass over area. The reason for using bone area instead of bone volume in the BMD equation is that DXA scans cannot measure bone depth, which is the measure that should be multiplied by bone area to calculate bone volume. Assuming that bone area is equivalent to bone volume and replacing bone volume with bone area can cause discrepancies in bones where bone area differentiates significantly from bone volume (Prentice et al., 1994). Additionally, the variables of bone thickness and bone length are not controlled when BMD is measured, so BMD is often overestimated in long bones and underestimated in short bones. As a result, BMD values are much more likely to be skewed during periods of growth (when bone size may be largely variable among the population and changing quickly in individuals) than during periods when bone size is relatively stable (Fewtrell, Gordon, Biassoni, & Cole, 2005; Holick & Dawson-Hughes, 2004; Katzman et al., 1991; Molgaard, Thomsen, Prentice, Cole, & Michaelsen, 1997).

Ultimately, the challenge that arises from using BMD as a measure of bone status in growing children is that it is difficult to assess whether changes in BMD are affected by changes in bone mineral content; changes in bone area, which may be influenced by changes in bone width or length; or changes in a combination of the two measurements. Additionally, changes in bone volume, which occur with growth, will not be reflected in BMD measurements (Fewtrell et al., 2005; Leonard et al., 1999; van Rijn et al., 2003). Several studies have demonstrated the low reliability of bone mineral density to predict children’s bone growth (Ausili et al., 2012; Faulkner, Davison, Bailey, Mirwald, & Baxter-Jones, 2006). These studies and others have revealed that bone mineral content is the most accurate measure to use to assess the bone status of the participants in our study, all of whom were assessed at the age of 13. In order to gain reliable insight into the bone health of the girls in the population we are studying, we will rely on bone mineral content as the primary indicator of bone status in our analyses of factors that influence bone mineralization.

Non-Modifiable Predictors of BMC

Genetics. To understand the influence of external factors on bone mineral content, it is crucial to first take into account the role of known non-modifiable factors that influence BMC. Genetic predisposition is the largest determining factor of bone mineral content, explaining 60-80% of the variance in bone mass in the population according to multiple research studies (French et al., 2005; Holick & Dawson-Hughes, 2004; New, 1999). Genetics may influence bone mineral content directly in the form of genes and Vitamin D receptors, and they may also indirectly influence behaviors like dietary choices among people with a greater genetic predisposition for osteoporosis. Attributing a large proportion of variation in bone mineral content to genetics helps us to narrow in on the strength of other predictors that may complement genetics. The role of these factors in bone development is less clearly understood and will be discussed later.

Significant differences in bone mineral density were observed between children with different genotypes (Holick & Dawson-Hughes, 2004). Twin studies and mother-daughter comparisons support the strong influence of genetics on bone accretion and loss throughout the life span (Matkovic, Fontana, Tominac, Goel, & Chesnut, 1990). The mechanism behind the link between genetics and bone mass can be explained by the action of dozens of modulator genes, each of which plays a relatively small role in bone accretion. For example, the gene LRP5 has been found to be associated with spinal BMD, size, and osteoporosis risk in men (Holick & Dawson-Hughes, 2004). TC1RG1 is another gene that has been linked to the development of autosomal recessive osteoporosis (Holick & Dawson-Hughes, 2004).

The most common genetic variants associated with bone mass are Vitamin D receptors (VDRs). Studies have inconsistently linked expression of VDRs with bone mass (Ferrari, Rizzoli, Manen, Slosman, & Bonjour, 1998; Lonzer et al., 1996). Dietary calcium can influence the action of Vitamin D receptors, as will be discussed later (Ferrari et al., 1998).

In addition to the direct action by genes on bone, genetics may also predispose people to dietary trends that may affect bone health. In a study on the relationship between dietary calcium intake and bone mineral density, children with a family history of osteoporosis had a significantly higher calcium intake than did their peers without genetic predisposition for low bone mass. One theory for this significant difference in dietary intake is risk prevention. Logically, children with a greater susceptibility to developing osteoporosis might increase their calcium intake as a preventative measure against poor bone health (Lonzer et al., 1996).

The established role of genetics as the dominant predictor of bone mineral content provides a solid foundation for a theoretical intervention that addresses non-modifiable factors such as lifestyle, diet, and BMI. In order to provide sound advice on prevention of low bone mass in girls, it is fundamental to understand exactly how individual non-modifiable factors interact with bone mineral content to tailor an intervention that addresses each of these factors adequately.

Modifiable Predictors of Bone Mineral Content

BMI and Body Composition. Body mass index (BMI) is associated with higher BMC because bone mineralization is promoted by carrying heavier loads (Bakker, Twisk, Van Mechelen, Roos, & Kemper, 2003; Kroke, Klipstein-Grobusch, Bergmann, Weber, & Boeing, 2000; Rocher, Chappard, Jaffre, Benhamou, & Courteix, 2008). A study by Lee and colleagues found that higher body weight positively correlated with BMC in 5-year-olds (Lee, Leung, Lui, & Lau, 1993). Body mass alone is not the only anthropometric measurement that affects bone health. Body composition also plays an inconsistent role in bone modeling.

The relationship between bone mineral content and obesity has been studied, but results have been mixed. In one study, BMC was measured in a group of obese children and in a group of non-obese peers with results adjusted for body weight. Before controlling for total body weight, obese children had higher total BMC measures. However, BMC relative to body weight and bone size was significantly lower in obese children than in children of a healthy weight (Rocher et al., 2008). Goulding and colleagues also found a link between obesity and poor bone mineralization. Their study revealed higher body fat levels in girls ages 3-15 who had bone fractures than in girls of the same age without fractures. Lower bone mineral density was also found among girls with fractures (Goulding et al., 1998). Fat mass negatively influenced whole-body BMC in a study of 10-19-year-old girls (Weiler et al., 2000). In contrast, Streeter and colleagues found that body fat was positively associated with increased bone area in girls, possibly because higher body fat levels trigger earlier puberty. In pubertal girls specifically, higher body fat levels are positively associated with larger and denser bones (Streeter et al., 2013). These mixed findings reveal that although BMI has a consistently positive relationship with bone mineral content, exceptions to this relationship may be present among children with extreme levels of body fat. We can be confident in hypothesizing that higher body weight will be associated with higher bone mineral content, but should remain aware that body fat is an interacting factor that may cause some outliers in subjects with extreme levels of body fat. The caveat that obesity may actually be a detriment to bone health reinforces the importance of promoting maintenance of a healthy weight when providing guidelines for optimal bone mineralization. As a result, encouraging a diet that promotes bone health should take precedence over merely promoting weight gain in children and adolescents, since excess weight that results in obesity can lead to a host of other health problems and may even be an antagonist in bone mineralization.

Role of Calcium in BMC

Total calcium intake. Dietary calcium plays a role in bone accretion because calcium makes up a large component of the bone mineral in the form of hydroxyapatite (Weaver, Peacock, & Johnston, 1999).

Low calcium levels in the blood, which can be triggered by low dietary calcium intake, signal bones to release calcium stores into the bloodstream and subsequently decrease the mineral stores in the bone. Calcium intake during growth has frequently been associated with better bone health outcomes. Many studies report that chronic calcium deficiency is highly associated with decreased bone mass and osteoporosis ("Optimal calcium intake," 1994; Ross et al., 2011). Experimental studies involving supplementation of calcium have yielded mixed results in bone health outcomes (Lloyd et al., 1992; Weaver et al., 1999).

The effectiveness of calcium in supporting bone growth and maintenance may be influenced by baseline calcium intake. A 1997 study by Bonjour and colleagues indicated that calcium supplementation is more effective on impacting BMC if baseline calcium intake is below the recommended intake. In girls with low intakes of dietary calcium (<855 to 880mg/day), BMC, BMD, BA, and bone width increases were significantly higher among participants in the supplementation group compared to the control group (Bonjour et al., 1997). This finding suggests that calcium intakes that are much higher than the daily recommended intake may have little impact on bone mineral content. This finding is not universally supported, however. Calcium supplementation was associated with a higher rate of bone density gains in prepubertal children whose baseline dietary calcium intake was already close to the daily recommended intake that corresponded to their age (Johnston et al., 1992).

Calcium intake during periods of growth. Achieving an adequate calcium intake is especially important during periods of growth because bone mass is accumulating. As a result, dietary calcium requirements increase during adolescence. The daily recommended intakes (DRIs) for calcium in children ages 4-8, children and adolescents ages 9-18, and adults are 1000 mg/day, 1300 mg/day, and 1000mg/day, respectively. Calcium absorption from the diet increases during growth, but calcium excretion also increases, along with bone retention because bones are increasing in size and in mineral content. These adaptations of the use of calcium in the body result in an increase in dietary requirements for maintenance of calcium balance in the extracellular fluid (Peacock, 1991).

Dietary recommendations for adolescents suggest that calcium intake should exceed calcium loss via storage and excretion because a positive calcium balance is needed to achieve maximal peak bone mass during growth and adolescence (Holick & Dawson-Hughes, 2004). Total calcium intake has been associated with higher bone mineral content in numerous studies (Bonjour et al., 1997; DeBar et al., 2006; "Human vitamin and mineral requirements," 2001; Lloyd et al., 1992; Valimaki et al., 1994; Weaver et al., 1999).

A meta-analysis of calcium studies supported the conclusion that one might draw from the previously mentioned statistics: that the proven effects of dietary calcium on bone mineral content are inconsistent. Twenty-seven of the 37 studies of calcium supplementation in this meta-analysis showed absolutely no relationship between calcium supplementation and bone health. The remaining relationships either had low significance or were confounded by other factors (Lanou, Berkow, & Barnard, 2005).

Despite theoretical evidence of the clear physiological role of calcium in bone accretion and demineralization, experimental studies indicate that there are stronger confounding factors such as pubertal status and initial calcium balance that may determine the degree to which calcium intake influences bone development. This lack of consistency in the research may translate to ambiguity in clinical recommendations due to individual differences among the clients. Further clarification on patterns between calcium intake and bone health are necessary to solve this problem, and a greater understanding of the role of calcium intake on bone mineral content may emerge from this study.

Calcium/phosphorus ratio and BMC A high ratio of calcium to phosphorus in the diet has been shown to be protective against bone fractures (Lutwak, Singer, & Urist, 1974; Wyshak & Frisch, 1994). Phosphorus is a component of calcium hydroxyapatite crystal in the bone (Wyshak & Frisch, 1994). Extremely low dietary calcium/phosphorus ratios are associated with higher intakes of cola and sugar sweetened beverages, as well as increased incidence of bone fracture (Wyshak & Frisch, 1994). However, balance in the diet is important for bone health, as an excessively high calcium/phosphorus ratio in the body may decrease phosphorus absorption and lead to reduced bone mineralization (Heaney &

Nordin, 2002). The calcium/phosphorus ratio of the typical American diet is lower than the ratio that is most conducive to bone health. Milk has a calcium/phosphorus ratio of 1.3; therefore, it would be logical to hypothesize that adequate milk intake would be associated with a higher calcium/phosphorus ratio that will maximize bone health (Wyshak & Frisch, 1994).

Evidence of the role of dairy products in bone mineralization Dairy products are excellent sources of calcium, and they also contain other nutrients that may promote bone mineralization including Vitamin D, protein, magnesium, phosphorus, and potassium. Thus, a high intake of dairy products may be protective against bone fracture. Several studies have linked higher bone mineral content measures with higher intakes of dairy products. Adding calcium-rich dairy products to the diets of a group of 11- to 18-year-old girls produced significantly higher gains in BMD of the lumbar spine and in total-body BMC compared to a control group (Chan, Hoffman, & McMurry, 1995). Another experimental study of 12-year-old girls found that daily consumption of a pint of milk produced significantly higher increases in bone mineral content in the treatment group compared to the control group. This study also found higher levels of insulin-like growth factor I, a hormone that promotes bone accretion, in the treatment group than in the control group (Cadogan, Eastell, Jones, & Barker, 1997). This evidence of milk and dairy products as promoters of bone mineral content indicates that growing girls may have an increased risk for poor bone development because of the decrease in dairy beverage consumption throughout adolescence observed by Fisher and colleagues (Fisher, Mitchell, Smiciklas-Wright, Mannino, & Birch, 2004).

Role of Nondairy Beverages in BMC The reported decrease in milk consumption throughout childhood and adolescence is accompanied by an increase in nondairy beverage consumption (Bowman, 2002; Guthrie & Morton, 2000; Harnack, Stang, & Story, 1999; Yen & Lin, 2002). Sugar-sweetened beverages and soft drinks have high levels of phosphoric acid; therefore, a high intake of nondairy beverages can further reduce the calcium/phosphorus ratio in an adolescent with an already low intake of milk and other dairy products (Wyshak, 2000; Wyshak & Frisch, 1994). Bone mineral content has been found to negatively correlate with intake of sugar-sweetened beverages in multiple studies.

Cola beverage intake was associated with increased fracture incidence in a study of adolescent girls with a median age of 14.3 years (Wyshak & Frisch, 1994). Fisher and colleagues found that girls who did not meet the recommended dietary intake for calcium had greater increases in their intake of sugar-sweetened beverages throughout childhood and adolescence (Fisher et al., 2004). Furthermore, girls who met the recommended dietary intake for calcium consumed significantly fewer sugar-sweetened beverages between the ages of 5 and 9 than girls who did not meet the calcium requirements (Fisher et al., 2004).

These findings that sugar-sweetened beverages correlate with lower calcium intake and higher risk for bone fracture (Fisher et al., 2004; Wyshak & Frisch, 1994) confirm that higher intakes of cola and other sugar-sweetened beverages create a dietary pattern that puts growing girls at risk for calcium deficiency and subsequent poor bone mineralization. It is important to note that given the inevitable interaction between total calcium intake, servings of dairy, and servings of sugar-sweetened beverages, a multifaceted approach should be taken to address dietary treatment to promote optimal bone mass accretion and maintenance.

Role of Puberty in BMC One predictor of bone mineralization that has been relatively consistent throughout the literature is earlier onset of puberty (Ausili et al., 2012; Berger et al., 2010; Carruth & Skinner, 2000; Galuska & Sowers, 1999; Katzman et al., 1991). The most consistent way to measure onset of puberty is the age of menarche. Higher body weight has been associated with earlier puberty (Streeter et al., 2013), indicating that BMI and early onset of puberty may interact with each other in their effects on bone mineral content. In a study of adolescent girls, later pubertal stages consistently predicted higher bone mineral content. These findings supported the hypothesis that bone mineralization increases throughout puberty because increased sex steroids promote bone mineralization (Katzman et al., 1991). Pubertal stages have also been shown to correlate more closely with bone mineral deposition than chronological age because of this variation in hormones among girls of the same age who have reached different pubertal stages (Novotny et al., 2004). This evidence suggests that menstruating girls will hypothetically have higher bone mineral content than their prepubertal peers (Ausili et al., 2012; Berger et al., 2010; Carruth & Skinner, 2000; Galuska & Sowers, 1999; Katzman et al., 1991; Novotny et al., 2004). We can expect the relative influences of other external factors to vary between girls who have reached menarche and girls who have not.

Role of Physical Activity in BMC Running, walking, jumping, weight-lifting, and other weight-bearing activities are defined as "impact load" activities and are positively associated with higher bone mineral content. Impact loading promotes activity of osteoblasts, the cells that create new bone tissue (Lee & Lanyon, 2004). Applying impact to bones, a process known as mechanical loading, can positively influence bone modeling, a process that affects the organization of the growth and development of bone tissue in growing children (Grimston, Willows, & Hanley, 1993). General physical activity, especially activity involving impact loading, has also been shown to correlate with higher bone mass when exercise is performed in moderation.

Total physical activity (including impact-loading and other categories of activity) has been linked to stronger bones in more than one study. Girls ages 8-16 with higher physical activity levels were at lower risk for bone fractures compared to their less active peers (Wyshak & Frisch, 1994). Physical activity throughout the lifespan has been associated with long-term bone health. A study of healthy premenopausal women ages 20-50 years indicated that four physical activity sessions of at least 45 minutes each week correlated with significantly higher bone mineral density and bone mineral content measures (Halioua & Anderson, 1989). These findings may be explained by a hypothesis that physical activity decreases cytokine levels, therefore inhibiting the action of osteoclasts, the cells that stimulate bone resorption (Dixon, 1992).

Just as the role of the calcium/phosphorus ratio in promoting bone mineralization is most effective when balanced, physical activity is a predictor that has been shown to produce positive effects on bone accretion in moderation. Since BMI and the presence of a regular menstrual cycle are two factors that play very strong roles in determining bone mineral content, it's important to recognize that excessive exercise is one of the factors that can cause decreased BMI and disruption of menstruation.

Weaver and colleagues confirmed that excessive exercise leading to excessive thinness or amenorrhea is associated with lower bone mineralization (Weaver et al., 1999).

When looking at trends between physical activity and bone mineral content in our study, the influence of exercise in moderation should be kept in mind. Based on the evidence that impact-loading exercises can increase bone mass, we can confidently predict that weight-bearing exercise will promote higher bone mineral status in our population (Dixon, 1992; Grimston et al., 1993; Halioua & Anderson, 1989; Lehtonen-Veromaa, Mottonen, Nuotio, Heinonen, & Viikari, 2000; Wyshak & Frisch, 1994). However, research does not indicate whether physical fitness has a direct influence on bone mineral status. Based on the evidence that moderate levels of weight-bearing exercise promote bone mass accretion as well as physical fitness, examining the relationship between bone mineral content and general physical fitness may yield results that can serve as the foundation for physical activity recommendations that promote bone health in adolescent girls.

Impact of Parenting Practices on BMC In addition to their genetic contributions, parents' attitudes and practices can have an overwhelming influence on the environment of their children, which may translate to long-term effects on their daughters' development. Maternal practices and attitudes regarding dairy are especially relevant to bone mineral status because dairy foods are high in calcium and because intake of dairy products may correlate with total calcium intake (Cadogan et al., 1997; Chan et al., 1995; Fiorito, Mitchell, Smiciklas-Wright, & Birch, 2006; Novotny et al., 2004). Studies on the factors that influence childhood obesity have examined the association between parenting styles, child intake of food, and obesity outcomes, including relationships between maternal child-feeding practices and obesity in children (Birch & Fisher, 2000). These studies have revealed that a child's food intake is dependent on many interacting factors including child temperament, parenting style, and specific parental feeding practices. Several studies yielded contradictory results about the effect of food restriction by a parent on the child's intake of that food, so more research is needed before we can classify parenting styles or specific parent feeding practices as positive or negative (Fisher, Mitchell, Smiciklas-Wright, & Birch, 2001; Vereecken, Rovner, & Maes, 2010).

This lack of strong evidence regarding the influence of feeding practices on child dietary intake leaves unanswered the highly specific question of the role of parenting practices regarding dairy in bone mineral development. Fisher and colleagues found a positive association between maternal milk intake and child milk intake in a study of 5-year-old girls and their mothers (Fisher et al., 2001). Although maternal intake has been found to correlate with child intake, little evidence exists on whether parental perceptions of a specific food influence intake of that food.

In our study, we will assess whether maternal perceptions influence child bone health, but maternal and child milk intake are not taken into account in either of these measurements. Results that show any relationship between maternal attitudes and child bone health would be highly beneficial for healthcare providers whose goal is to promote parenting behaviors that will improve bone health among young girls.

Summative Statement

In this study, we predicted that genetics, BMI, and pubertal status play chief roles in determining bone mineral content. The remaining factors may play supporting roles whose significance could vary greatly due to mixed research findings. Total calcium intake and, more specifically, intake of dairy and non-dairy beverages like sugar-sweetened beverages may influence bone mineral content, but the degree of their influence is difficult to predict based on the codependence of these three variables. One finding regarding calcium that we expected to see replicated in this study is the decrease in calcium intake over time. Whether this decrease in calcium intake would be accompanied by a trend toward lower bone mineral content was difficult to predict. We predicted that girls in the study who are more physically active and therefore more physically fit would be prone to higher bone mineral content measures, unless physical activity is excessive enough to have debilitating effects on menstrual cycles or maintenance of a healthy weight. Finally, maternal attitudes toward milk may influence child intake of calcium-rich dairy products, but once again the existing debate on calcium's role in bone mineralization indicates that maternal attitudes may not impact bone mineral content, even if it does promote child milk intake.

The overall objective of our study was to examine the influence of diet, physical fitness, and maternal perceptions of dairy at age 11 and physical activity at age 13 on bone mineral content at age 13.

In this study, the author of this paper made contributions to the secondary data analysis process. This author was responsible for working closely with the lab statistician to identify research questions, choose variables, and run secondary data analyses on the chosen areas of research. The overall objective of our study is to examine the influence of growth and development measures, diet, physical fitness, and maternal perceptions of dairy at age 11 and of physical activity at age 13 on bone mineral content at age 13.

Research Aims

Aim 1: To confirm preexisting findings that BMI, height velocity, and menstruation positively impact BMC.

Hypothesis 1: Based on the literature on non-modifiable factors that influence bone mineral content, bone mineral content at age 13 will be positively associated with a higher BMI at age 11, greater height velocity from age 9-11, and starting menstruation by age 13.

Aim 2: To determine the association between dietary intake and bone mineral content by testing correlations between bone mineral content and total calcium intake, daily servings of dairy, daily servings of non-dairy beverages, and maternal attitudes toward milk.

Hypothesis 2: Based on the role of calcium in bone mineralization and the evidence of the effect of calcium supplementation on bone mineralization, total calcium intake at age 11 is predicted to be associated with higher BMC at age 13. Specifically, higher intake of servings of dairy at age 11 will predict for higher BMC at age 13. Since non-dairy beverage intake increases throughout adolescence as dairy intake decreases, bone mineral content at age 13 will be inversely associated with non-dairy beverage intake at age 11. Although research on the relationship between parenting styles and child health outcomes is inconclusive, we expect to see an association between positive maternal perceptions of dairy at age 11 and higher bone mineral content in daughters at age 13.

Aim 3: To evaluate the relationships between bone mineral content and energy expenditure factors, which are measured by physical activity and physical fitness levels.

Hypothesis 3: Based on literature that indicates that the benefits of bone modeling associated with physical activity outweigh the risks for fracture, girls with higher levels of moderate and vigorous physical activity at age 13 will have higher bone mineral content at age 13. Additionally, girls with better performance on shuttle run fitness tests at age 11 will have higher BMC at age 13.

Methods

Participants

Participants were part of a longitudinal study of the health and development of young girls living in central Pennsylvania. At study entry, participants included 197 5-year-old girls and their parents; families were reassessed every 2 years (ages 7, 9, 11, and 13 years). . Families were recruited for participation in the study using flyers and newspaper advertisements. In addition, families with age-eligible female children within a 5-county radius received mailings and follow-up phone calls (Metromail Inc.). Eligibility criteria for girls' participation at the time of recruitment included living with both biological parents, the absence of severe food allergies or chronic medical problems affecting food intake, and the absence of dietary restrictions involving animal products; families were not recruited based on weight status or concerns about weight. For the current study, only girls with complete data on anthropometrics, dietary intake, bone mineral content, menstrual history, physical fitness, and parental dairy attitudes at measurements at age 11 and age 13 were included, resulting in a final sample of 134 girls. Attrition was primarily due to family relocation outside of the study area.

Families were predominantly non-Hispanic, white, and the average income for the sample ranged between $50,000 and $75,000, representing the demographics of the area surrounding the study site (Census 2000). Parents were relatively well educated, with fathers having a mean SD of 14.9 +/- 2.7 years of education and mothers having 14.8 +/- 2.3 years. Parents were on average slightly overweight at the first time of measurement with a mean body mass index score (BMI) of 26.4 +/- 6.05 for mothers, and 28.0 +/- 4.35 for fathers. Mean BMI of girls at entry was 15.8 +/- 1.4, and 18% and 5% were "overweight" and "obese", respectively classified as "overweight" if their BMI percentile was >85 and "obese" if their BMI was >95 (Kuczmarski, Ogden, Grummer-Strawn, & al., 2000; Ogden & Flegal, 2010). The Pennsylvania State University Institutional Review Board approved all study procedures, and parents provided consent for their family's participation before the study began. At ages 11 and 13, girls also provided verbal assent prior to data collection.

Measures

24 Hour Dietary recall

Girl's energy, macronutrient, micronutrient, and dairy intakes were measured using three, 24-hour recalls. Girls' dietary intake was measured at age 11y. At age 11 years, girls were the primary reporters with mothers participating in the interview as needed. Three recalls were obtained per respondent at each time of measurement; two weekdays and 1 weekend day during the summer and fall months were randomly selected over a 2-week period. Interviews were conducted at the Dietary Assessment Center at the Pennsylvania State University at each occasion by trained staff using the computer-assisted Nutrition Data System for Research (NDS-R) software (database version 4.01_30, 2000, Nutrition Coordinating Center, University of Minnesota, Minneapolis). The NDS-R software itself provides a structured, guided controlled platform where questions and probes are standard, and the process of conducting the 24-hour recall is standardized. The NDS-R time-related database updates analytic data annually while maintaining nutrient profiles true to the bastion used for data collection. Interviewer reliability was assessed (Jonnalagadda et al., 2000). Nutrient Data System for Research (NDS-R) was used when the girls were 7, 9, and 11 years old. At age 11 we used version 4.06_34 (2003). Food portion posters (2D Food Portion Visual, Nutrition Consulting Enterprises, Framingham, Ma) were used to assist in the estimation of food amounts.

Nutrient data were averaged across 3 days to obtain an estimate of energy and nutrient intakes. For calcium, mean intakes were compared with Adequate Intake (AI) recommendations (Institute of Medicine & Food and Nutrition Board, 1997). Girls' calcium intakes at age 11 were compared to dietary calcium recommendations for 9-13 year old girls according to Institute of Medicine guidelines. Calcium intake was based solely on food and beverage intake and excluded any source of calcium from multivitamin-mineral supplements.

The mean number of servings consumed from the dairy food group of the USDA Food Guide Pyramid was calculated from the 24-hour recall data using the NDS-R methodology previously described (Mitchell, 2001). To do this, the gram weights of all foods consumed were summed, including those contained in mixed dishes. Mixed dishes were disaggregated into the corresponding ingredient gram weights and the ingredient gram weights were summed into single whole food weights that were assigned to the dairy food group according to the Food Guide Pyramid. The number of servings was calculated from gram weights of whole foods consumed and were primarily based on serving sizes as defined by the Food Guide Pyramid.

Dairy output files were used to calculate average 3-d dairy consumption into three categories: total dairy, total milk and other dairy. Total dairy included total milk, cheese, yogurt and dairy desserts. Total milk intake was that consumed as a beverage and milk consumed with other foods (i.e. cereal) or as part of a recipe. Other dairy included cheese, yogurt and dairy desserts. Consumption in each category was expressed in servings, and as a percent of total daily energy intake.

Beverage output files were used to calculate average of 3 days' beverage consumption into two intake categories: milk and nondairy beverage. Milk included whole and reduced-fat plain or flavored milk and was consumed as a beverage. Nondairy beverages included fruit juice, fruit drinks, sodas, tea and coffee. Fruit juice was defined as containing 100% fruit juice. Fruit drinks included any sugar-sweetened and artificially sweetened fruit flavored drinks, sport-ades, and drinks that contained <100% fruit juice. Sodas included carbonated sugar sweetened or artificially sweetened beverages, such as colas and flavored carbonated beverages, caffeinated or decaffeinated. Tea and coffee were defined as caffeinated or decaffeinated, sugar sweetened or artificially sweetened, with sugar or artificially sweetened additions such as cream, milk, or creamer. Unsweetened tea and coffee with no additions were included. Girls' intake of water was not assessed. Amounts consumed in each category were expressed in servings (1 serving = 8 fluid ounces).

Weight Status

Height and weight were measured at 11 years old by a trained staff member following procedures described by (Lohman, 1988). Children were dressed in light clothing and measured without shoes. Height was measured in triplicate to the nearest 10th of a cm using a Shorr Productions stadiometer (Irwin Shorr, Olney MD). Weight was measured in triplicate to the nearest 10th of a kg using a Seca Electronic Scale (Seca Corp., Burmingham, UK). Age and gender specific BMI percentiles were calculated using growth charts from the Centers for Disease Control (Kuczmarski et al., 2000).

Height and weight can be used to calculate BMI, a measure of weight status. The BMI calculation assumes that ratios of weight/height are highly correlated with obesity or fatness. These values can then be compared against reference data in order to place participants' weight statuses in the context of normative data specific to their age and sex. In our study, girls' BMI percentiles and z-scores were calculated using the NCHS Growth Charts (Kuczmarski et al., 2000). BMI z-scores with a higher absolute value indicate that the participant's BMI is further from the mean for girls her age, with negative values indicating that she is below the mean and positive values indicating that she is above it.

Height velocity

Girls' height velocity was calculated by subtracting height in centimeters at age 11 from height at age 9. Height velocity is an indicator of somatic maturity and reflects growth in stature.

Bone mineral content

Girls' total body bone mineral content (TBBMC) was assessed at age 13 using Dual energy X-ray Absorptiometry (DXA). A trained technician obtained measurements with children in a supine position, in light clothing without shoes. Whole body scans were obtained using a Hologic QDR 4500W (S/N 47261) instrument in the array scan mode. Scans were analyzed using whole body software, QDR4500 Whole Body Analysis. Bone mineral content was expressed in grams.

In this study, BMC and not bone mineral density (BMD) was used as the outcome measure to assess the relation between calcium intake and bone mass. Because BMD is BMC divided by bone area, density is related to mass and is not a sensitive measure of bone accumulation associated with growth and increase in skeletal size (Fiorito et al., 2006; Heaney, 2003).

Maternal milk attitudes

Mothers' attitudes about child milk consumption were measured when children were 11 years old. The Mothers' Dairy Attitudes questionnaire was used to assess this measure. This questionnaire contained 10 items asking the importance of child's milk consumption (e.g. "It is essential for my child to drink milk at dinner"; "It is important for me to set a good example for my child by drinking milk"). This questionnaire was developed as part of a project initiated by the National Dairy Council. This instrument was designed to attempt to understand whether mothers' attitudes on child's milk intake are associated with actual milk intake in girls. Variables were coded categorically (as 1 or 0) and summed to create a total milk attitudes score. Higher scores indicated more positive attitudes and scores ranged from 0-10.

Menstruation

A 5-item questionnaire was modified from an existing measure from Liz Susman (Dorn et al., 1999) and was administered at age 13 to assess the status of the girls' menstrual cycles. This questionnaire included five items that assessed menstrual status: whether menstruation had begun, the grade in school of the first period, the age of the first period, the month and year of the first period, and the day that the most recent period began. For the purpose of this study, the categorical data on whether menstruation had begun by age 13 (0=no, 1=yes) was used as a tool to determine whether girls had reached puberty and started their menstrual cycles.

Physical fitness

Girls' physical fitness was assessed using the Progressive Aerobic Cardiovascular Endurance Run (PACER) (Leger & Lambert, 1982; Leger, Mercier, Gadoury, & Lambert, 1988). This is a progressive test providing an index of aerobic fitness and is suitable for children of all ages. Children run back and forth between markers spaced 20 m apart at a specified pace that progressively increases. The more “laps” completed and an ability to maintain the specified pace indicate a higher level of aerobic capacity and in turn a higher level of fitness. Previous research illustrates the test-retest reliability of the PACER and has shown that the number of laps completed is significantly and positively correlated with measured VO_{2max} ($r = 0.69$ boys and $r = 0.51$ for girls) (Liu, Plowman, & Looney, 1992).

In this sample, the number of laps completed in the PACER fitness test was negatively related to weight status ($r = -0.41$, $p<.0001$) (Figure 1) and positively to the tendency to participate in physical activity ($r = 0.22$, $p<.01$).

Physical activity

The Children's Physical Activity scale (CPA) was used to measure girls' self-reported physical activity at age 11. In a self-administered survey, girls responded to 15 questions such as “I participate in sports almost every day” with a 4-point scale ranging from 1 = completely false to 4 = completely true. Scores on the 15 items were averaged to create a score ranging from 1 (low activity) to 4 (high activity). In previous studies, scores on the CPA have been correlated in the expected direction with 1-mile run/walk time ($r = −.43$, $P < .0001$), body fat percentage ($r = −.41$, $P < .0001$), and BMI ($r = −.32$, $P < .0001$) (Tucker, Seljaas, & Hager, 1997). The internal consistency coefficient for the CPA in this study was $\alpha = .73$, indicating acceptable internal reliability.

In addition to self-reported physical activity, objective assessments of physical activity were also obtained with the ActiGraph 7164 accelerometer (Shalimar, FL). The ActiGraph is a uniaxial accelerometer designed to detect vertical accelerations ranging in magnitude from $0.05g$ to $2.00g$ with a frequency response of 0.25 to 2.50 Hz. These measures allow for the detection of normal human motion and will reject high-frequency vibrations encountered during activities such as operation of a lawn mower. The Actigraph 7164 has been shown to be a valid and reliable tool for assessing physical activity in children and adolescents (Trost et al., 1998).

After receiving detailed instructions regarding the care and use of the accelerometers, girls were instructed to wear the ActiGraph at all times, except when bathing and swimming, for 7 consecutive days. The ActiGraph was worn on the right hip (mid-axilla line at the level of the iliac crest). Non-wearing time for each monitoring day was calculated by counting the number of zero counts accumulated in strings of 20 minutes or longer. Girls were included in the analyses if they had 4 or more days with 10 or more hours of wearing time (Masse et al., 2005). Previous work has shown that 4 days of monitoring provides reliable estimates of usual physical activity in adolescent youth (Trost, McIver, & Pate, 2005). In this study, 75.2% of girls had 7 valid monitoring days, with 14.3%, 6.8%, and 3.8% providing 6, 5, and 4 valid days, respectively. Among the participants with 4 or more valid monitoring days, daily wear time ranged from 763.4 minutes to 1282 minutes, with an average of 1086 ± 116 minutes.

Raw accelerometer counts were uploaded to a customized software program for determination of total daily counts, and daily time spent in moderate (MPA), vigorous (VPA), and moderate-to-vigorous (MVPA) physical activity. The age-specific count thresholds corresponding to the aforementioned intensity levels were derived from the metabolic equivalent prediction equation developed by Freedson et al (Freedson, Pober, & Janz, 2005; Trost et al., 2002). To accommodate the 30-second epoch length, count thresholds were divided by 2 (Nilsson, Ekelund, Yngve, & Sjostrom, 2002).

Statistical Analyses

All descriptive statistic and data analyses were completed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). In all statistical analyses, significance was indicated by $p \leq .05$.

Analyses first aim: The goal of the first aim is to confirm existing research findings that girl BMI at age 11, her height velocity from age 9 to 11, and menstruation at age 13 are associated with higher BMC at age 13. Regression analysis was used to examine the relationship between the following predictors: BMI at age 11, height velocity from 9 to 11, and menstruation at age 13 and the outcome measure: BMC at age 13 (Model 1A). This model was used as the baseline and was designed for factors to be added to it to test for significance in relationship to bone mineral content.

Analyses second aim: The goal of the second aim is to determine whether a significant difference in BMC at age 13 existed among girls in the sample based on their dietary calcium intake at age 11 and also on their mothers' perceptions of dairy products at this point in time. Calcium intake was added to the baseline model to determine the effect of dietary calcium intake bone mineral status. A partial correlation was conducted with calcium in the model. Calcium was then replaced with the variables that measure daily servings of dairy products and daily servings of sugar-sweetened beverages. Finally, dairy predictors were replaced with maternal milk attitudes to determine the influence of maternal attitudes toward milk, a potential contributor to dietary habits, on bone mineral content at age 13. Regression analysis was used to develop three models. These models retained the factors assessed in the baseline model and each of the three additionally examined the impact of total calcium intake at age 11 (MODEL 2A), daily servings of dairy products and nondairy beverages at age 11 (MODEL 2B), or maternal attitudes toward milk at daughter age 11 (MODEL 2C) on BMC at age 13.

Analyses third aim: The third aim sought to determine the influence of physical fitness at age 11 and physical activity at age 13 on bone mineral content at age 13. A regression analysis was conducted, adding each of these variables separately to the baseline model formed in the first analysis. Two models were identified from addition of variables to the baseline stepwise regression model used in the first aim. One model assessed the influence of physical fitness at age 11 on bone mineral content at age 13 (MODEL3A). Fitness was assessed by a shuttle run test, and the number of lines run by each participant was used as a continuous measure of physical fitness in this analysis. The second model replaced the physical fitness variable with daily minutes of moderate-to-vigorous physical activity at age 13, a variable which correlated with moderate physical activity at age 13 (MODEL 3B). Physical activity data from only half of the participants was collected at age 11, so physical activity at age 13 was a more representative measure of the entire sample's physical activity habits than activity at age 11. Physical activity was categorized by intensity by a software program that evaluated the information collected from the ActiGraph accelerometer worn by each participant to specifically document minutes of moderate-to-vigorous physical activity each day.

Results

Descriptives

Descriptives are outlined in Table 1 and Table 2. Almost one-third (31.34%) of participants had a BMI that, based on the 2000 National Center for Health Statistics criteria, classified them as overweight at age 11. Eighteen (13.43%) were obese according to NCHS standards. Of the 134 girls with menstruation data at age 13, 93 (69.40%) had started their period. Height velocity from age 9 to age 11 was recorded for the 134 participants. The mean height velocity over this time span was 13.12 centimeters (min=8.77 cm, max=24.43 cm).

The mean 24-hour dietary calcium intake at age 11 was 927.10 mg (min=293.39, max=2534.61). This group averaged .85 servings of dairy beverage each day (min=0, max=6.33) and 2.15 servings of nondairy beverages daily (min=0, max=7.42). Dairy beverage intake tended to be low at age 11, with a majority of participants falling short of the 3 servings of dairy recommended by the Institute of Medicine to optimize calcium intake (Figure 2). Nondairy beverage consumption, on the other hand, was more popular (Figure 3). This tendency to drink nondairy beverages rather than dairy beverages could explain the large proportion of participants who failed to meet the daily recommended intake for calcium of 1300 milligrams per day (Figure 4).

Ninety-seven participants (63.82%) were classified as physically fit. The mean number of lines run in the shuttle run fitness test for the participants at age 11 was 17.64 lines (min=4.0, max=48.0). At age 13, only 115 of the girls had complete measurements from the physical activity assessment. These girls participated in an average of 36.14 minutes of moderate-to-vigorous physical activity (min=9.86, max=99.6) each day. Bone mineral content in the girls at age 13 averaged 1756.55 grams (min=954.38, max=2841.17).

The interaction between bone mineral content and BMI-z score, menstruation status, and height velocity in our sample of 134 girls is shown in Model 1. BMI z-score at age 11 was a very strong predictor of bone mineral content at age 13 ($p<.0001$), indicating that carrying more body weight is likely to promote bone accretion. Girls whose menstrual cycles had already begun by the time they were 13 also had significantly higher bone mineral content than their peers who had not yet achieved menses ($p=.0062$). This finding suggests that as bone mass accumulates in girls throughout adolescence, pubertal status plays a strong role in determining the timing and magnitude of bone acquisition. Rate of growth between the age of 9 and 11 also predicted bone mineral content at age 13 ($p=.0056$), suggesting that girls who have already experienced their growth spurts have likely also experienced rapid bone mineralization, which generally occurs after a period of rapid height velocity (Ausili et al., 2012). The model that assessed these three predictors accounted for a considerable amount of the variation in bone mineral content at age 13 ($r^2=.4016$) (Table 3: Model 1).

After confirming the findings of previously conducted research, measures related to dietary intake were added to the baseline model. Calcium intake at age 11, measured by a 24-hour recall questionnaire, was assessed in terms of its effect on bone mineral content when tested alongside BMI, menstruation, and height velocity measurements (Table 4: Model 2A). BMI-z-score remained significant in predicting bone mineral content ($p<.0001$), as did the incidence of menstruation by age 13 ($p=.0066$) and height velocity between age 9 and 11 ($p=.0059$). Calcium intake at age 11, however, showed no correlation with bone mineral content at age 13 ($p=.9470$). Adding calcium intake to the baseline model resulted in no change in the baseline R-squared value ($r^2=.4016$). We can confidently conclude that in this particular study, recent 24-hour estimates of calcium intake played no role in bone health, but this finding does not rule out the possibility that a longitudinal study measuring cumulative calcium intake may reveal a relationship between calcium intake over several years and bone mineral content.

Although the data revealed no correlation between 24-hour calcium intake from foods and bone mineral content, assessment of intake of specific foods as opposed to raw nutrient measures may yield a more complete understanding of the role of diet in bone development. Dairy foods in particular contain bioavailable calcium, phosphorus, and Vitamin D, all of which can influence calcium absorption and subsequently bone mineral content (Heaney & Nordin, 2002; Wyshak & Frisch, 1994). Since diets high in nondairy drinks such as sugar-sweetened beverages can lack nutrient-dense milk and other dairy beverages, one might expect to see a trend toward higher bone mineral content among girls who consume more dairy and fewer nondairy beverages during their childhood and adolescence (Fisher et al., 2004). The analysis that focused on beverage intake looks specifically at the average number of servings of dairy beverages as well as the number of nondairy beverages documented by three 24-hour recall questionnaires (Table 4: Model 2B). The sum of the factors in this model increased slightly in contributing to the variation in bone mineral content compared to the baseline model or the model including calcium (r^2=.4024; Δr^2=.0008). BMI-z-score retained its significance (p<.0001), and menstruation at 13 (p=.0062) and rate of growth between age 9 and age 11 (p=.0069) also remained significant predictors of bone mineral content at age 13. In this model, menstruation had a slightly higher level of significance and height velocity a slightly lower level of significance than the p-values in the baseline model indicated. The hypotheses that dairy beverage intake would have a positive association and nondairy beverage intake a negative association with bone mineral content were not supported in this study. Dairy beverage servings (p=.7958) and nondairy beverage servings (p=.8342) at age 11 revealed no significance in their relationship with bone mineral content at age 13.

Although the dietary intake measures of calcium, dairy, and nondairy beverages recorded in this study showed no association with bone mineral content, the influence of parental attitudes toward diet on bone development was assessed to see if the theoretical relationship between maternal milk attitudes and long-term milk intake influenced bone mineral content in the girls in this study. We removed both intake measures from the model and replaced them with maternal milk attitude scores (Table 4: Model 2C). Higher scores indicated more positive attitudes toward consumption of milk and other dairy products among participants' mothers. This new model, which assessed the role of BMI, height velocity, menstruation, and maternal milk attitudes in affecting bone mineral content, explained more of the variation in bone mineral content than the baseline model did (r^2=.4256; Δr^2=.024). BMI-z-score remained significant (p<.0001) in predicting bone mineral content. The relationship between height velocity between age 9 and 11 and bone mineral content at age 13 actually increased in significance (p<.0001), and the onset of menstruation by age 13 also remained a significant predictor of bone mineral content (p=.0062). More positive maternal attitudes toward milk when the girls were 11 years old also significantly predicted higher bone mineral content at age 13 (p=.0216). This positive association indicates that despite the lack of relationship between short-term dietary intake and bone health, parental attitudes toward intake may influence long-term intake, which may in turn have an effect on bone mineral content.

The third category of potential predictors of bone mineralization involves measures related to energy expenditure. To test the relationship between fitness and bone mineral content, a continuous measure of physical fitness at age 11 was added the baseline model of BMI, menstruation, and height velocity (Table 5: Model 3A). This fitness measure ranked participants' fitness levels based by the number of lines run by each in a shuttle run. This model accounted for more of the variation in bone mineral content than did the initial baseline model (r^2=.4467; Δr^2=.0451). BMI z-score at age 11 retained its significance (p<.0001), and onset of menstruation at age 13 had a weaker, albeit still significant, relationship with bone mineral content at age 13 (p=.0149). Height velocity between age 9 and age 11 played an even stronger role in predicting bone mineral content than it did in the baseline model (p=.0026). Physical fitness level was also a significant predictor of bone mineral content at age 13 (p=.0015). This finding was consistent with the hypothesis that girls who are more physically fit will have higher bone mineral content, which is likely a function of more frequent participation in bone-building exercise (Grimston et al., 1993; Lehtonen-Veromaa et al., 2000).

The second measure related to energy expenditure, physical activity level at age 13, was tested in a sample of only 115 of the original 134 participants. The amount of time spent participating in moderate-to-vigorous physical activity was added to the baseline model and tested (Table 5: Model 3B), resulting in a model that accounted for even more variation in bone mineral content at age 13 (r^2=.4292; Δr^2=.0276). This change could be attributed to the smaller sample size, because significance of individual factors was not greatly changed. Once again, BMI z-score remained a significant predictor of bone mineral content (p<.0001) and menstruation at age 13 remained significant (p=.0194). Height velocity between age 9 and 11 was significant in determining bone mineral content (p=.0082) albeit marginally less so than in the baseline model. The average amount of time spent in moderate-to-vigorous physical activity at age 13, however, did not correlate at all with bone mineral content at age 13 (p=.2325).

Discussion

The strong relationship between indicators of growth (BMI, onset of menstruation, and rapid height velocity) and bone mineral content in this study suggests that gains in both height and weight are associated with healthy bone development in girls. Girls who have started their menstrual cycles early are also more likely to have higher bone mineral content, whether because of rapid growth associated with puberty or because of changes in bone accretion-inducing hormones that occur during puberty. The puzzling lack of relationship between dietary calcium and bone mineral content, as well as beverage intake and bone mineral content, could be explained by the limited number of dietary recalls that were collected from participants, the two-year lapse between dietary data collection and bone mineral content measurement, or inaccuracies and bias observed in any measure of self-report. Additionally, calcium supplementation was not included when calculating total dietary calcium intake, and variations in calcium absorption among the participants could have also influenced the degree to which dietary calcium influenced bone mineral deposition. However, the correlation between maternal milk attitudes and daughter bone mineral content may indicate an indirect relationship between milk attitudes and bone accretion that is facilitated by consistently adequate long-term dairy intake. Physical fitness levels among the girls in the study strongly predicted bone mineral content, probably as a function of habitual weight-bearing exercise, supporting the theory of the role of mechanical loading in bone accretion (Grimston et al., 1993; Lehtonen-Veromaa et al., 2000). Physical activity, on the other hand, did not correlate at all with bone mineral content, possibly because the measure of physical activity used in this study did not differentiate between impact-loading activities that have been shown to promote bone accretion and other forms of activity. Additionally, excessively high levels of physical activity may result in a caloric deficit that hinders growth, development, and bone mineral accretion (Swift, Swift, & Bloomfield, 2014; Warren et al., 2002).

Aim 1 Factors

True to our hypothesis, in this study bone mineral content at age 13 was significantly higher among girls with higher BMI at age 11, girls with greater height velocity from age 9 to age 11, and girls whose menstrual cycles had already begun at age 13. These positive relationships indicated that bone mineral content is highest after girls have achieved a certain level of growth and development, highlighting the importance of ensuring a normal growth trajectory and attainment of puberty in a timely fashion. Our findings supported evidence from previous studies that mechanical loading plays an important role in bone mineral deposition, and that a skeleton carrying a higher body mass will be more prone to bone mineral accretion because of the weight it must support (Bakker et al., 2003; Kroke et al., 2000; Rocher et al., 2008).

Hormonal changes appear to amplify the impact of elevated body mass on bone mineral deposition during puberty. Increased bone mineral content after menses may be partially attributable to puberty-associated stimulation of hormones, particularly insulin-like-growth-factor I (IGF-I). IGF-I directly promotes bone mineralization by acting on osteogenic cells that build bone tissue. The increase in bone mass observed in the girls who had already begun to menstruate may be explained by the role that IGF-I plays in increasing intestinal absorption and renal reabsorption of calcium and phosphate in the intestine. Both of these processes make these nutrients available to participate in bone mineralization (Bonjour & Chevalley, 2014).

Height velocity adds another element to the promotion of bone accretion. Studies that demonstrate the succession of rapid growth by rapid bone mineralization support the theory that during peak height velocity, bone area increases faster than bone mineral content, resulting in a temporary low BMD (Ausili et al., 2012; Faulkner et al., 2006). The girls in this study who experienced growth spurts between ages 9 and 11 had surpassed this phase of relative low BMC per bone area and had accumulated more bone mass than their peers who had not experienced significant growth by the age of 13. A probable explanation of this is that the girls who had already completed their growth spurts had also gained weight and started their menstrual cycles. Thus, these three factors are linked and collectively, as well as individually, promote higher bone mineral content in the girls in this study.

Aim 2 Factors

In partial support of our hypothesis, the analyses of dietary factors in predicting bone mineral content at age 13 yielded no significant results for any intake measures (total calcium, dairy and nondairy beverage intake) as assessed by 24-hr recall, but a correlation between positive maternal milk attitudes and higher bone mineral content at age 13 was observed. The lack of relationship between dietary intake and bone mineral content in this study mirrored findings of previous cross-sectional, retrospective, prospective, and randomized controlled studies (Lanou et al., 2005; W. T. Lee et al., 1993; Lehtonen-Veromaa et al., 2000; Matkovic et al., 1990). The null relationship, however, does not necessarily discount calcium's role in bone accretion. Unlike a study by Fiorito and colleagues, calcium from multivitamin-mineral supplements was not included in participants' dietary calcium intake (Fiorito et al., 2006). The insignificant role that dietary calcium appears to play in bone development in this study may be explained by the variation in calcium absorption among individuals due to genetics and other dietary influences. Physiological levels of Vitamin D and intake of other minerals such as phosphorus, magnesium, and potassium, as well as protein status and energy balance, can stimulate bone resorption or modeling and override the effect of total dietary calcium intake (Bonjour & Chevalley, 2014; Ferrari et al., 1998; Weaver, Heaney, Nickel, & Packard, 1997). Vitamin D, protein, and these additional minerals are all found alongside bioavailable calcium in dairy products, but the hypothesis that intake of dairy products is associated with bone mineral content, which has been evident in some randomized controlled trials (Cadogan et al., 1997; Chan et al., 1995) was not supported in this study. Similarly, evidence suggesting that nondairy beverage intake is detrimental to bone development in growing girls (Wyshak, 2000; Wyshak & Frisch, 1994) was not supported in this study, possibly because there was no trend indicating a shift in beverage choices among the participants to support evidence that dairy beverage intake decreases with age as nondairy beverage increases (Fisher et al., 2001). Although dietary recall data at age 11 did not appear to have any impact on bone mineralization two years later, it remains possible that bone mineral content at age 13 might reflect undocumented changes in dietary patterns that could have occurred during the two-year lapse in time between dietary data collection and bone mineral content measurement.

Since dietary data was only collected on three occasions and retrospective methods of data collection have potential for recall error, the role of dietary calcium in bone development may be more significant than results indicate. The theory that milk intake promotes bone health is supported by the significant relationship between maternal attitudes toward milk and daughter bone mineral content, revealing that parents who encourage milk intake have daughters with higher bone mineral content. Positive perceptions of dairy products can create an environment conducive to higher habitual long-term dairy calcium intake, which is a measurement that this study did not assess. A positive relationship between maternal milk attitudes and long-term milk intake would support the theory that girls who regularly drink more milk throughout childhood due to parental encouragement will be more likely to achieve maximal bone mineral development throughout adolescence.

Aim 3 Factors

Our hypothesis was supported in one of the two factors tested in Aim 3. The correlation between fitness level at age 11 and bone mineral content at age 13 supports the theory that physical fitness leads to stronger bones. Girls with higher fitness levels at age 11 had significantly lower BMIs at age 13 (Figure 2). This study supports other evidence that BMI is a strong predictor of bone mineral content (Katzman et al., 1991; Lloyd et al., 1992; Lonzer et al., 1996; Rocher et al., 2008) but despite the negative correlation between BMI and physical fitness, the relationship between fitness at age 11 and bone mineral content at age 13 was significant. Strangely, these two contradictory factors are each independently associated with the outcome of bone mineral content. This counterintuitive relationship suggests that fitness plays such a vital role in bone modeling that fitness level may override the effect of BMI in determining bone mineral content. The strength of the correlation between physical fitness and bone mineral content would suggest a similar relationship between bone mineral content and physical activity, which has been observed in previous research (Dixon, 1992; Grimston et al., 1993; Halioua & Anderson, 1989; Wyshak & Frisch, 1994). In this study, moderate-to-vigorous physical activity at age 13 did not affect bone mineral content. However, it is important to remember that while physical activity is generally the route to physical fitness, other factors such as genetics, growth, and pubertal status can influence fitness level, as well as environmental influences such as altitude, exposure, and overall health status (Chilton, Haas, & Gosselin, 2013; Maridaki, 2006; Scrase et al., 2009).

Limitations and Suggestions for Future Studies

Future studies that examine the influence of growth and pubertal development on bone mineral content might benefit from categorizing participants by body fat percentage as well as BMI to determine whether BMI continues to be a strong predictor of bone mineral content in participants with excess body fat. However, since this particular study had such a low prevalence of obesity among its participants, this anthropometric measure would be unlikely to change the significance in the results collected. Additionally, tracking levels of the hormone insulin-like growth factor 1 (IGF-1) may help to determine whether increases in bone mineral content during puberty are triggered by hormonal changes that occur during menses or whether bone accretion merely coincides with menses as a result of bone modeling that occurs during simultaneous growth and weight gain.

Dietary data collection via 24-hour recall was a reliable form of data collection because the multiple-pass method was used. However, future studies might provide a more accurate estimate of typical dietary intake by assessing intake on more than three days. Creating more opportunities for points of data collection would increase the reliability of the dietary data, and observing dietary patterns more frequently than once every two years could give a more accurate estimation of dietary habits between data collection points.

When it came to physical fitness assessment, this study assessed purely aerobic fitness. Physical fitness assessments that involve qualitative as well as quantitative measures could provide a more comprehensive perspective on categorical fitness measurements including resting heart rate, blood pressure, and specific assessments for strength, flexibility, and aerobic fitness. Physical activity data collection was reliable because it assessed both self-reporting and objective reporting methods, but it was also only measured quantitatively in terms of duration and intensity. Thus, an impact-loading activity that may contribute to bone mineralization (e.g. jumping) (Grimston et al., 1993; Lehtonen-Veromaa et al.,

2000) was recorded indiscriminately from other activities, such as swimming, that involve no stress on the skeleton and do not promote osteoblast activity in the bone (Lee & Lanyon, 2004; Swift et al., 2014). Future studies would benefit from categorizing physical activity measures into impact-load activities and non-impact-load activities to determine which specific activities are the greatest predictors of bone mineralization. Finally, the accelerometers used to measure physical activity levels could not be worn during swimming or bathing. Physical activity from swimming would not be recorded in this study, so an alternative method of recording activity from swimming should be used in future studies.

Translating Findings to Clinical Recommendations

The complexity and the various interpretations of these findings indicate that there is no clear-cut route to promoting optimal bone development in growing girls. Instead, a holistic approach that encourages healthy growth, pubertal development, and weight gain appropriate to the individual's growth is ideal. Lifestyle interventions should promote strength-based physical activity and a healthy, balanced diet that contains nutrients known to play a role in bone mineralization and factors that aid in the body's utilization of these nutrients. An optimal diet includes all food groups and plenty of variety, but dairy products are prime examples of foods that provide an environment in the body that encourages bone growth and overall health (Heaney & Nordin, 2002; Holick & Dawson-Hughes, 2004; Wyshak & Frisch, 1994). As we discovered in this study, mothers with positive attitudes toward milk were more likely to have daughters with higher bone mineral content, suggesting a correlation between positive dairy perceptions, long-term dairy intake, and bone mineral content. Promoting dairy intake throughout the life span may also lead to less frequent consumption of sugar-sweetened beverages and other drinks that offer minimal health benefits, since dairy beverage intake tends to decrease among adolescents whose nondairy beverage intake increases (Bowman, 2002; Guthrie & Morton, 2000; Harnack et al., 1999; Yen & Lin, 2002).

The significance of physical fitness in predicting bone mineral content regardless of BMI supports the promotion of physical fitness among growing girls as an appropriate method to maximize bone development during growth (French et al., 2005; Grimston et al., 1993). It would be logical to assume that the most effective path to physical fitness would involve increased physical activity, and a healthcare provider might recommend physical activity to parents as a way to promote bone building in their daughters. However, excessive physical activity can result in energy deficit that can delay growth and development and trigger unwanted weight loss, putting girls at risk for poor bone development and even early bone loss (Weaver et al., 1999). Healthcare providers should educate parents and their daughters on the benefits of bone-building exercise such as jumping, weight lifting, and running and encourage these exercises in moderation while stressing the importance of maintaining a diet that is healthy, balanced, and adequate (De Craemer et al., 2013; French et al., 2005; Grimston et al., 1993; Lee & Lanyon, 2004). Increases in exercise levels, as well as the type of exercise performed, should be carefully monitored to ensure that the girls maintain growth patterns, BMIs, and pubertal progressions that are healthy and appropriate for their individual needs.

Promoting maintenance of a healthy BMI, not necessarily weight gain, is an appropriate strategy to optimize bone mineral content. Excessive weight gain resulting from poor dietary habits or low physical activity levels can exacerbate fat deposition and even lead to obesity, which can have serious consequences in all aspects of long-term health (Rocher et al., 2008). In fact, weight gain resulting in obesity might even negate BMI’s supporting role in bone development and increase risk for earlier onset of osteoporosis, as some studies have suggested (Weaver et al., 1999). The findings of this study suggest that balance is key in maintaining growth and development patterns, dietary intake, parenting practices, and exercise habits that will support bone growth and maximize a healthy skeleton during adolescence and throughout the life span.

Table 1 – Descriptives: Daughter and Parental Predictors of Daughter BMC

Daughter Predictors at Age 11 (n=134)	Mean ± Standard Deviation
	(Min Value-Max Value)
BMI-for-age z-score	.54±.957
	(-2.06-2.74)
BMI percentile	64.1±.27.2
	(2.0-99.1)
Height velocity (age 9-11) (centimeters)	13.12±2.51
	(8.77-24.43)
Total 24-hour calcium intake (mg/day)	924.21±392.53
	(293.39-2534.1)
Daily servings of dairy beverage	0.85±.889
	(0-6.33)
Daily servings of nondairy beverage	2.2±1.424
	(0-7.41)
Physical fitness (number of lines run in shuttle run test)	17.64±8.27
	(4-48)
Parental Predictors	
Maternal milk attitudes (girl age 11)	6.79±2.38
	(1-10)
Daughter Predictors at Age 13	
Average moderate-to-vigorous physical activity (minutes per day)	36.14±14.41
	(9.86-99.6)
Outcome Measures (Age 13)	
Bone mineral content (g)	1756.55±306.54
	(954.38-2841.17)

Table 2 - Daughter Descriptives

Categorical Predictors (n=134)	**Number of Participants**	**Percent of Sample**
Girls Menstruating by Age 13	93	69.4
Girl BMI Classification Based on BMI percentile (age 11)		
Normal weight	92	68.66
Overweight (BMI>85th percentile)	42	31.34
Obese (BMI>95th percentile)	18	13.43
Physical Fitness		
Participants classified as physically fit (age 11)	97	63.82

Table 3 (Model 1) Baseline Parameters that Predict BMC at Girl Age 13 (n=134)

R-Square		**Pr > F**
0.4016		<.0001
Variable	**Parameter Estimate**	**Pr > F**
	(Standard Error)	
Intercept	1233.22375	<.0001
	(113.04005)	
BMI at age 11*	129.77748	<.0001
	(23.03689)	
Menstruating by age 13*	148.33533	0.0062
	(53.28277)	
Height velocity from age 9-11*	26.52875	0.0056
	(9.41679)	
**Significance indicated by p<.05*		

Table 4 (Model 2) Dietary Parameters that Predict BMC at Girl Age 13

A. Addition of Total Calcium Intake at Age 11 (n=134)		
R-Square	**Change in R-Square from Baseline**	**Pr > F**
0.4016	0	<.0001
Variable	**Parameter Estimate**	**Pr > F**
	(Standard Error)	
Intercept	1229.35	<.0001
	(127.55)	
BMI at age 11*	129.89	<.0001
	(23.18)	
Menstruating by age 13*	148.09	0.0066
	(53.62)	
Height velocity from age 9-11*	26.58	0.0056
	(9.49)	
24-hour recall calcium intake at age 11 (mg)	.00356	0.947
	(.05352)	
B. Addition of Daily Servings of Dairy and Nondairy Beverages at Age 11 (n=134)		
R-Square	**Change in R-Square from Baseline**	**Pr > F**
0.4024	0.008	<.0001
Variable	**Parameter Estimate**	**Pr > F**
	(Standard Error)	
Intercept	1235.27	<.0001
	(122.86)	
BMI at age 11*	128.88	<.0001
	(23.62)	
Menstruating by age 13*	150.46	0.0062
	(54.07)	
Height velocity from age 9-11*	26.15	0.0069
	(9.53)	
Daily servings of dairy beverage	-6.71	0.7958
	(25.87)	
Daily servings of nondairy beverage	3.45	0.8342
	(16.44)	

C. Addition of Maternal Milk Attitudes at Girl Age 11 (n=134)		
R-Square	**Change in R-Square from Baseline**	**Pr > F**
0.4024	0.024	<.0001
Variable	**Parameter Estimate**	**Pr > F**
	(Standard Error)	
Intercept	1235.27	<.0001
	(122.86)	
BMI at age 11*	128.88	<.0001
	(23.62)	
Menstruating by age 13*	150.46	0.0062
	(54.07)	
Height velocity from age 9-11*	26.15	0.0069
	(9.53)	
Maternal milk attitudes at age 11*	20.74	0.0216
	(8.92)	
*Significance indicated by p<.05		

Table 5 (Model 3) Exercise Parameters that Predict BMC at Girl Age 13

A. Addition of Physical Fitness at Age 11 (n=134)		
R-Square	**Change in R-Square from Baseline**	**Pr > F**
0.4467	0.0451	<.0001
Variable	**Parameter Estimate**	**Pr > F**
	(Standard Error)	
Intercept	1047.02	<.0001
	(123.30)	
BMI at age 11*	170.64	<.0001
	(25.56)	
Menstruating by age 13*	127.93	0.0149
	(51.82)	
Height velocity from age 9-11*	27.90	0.0026
	(9.10)	
Number of lines run in a shuttle run assessment (age 11)*	9.08	0.0015
	(2.80)	
B. Addition of Physical Activity at Age 13 (n=115)		
R-Square	**Change in R-Square from Baseline**	**Pr > F**
0.4292	0.0276	<.0001
Variable	**Parameter Estimate**	**Pr > F**
	(Standard Error)	
Intercept	1090.71	<.0001
	(151.08)	
BMI at age 11*	154.02	<.0001
	(25.88)	
Menstruating by age 13*	141.74	0.0194
	(59.73)	
Height velocity from age 9-11*	31.45	0.0082
	(11.68)	
Minutes spent in moderate-to-vigorous physical activity (age 13)	1.93	0.2325
	(1.61)	
**Significance indicated by p<.05*		

Figure 1: Relationship Between BMI at Age 13 and Fitness (Number of Shuttle Lines Run) at Age 11

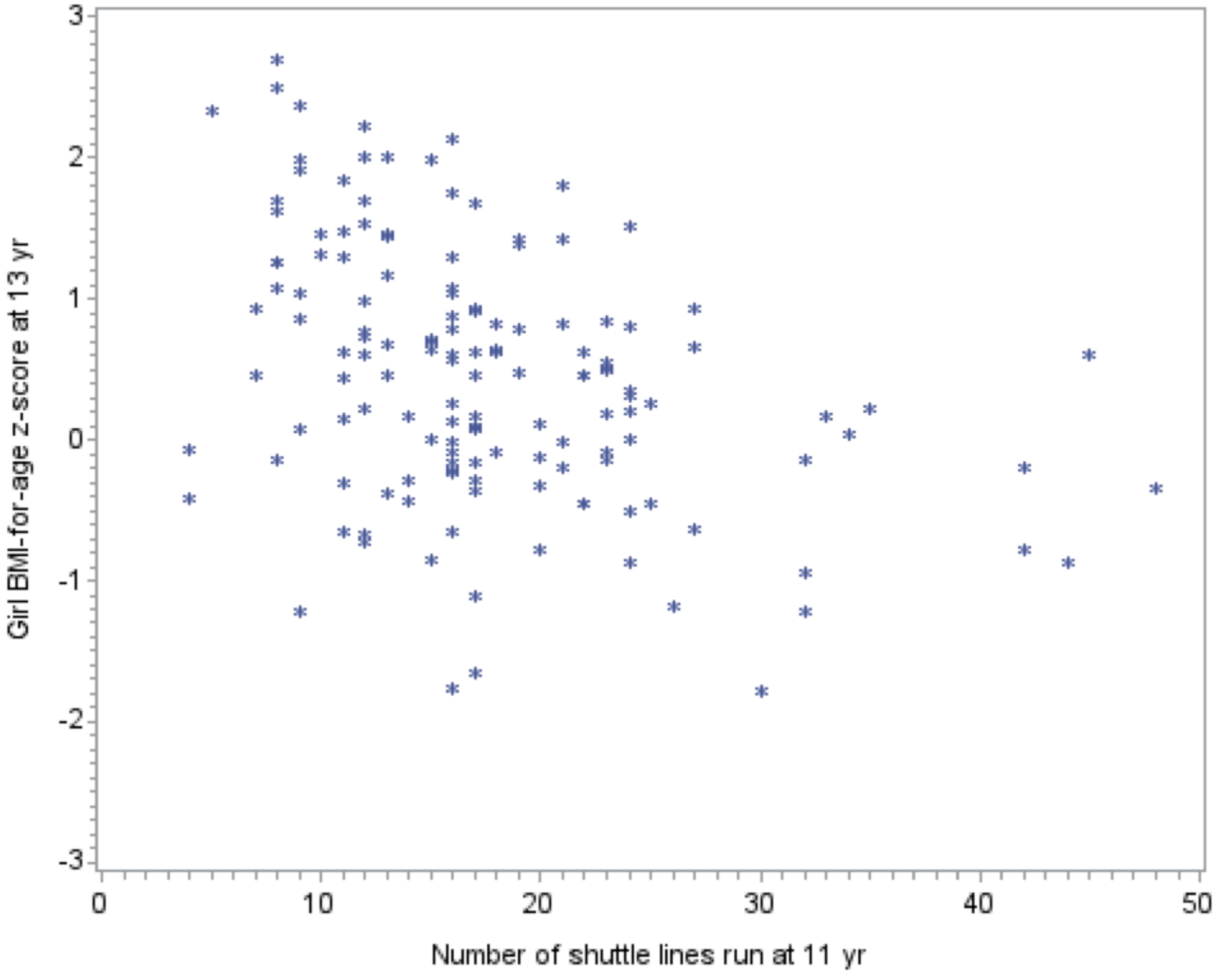


Figure 2: Frequency of 8-Ounce Servings of Dairy Beverage Intake

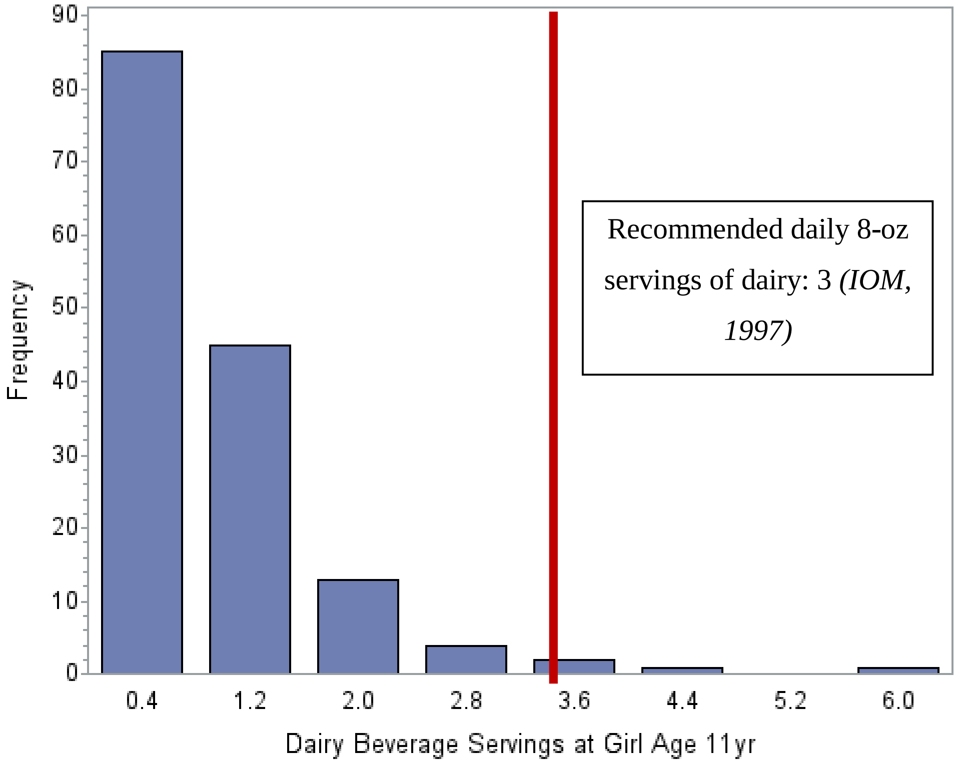


Figure 3: Frequency of 8-Ounce Servings of Non-Dairy Beverage Intake

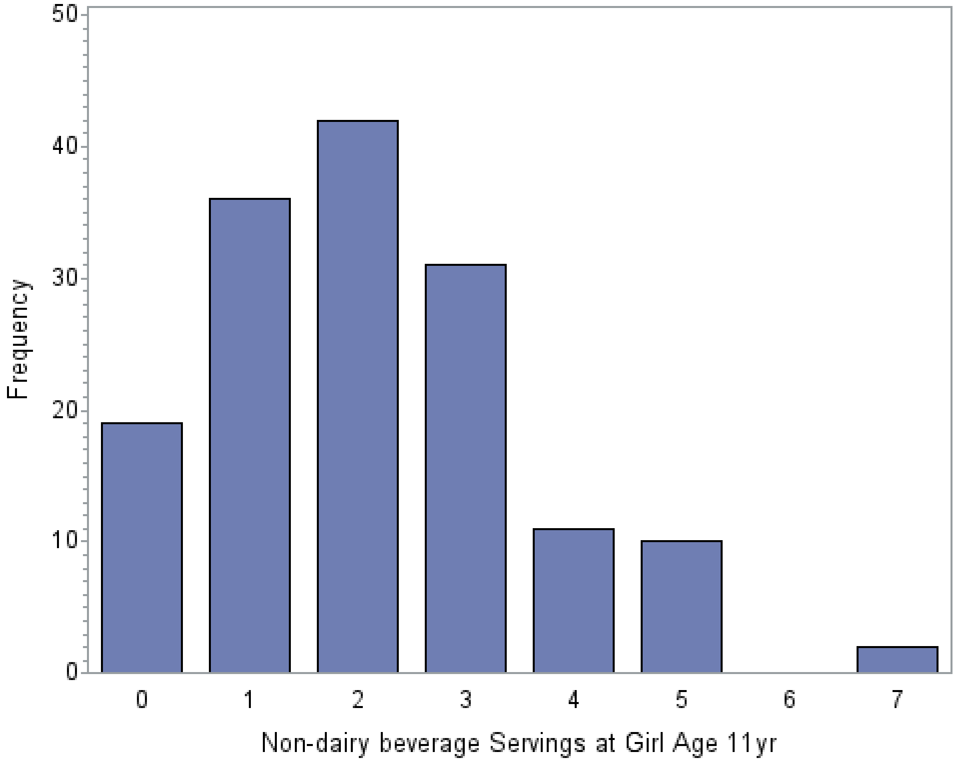
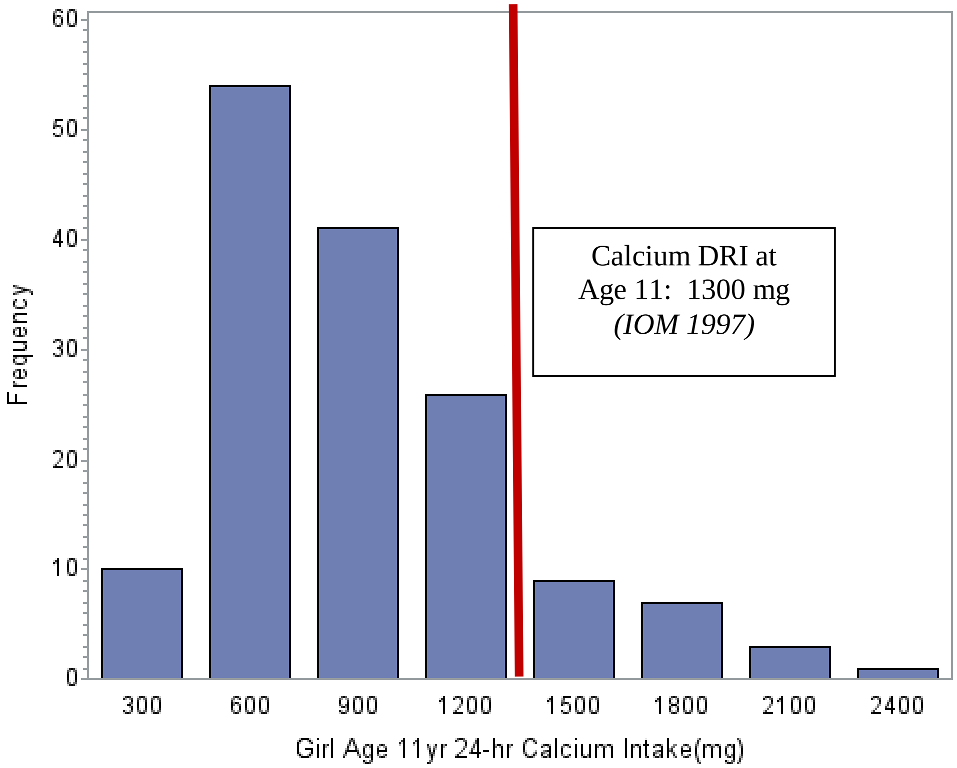


Figure 4: Frequency of Total Calcium Intake at Age 11



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Megan Siverling

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Education

The Pennsylvania State University, *Schreyer Honors College* University Park, PA
Bachelor's in Science: Nutritional Sciences – Dietetics Option Projected Graduation: May 2015

Experience

Mount Nittany Medical Center, State College, PA January 2015-present
Volunteer, Nutrition Department

Geisinger Medical Center GI Nutrition Clinic, Grays Woods, PA May 2014-present

- Shadow a registered dietitian in collecting dietary recall, interpreting symptoms, and educating patients

The Center for Childhood Obesity Research, Penn State University July 2013-present
Research Assistant

- Wrote a thesis on factors affecting bone mineral content in 13-year-old girls
- Assisted in data collection and data entry for a study examining patterns of candy intake in children
- Memorized and followed interview scripts to ensure consistent data collection
- Created lesson plans for overweight pregnant women on eating habits that optimize gestational weight gain

Camp Setebaid at Swatara, Bethel, PA July 2014
Diabetes Camp Counselor

- Managed the health and well-being of children with Type 1 diabetes
- Routinely tested blood sugars and provided treatment for low blood sugar
- Educated and supervised campers in carbohydrate counting and insulin administration

Pregnancy Resource Clinic, State College, PA
Volunteer Parent Educator November 2013-present

- Provide printed materials and give presentations on healthy recipes, prenatal nutrition, breastfeeding, and child feeding

Chester County Food Bank, Downingtown, PA July 2012
Intern

- Assisted in organizing distribution of free lunches to children at a local park
- Coordinated volunteer activities, communicated with food providers

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Activities and Achievements

Penn State University Varsity Swimming 2011-present

- Year-round commitment of twenty or more hours of training per week and frequent travel for competitions

Academic All-American, Academic All-Big Ten, All-American Honorable Mention 2013, 2014
Scholar All-American, College Swimming Coaches Association of America 2013, 2014
Big Ten Sportsmanship Award Winner 2015

Spiritus Leoninus April 2014-present

- Rising seniors honored for excellence in athletics, academics, leadership, and community service

Athletic Director's Leadership Institute August 2013-present

- Participate in activities to personalize, develop, and apply leadership skills in all areas of life

Penn State Christian Athletics 2011-present
Secretary August 2012-present

- Mentor a fellow student, coordinate weekly Bible studies, organize community service activities

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Skills

CPR certified
ServSafe certified
CulinarE Companion Software
Diet Analysis Plus Software
Microsoft Office Suite
EndNote