Increasing evidence suggests that adults and adolescents throughout the United States are at risk for poor vitamin D status, but vitamin D levels of young children in the United States have not been assessed. Serum 25(OH)D was assessed in prepubertal females, aged four to eight years (n=168) living in southeast United States and relationships were examined with bone and body composition. Serum 25(OH)D was assessed using radioimmunoassay (DiaSorin Laboratories), and bone area (BA), bone mineral content (BMC), and areal bone mineral density (aBMD) was measured using dual-energy X-ray absorptiometry (Hologic QDR-1000W). Data were analyzed using ANOVA, ANCOVA, stepwise multiple regression, and partial correlations. Mean serum 25(OH)D was 93.8 nmol/l (SD 28.1, range 31.1-181.4 ). The multiple regression model identified race and season as the strongest predictors of vitamin D status. Black girls had significantly lower mean 25(OH)D values than white girls (p <0.01), and mean values were significantly different between seasons in the total sample (F=11.87, p<0.001), ranging from 74.4 nmol/l in participants tested in the winter months to 107 nmol/l in those tested in the summer. Furthermore, after adjusting for season, age, race, and BMI, 25(OH)D values had no significant associations with bone variables, except for a
negative partial correlation with forearm BMC ($r=-0.182; p=0.021$). In black girls only, age, calcium intake, and household income were significantly associated with 25(OH)D ($r=-0.41, 0.36, 0.31$, respectively). Unlike some prior reports of adults and adolescents living in the southeast United States, vitamin D status was adequate among the prepubertal girls in this study. Moreover, 25(OH)D levels were not positively associated with higher bone mineral in these children. This project was funded by National Institute on Child Health and Human Development grant 1 RO1 HD 35592-01A1.

INDEX WORDS: BONE MINERAL DENSITY, GEORGIA, PREPUBERTAL GIRLS, RACE, SEASON, SERUM 25-HYDROXY VITAMIN D, VITAMIN D
SERUM 25(OH)D CONCENTRATIONS IN GIRLS AGED 4-8 IN THE SOUTHEAST UNITED STATES

by

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B.A., New York University, 2001

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTERS OF SCIENCE

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2005
DEDICATION

This thesis is dedicated to my family for their support, love, and advice (whether I asked for it or not). To my parents, thanks for raising me to love learning and for telling me that I can be anything I want to be when I grow up, even the next Picasso. Maybe you were exaggerating, but it allowed me to dream. To my sister Renee, thank you for imparting to me your personal experience with your own thesis and dissertation and for encouraging me. To my brother Jimmy, thank you for your frequent cell phone calls. Your humor and warmth has always been my favorite study break. And, of course, thank you Dave for being my solace for the past year and a half.
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CHAPTER 1
INTRODUCTION

The hormone action of vitamin D plays an essential role in maintaining skeletal integrity by facilitating calcium absorption in the intestine, thereby sustaining circulating levels of calcium and suppressing parathyroid hormone (PTH). Vitamin D deficiency results in secondary hyperparathyroidism and accelerated calcium resorption from bone, leading to the acute bone disease rickets in children and osteomalacia in adults. Health professionals once characterized vitamin D sufficiency as the mere absence of acute disease, not recognizing the potential health risks of hypovitaminosis D, an insufficient range of 25-hydroxyvitamin D (25[OH]D) concentration just above values considered deficient. In the past two decades, however, researchers have found that adults may require a higher amount of vitamin D for the prevention of the chronic bone disease osteoporosis. Using functional indices such as secondary hyperparathyroidism, calcium absorption, and glucose tolerance, many vitamin D experts estimate that serum 25-hydroxyvitamin D [25(OH)D] ≥ 80 nmol/l indicates vitamin D sufficiency in adults. Due to predominately indoor lifestyles and low dietary vitamin D intake, nearly all American adults have wintertime hypovitaminosis D by this criteria. These reports of poor vitamin D status in adults parallel breaking discoveries of the role of vitamin D in preventing the onset of diseases such as osteoporosis, cancer, multiple sclerosis, diabetes, and schizophrenia. In addition, researchers have recently discovered vitamin D receptors on a multitude of cell types.
In light of these findings, scientists and health professionals no longer view vitamin D as a nutrient necessary to prevent bone loss in older adults; rather, they regard vitamin D as a hormone with multi-faceted functions in human health at all stages of development. Because of the early onset of some of vitamin D-associated diseases, childhood could be an opportune time to optimize vitamin D status and ensure better health across the lifespan. For instance, proper diet and hormonal status could optimize peak bone mass, reducing the potential for osteoporosis in later life. In fact, studies of adolescent girls in Finland are consistent with this belief, reporting that poor vitamin D status correlated with smaller gains in areal bone mineral density (aBMD) and lower measures of volumetric BMD (vBMD).

Despite these recent developments in vitamin D research, insufficiency ranges for children have not been established, and evidence of which 25(OH)D range best suppresses parathyroid hormone (PTH) is conflicting. Most have observed suppression of PTH at 25(OH)D concentrations between 30 and 50 nmol/l in children (aged seven to 10 years) and 40 to 60 nmol/l in adolescents. Although reports of adolescent vitamin D status span the globe, few studies have measured 25(OH)D concentrations in young children. Researchers have found a high prevalence of vitamin D insufficiency in adolescent populations, with the highest risk noted in individuals with dark skin and with less exposure to direct sunlight. These findings heighten concerns that the vitamin D status of younger populations of children could be comparably poor.

Three key studies in Northern Spain, Brazil, and Tasmania have investigated vitamin D status in prepubertal populations. To our knowledge, no studies have assessed vitamin D status in young children in the United States. The purpose of this study was to assess serum 25(OH)D concentrations of four to eight year old prepubertal girls living in northeast Georgia
(34°N), an area in the southeast United States with sunlight available year-round. Secondly, the study aimed to identify predictors of vitamin D status and to investigate associations between 25(OH)D and bone area (BA), bone mineral content (BMC), and aBMD.

REFERENCES


Much history and controversy surrounds the vitamin D research discussed in this review. Until recently the health community maintained the long-standing opinion that the prevention of rickets and osteomalacia indicates sufficient vitamin D status and that ultraviolet (UV) exposure leads people to synthesize adequate levels of the “sunshine vitamin.” Now, researchers are arguing that acute disease states only present the tip of the iceberg, recognizing that preclinical levels of vitamin D deficiency contribute to the development of osteoporosis and that insufficient vitamin D may compromise bone mineral accrual during periods of growth. In addition, discovery of vitamin D-receptors in over thirty different tissues (including brain, lymphocytes, skin, and malignant tissues) and evidence from animal and epidemiologic research suggest that insufficient vitamin D levels cause many other diseases unrelated to bone metabolism. These studies support the idea of a vitamin D cutoff for optimal status as high as 50-80 nmol/l; thus, researchers worldwide have begun gathering data on vitamin D status of different populations. Studies have found a high prevalence of vitamin D insufficiency in adolescents and adults in all locations by these new standards, especially at northern latitudes. Health professionals once assumed that children have sufficient vitamin D status in areas where rickets is rare, but, with new concepts for what constitutes optimal status, children could be at risk for diseases preventable with higher vitamin D concentrations. However, few studies have measured the vitamin D status of children in the United States.

This review discusses recent studies of vitamin D and background information surrounding pediatric bone health. Bone health in children requires knowledge of bone anatomy, growth, and
measurement, while understanding the role of vitamin D in child health calls for knowledge of vitamin D synthesis, dietary sources, and predictors of poor status.

**Bone Health**

Although genetics and body composition determine 80% of the variance in bone mass of healthy individuals, environmental factors have enough influence to increase or decrease osteoporosis risk.⁸ Research has recognized physical activity and diet as the non-genetic variables that play the largest role in achieving optimal bone mass.⁹-¹¹ Of the dietary factors, calcium has been most studied by bone researchers; however, experts have long recognized the need for adequate vitamin D for the intestinal absorption of calcium. Early studies discovered the connection between vitamin D deficiency and the acute bone diseases Rickets and osteomalacia, and more recent studies have investigated the role of vitamin D in the chronic bone disease osteoporosis.¹²-¹⁴ In order to better understand the influence of vitamin D on bone health, scientists have also studied its effects on different types of bone at different skeletal sites.

**Anatomy of Bone**

Bones are living tissues that not only provide the body with mechanical support and functional movement but, also, offer the body a reservoir for calcium and a site for forming red blood cells.¹⁵, ¹⁶ Bone tissue, collagen, and non-collagen protein form a matrix structure, providing a site for mineral deposits.¹⁷ Two types of bone tissue, cortical (compact) and trabecular (cancellous) make up each bone in proportions that differ depending on the bone site (as shown in Figure 1).¹⁸, ¹⁹

Cortical bone accounts for 80% of the skeleton’s total mass and makes up most of the shaft in long bones.¹⁸, ¹⁹ This densely-packed, compact bone made mostly of mineralized collagen provides strength and rigidity to the bone.²⁰ While it has a slow turnover rate of approximately 3% per year, it provides a temporary supply of calcium during times of growth.¹⁶, ²¹
Trabecular bone makes up the ends of long bones and the vertebral core and has a spongy and soft appearance. Unlike the tight layers of cortical bone, trabecular bone forms a cross-linked structure that provides a higher surface area. The increased surface area makes it more suitable for metabolic activity than cortical bone, and, indeed, it’s higher turnover rate of about 26% per year accounts for this. 21, 22

![Figure 2.1. Inner Structure of Bone](image)

**Bone Modeling**

Over a lifetime, bones change in composition, shape, and size. As early as the 5th week of embryonic development, skeletal calcification begins,24 and bone continues the modeling process into young adulthood. Bone modeling involves both formation and resorption, where bone from one area may removed and added to another area.25 In infants and young children, cartilaginous areas make up a large part of the skeleton and act as some of the first sites of resorption. In the process of endochondral ossification, bone replaces the resorbed cartilage, forming osteoid sites of unmineralized bone. During growth, these areas accrue mineral as bones strengthen and mature.26

In order for bones to grow and change shape, bone resorption occurs at select sites, allowing for bone formation at other sites. During bone formation, osteoblast cells differentiate from stem cells and mature into osteocytes. Eventually, osteocytes lose cell organelles and incorporate into the
bone matrix, allowing increased density and mineralization.\textsuperscript{27} Osteoclasts, on the other hand, induce periods of bone resorption by attaching to bone surfaces and secreting digestive enzymes. This causes breakdown of the bone matrix and results in the release of calcium from the skeleton.\textsuperscript{28} Although both formation and resorption occur in healthy bones, growing children must have more formation activity than resorption. In adults, balance of the two states is key in maintaining strong bone strength and preventing loss.\textsuperscript{29}

\textbf{Assessing Bone Health}

In recent vitamin D research, scientists have commonly used two different technologies for bone measurement: dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computer tomography (pQCT). To assess an individual’s bone health inexpensively and non-invasively, scientists and practitioners frequently use DXA. DXA can scan the skeleton in its entirety or at specific sites, measuring bone mineral content (BMC; g). DXA provides a two-dimensional projection of bone mineral density (BMD) by dividing BMC by the site’s area, giving a measurement known as areal BMD (aBMD, g/cm\textsuperscript{2}). In adult studies, researchers usually report aBMD to eliminate the need to correct for body size.\textsuperscript{30-32} However, most pediatric bone researchers agree that prospective measures of aBMD does not appropriately assess bone mineral in children because the growing skeleton increases in size and mass without uniform bone mineralization.\textsuperscript{33-35} In studies of growing children and young adults, scientists often report changes in BMC while adjusting for body size.\textsuperscript{36}

Although many studies in adults have found a strong inverse correlation between aBMD or BMC and fracture rate, bone experts emphasize that aBMD does not independently describe bone health.\textsuperscript{35} Rather, fracture risk of a particular bone depends on the bone’s strength.\textsuperscript{37} The strength of a three-dimensional object, like bone, depends on its mass, composition, and spacial arrangement.\textsuperscript{38}
Thus, a two-dimensional DXA scan can determine the mass and composition properties of strength, but only a volumetric analyzer can assess the true geometric aspect of bone strength. More studies are beginning to measure bone properties by pQCT, an analyzer that measures three-dimensional cross-sections of bone, differentiating between cortical and trabecular bone tissue. The pQCT is of particular interest to pediatric bone researchers because each compartment of a child’s bone grows at different rates. In addition, vitamin D researchers can use pQCT to better understand the effect of vitamin D on bone strength in addition to bone mass.

**Determinants of Bone Development in Young Females**

Studies in adults have found that compromised aBMD, although not an independent measure of bone strength, strongly correlates with fracture incidence and is used to define osteoporosis. Two major factors contribute to the development of osteoporosis in later life: the level of peak bone mass reached in young adulthood and the subsequent loss of bone mass during adult years. Thus, reaching maximal peak bone mass allowed by an individual’s genetics plays a major role in osteoporosis prevention. The level of peak bone mass that an individual achieves is influenced by puberty, diet, physical activity, body composition, and race.

**Puberty**

Bones researchers often describe sexual maturation by stages of puberty defined by Tanner. These stages are represented pictorially for health professionals to use as guides when classifying patients and research participants. Stage I describes prepubertal girls, with the early development of secondary sex characteristics indicating Stage II. In Stage III, secondary sex characteristics continue developing, and height increases at a peak rate of 8 cm/yr. Growth and development decelerate during Stage IV (late-puberty) and terminate at Stage V (post-puberty).
Peak bone mass refers to the time in an individual’s lifespan when BMD reaches its maximum potential. Different sites of the skeleton attain peak bone mass at different points during the lifetime, with the femur reaching its highest level of BMD at around 20 years of age and the spine BMD continuing to increase into a woman’s thirties. Children attain nearly half of adult bone mass before puberty, but the rate of bone accrual and modeling increase during pubertal years due to surges in estrogen and growth hormone. Females attain as much as 86% of BMD before 14 years of age, with the highest rates of mineralization occurring during pubertal stage II and III. Although genetics accounts for 40 to 80% of the variance in aBMD, factors such as calcium intake, hormonal state, and physical activity can affect the amount of peak BMD attained. During pubertal stages II and III, in particular, inactivity and inadequate nutrient intake can reduce bone mineral accrual, with the long-term consequence of increasing the risk of osteoporosis.

**Diet**

Many nutrients affect bone metabolism, but studies have identified calcium along with vitamin D as the most influential. Bone studies of children and adolescents suggest that calcium is a threshold nutrient, meaning that up to the optimal intake level, calcium has a positive effect on measures of BMD and calcium retention, but above this level, the effect of calcium plateaus. In children under nine years of age, calcium has a threshold intake of 800 mg (1200 mg in girls aged nine to 15 years).

Normally, the small intestine absorbs 30% of dietary calcium, increasing to 80% during periods of growth, pregnancy, and lactation. Calcium absorption occurs in the small intestine by both active transport and passive diffusion. Passive diffusion only occurs with high calcium intake, when the proteins of the active transport mechanism become saturated. The biologically active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D3), facilitates active transport by
increasing calcium uptake at the intestinal mucosal cell’s brush border. Without vitamin D, only 10 to 15% of ingested calcium would be absorbed. Thus, individuals with vitamin D insufficiency may suffer the effects of poor bone mineralization, even with a diet adequate in calcium.

**Physical Activity**

Weight bearing physical activity induces mechanical loading and strain on the skeleton, which stimulates bone remodeling and increases the rate of bone mineral accrual. Studies have shown that inactive adults have skeletons favoring bone resorption over formation; however, enough strain on the skeleton maintains a balance of metabolic bone activity, preventing bone mineral loss. In children and adolescents, physical activity can increase the rate of mineralization in the already growing skeleton, resulting in increased peak bone mass relative to inactive children. For example, in studies of adolescent female gymnasts, Laing et al. and Jaffre et al. found that these groups had higher measures of aBMD relative to controls, especially at the lumbar spine. In addition, a six-year longitudinal study of pubertal children demonstrated that participants with the highest level of physical activity showed BMC gains that were 17% higher than the most inactive participants. Bone experts agree that the early pubertal years prior to attainment of peak bone mass provide a window of opportunity for physical activity to enhance the already growing skeleton.

**Body Composition**

Like weight-bearing activity, added body weight places mechanical stress on bone, resulting in increased bone modeling and possibly greater bone strength. Studies of both adults and children support this theory, finding a positive association between body weight and aBMD at the total body
and at regional sites (hip and spine). More recent studies of children and adolescents have proposed that lean body mass, in particular, predicts measures of bone mineral better than fat mass.

**Race**

Studies have consistently shown that blacks have higher aBMD than whites across the lifespan and have lower rates of fracture. Although racial differences in muscle mass and body size account for one-fifth of the variance in aBMD, bone researchers have identified differences in bone metabolism and pubertal hormones as additional contributors. For example, Bryant et al. studied calcium metabolism in black and white adolescent females of similar pubertal status and body composition and found that black females had greater rates of calcium absorption and retention despite lower dietary calcium intake and comparable serum 25(OH)D. The authors concluded that the black children had more efficient calcium metabolism than whites.

**Vitamin D Metabolism**

Vitamin D’s reliance on UV radiation exposure for synthesis and its later conversion into a hormone sets it apart from other vitamins. During periods without adequate exposure to solar UVB radiation, humans must rely on exogenous dietary sources of vitamin D; otherwise, they lack the precursor for 1,25(OH)₂D₃, the steroid hormone made from vitamin D that plays a pivotal role in calcium metabolism and cell development.

**Synthesis in the Skin**

Skin synthesis is achieved through a cholesterol biosynthetic pathway in which the cholesterol precursor to vitamin D, 7-dehydrocholesterol (provitamin D₃) absorbs UV radiation at wavelengths of 290-315 nm. This radiation causes a rearrangement in the molecular structure of provitamin D₃, resulting in the synthesis of vitamin D₃. In a recent study of Caucasian adults, Vieth measured the amount of sun exposure needed for vitamin D synthesis in the skin to reach a plateau.
He found that adults who spent only 15 to 20 minutes in the direct sunlight wearing bathing suits produced the equivalent of 10,000 IU of vitamin D, which is 50 times greater than the recommended adequate intake and five times greater than the level that the Food and Drug Administration considers toxic.

**Dietary Sources**

Dietary and supplemental vitamin D exists in two forms: ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Chemically-equivalent to vitamin D synthesized in the skin, vitamin D₃ is the most biologically active form of dietary vitamin D, and most vitamin D-fortified foods and supplements use form.⁷⁷ In fact, vitamin D₃ is more bioavailable than vitamin D₂, and ingestion of vitamin D₃ results in 70% higher serum 25(OH)D concentration compared with vitamin D₂.⁷⁷

In the United States, vitamin D-fortified milk and juices provide a major source of vitamin D, although the amount reported on the nutritional information label is often unreliable.⁷⁸ One study sampled milk in the United States and Canada, finding that half of the milk sampled did not contain within 50% of the amount reported by the label.⁷⁹ Other sources include fish, fortified cereals, and fortified margarine.¹³ Two glasses of milk provide the recommended adequate intake of vitamin D for children and adults under 50 years of age (200 IU); however, most experts argue that without additional vitamin D from UV radiation, 200 IU of vitamin D does not maintain sufficient levels in serum.⁸⁰

**Metabolism of Vitamin D₃ After Synthesis or Ingestion**

Both dietary and UV-induced forms of vitamin D are mobilized to the liver for hydroxylation into 25-hydroxyvitamin D. From there, 25(OH)D enters the circulation. An additional hydroxylation of vitamin D occurs in the kidney, converting it into the hormone 1,25(OH)₂D₃, the biologically active form of vitamin D.²¹
Assessing Vitamin D Status

Although 1,25(OH)₂D₃ is the most biologically active form of vitamin, its half-life in circulation of only four to six hours makes it a poor marker of vitamin D status. The storage form of vitamin D, 25(OH)D, serves as a more valuable index, with a half life of 10 days to three weeks. The measurement of serum or plasma 25(OH)D combines dietary vitamin D with vitamin D synthesized in the skin, serving as a useful assessment of vitamin D status. Most scientists measure 25(OH)D by radioimmunoassay (RIA) or by competitive binding protein (CBP); however, a study by Lips et al. compared the two methods, finding that CBP estimates 25(OH)D 30% higher than RIA. Because the majority of vitamin D studies used RIA to assess 25(OH)D, this review will note any study that used CBP and give an estimate of what the RIA measure would be.

Vitamin D and Disease

Vitamin D status can be viewed as a continuum, with severe vitamin D deficiency (25(OH)D < 20 nmol/l) causing acute diseases and chronic vitamin D insufficiency (25(OH)D > 20 and less than optimal) contributing to diseases with long-term latency. Young children with severe vitamin
D deficiency suffer from rickets, while adults present with symptoms of osteomalacia. Chronic vitamin D insufficiency leads to osteoporosis in later life and possibly relates to the development of other non-osseous diseases.

**Vitamin D Deficiency and Rickets**

The pediatric bone disease rickets develops in infants and young children when the newly form osteoid fails to mineralize due to hypocalcaemia. This results in bone abnormalities such as bowing of the legs, knock-knees, swelling at the end of long bones, and poor growth. In addition, non-osseous symptoms present in the early stage of rickets due to lack of calcium and include convulsions, tetany, and even cardiac failure.

In nutritional rickets, the etiology of hypocalcaemia begins with vitamin D deficiency. In the 19th century, industrialization combined with unfortified food resulted in vitamin D deficiency and rickets outbreaks. When physicians identified vitamin D deficiency as the cause, cod liver oil (a food rich in vitamin D) was given to infants and young children, and rickets seemed to vanish in most developed countries. However, across the globe, rickets has reemerged in the last decade, with several cases appearing in the southeast United States. Experts suggest multiple causes such as the immigration of dark-skinned people to northern latitudes, sunscreen use and sun avoidance practices, unfortified soy milk, and exclusive breastfeeding by vitamin D deficient mothers.

**Vitamin D Insufficiency and Bone Health**

Even with sufficient intake of calcium, dietary calcium cannot function optimally in bone metabolism without adequate 1,25(OH)_{2}D_{3} aiding its absorption. The body responds to this low vitamin D state by increasing parathyroid hormone (PTH), which then mobilizes the necessary amount of calcium from bone. Therefore, a low level of vitamin D results in secondary hyperparathyroidism (SHPT, defined as PTH > 85 nmol/l), leading to increased bone turnover and a
subsequent decrease in bone mineral accretion. This finding has encouraged scientists to consider SHPT a primary marker of vitamin D deficiency.

**Effect of PTH**

Studies have found an inverse relationship between 25(OH)D and PTH in children, adolescents, and adults of all races and gender. The negative correlations have varied from $r = 0.2$ to $r = 0.3$. Although poor 25(OH)D status inarguably results in SHPT, researchers disagree on the precise concentration where 25(OH)D optimally suppresses PTH. Some studies found that 25(OH)D concentrations up to 100 nmol/l suppresses PTH, while other researchers made more conservative estimates at 25(OH)D concentrations up to 40 nmol/l. These ambiguities, resulting from different measurement techniques and different population samples, fuel the ongoing debate among scientists and nutrition boards over which range of 25(OH)D should be considered insufficient. Studies of PTH in children and adolescents further complicate the debate. For example, Cheng et al. measured 25(OH)D and PTH in 10 to 12 year old girls and found an inverse relationship between the two measures up to the 25(OH)D level of 60 nmol/l, but above 60 nmol/l, this relationship became positive. In addition, Harkness et al. studied the PTH-25(OH)D relationship in American adolescents and noted the same curvilinear relationship turning from negative to positive at the comparable 25(OH)D measure of 90 nmol/l (measured by CBP, approximately 63 nmol/l by RIA). This positive correlation above 90 nmol/l was weak ($\beta = -0.005$) compared to the stronger negative correlation under 90 nmol/l ($\beta = 0.231$).

The level at which 25(OH)D optimally suppresses PTH in different demographics warrants further investigation because studies have shown that even a small rise in PTH can have a negative impact on bone health. Table 2.1 summarizes studies of PTH in young populations. In older adults, increased PTH resulted in increased fracture rates and accelerated bone loss.
subjects with higher PTH showed lower measures of BMD. Other studies of adults with primary hyperparathyroidism reported results that provide a more complex role of PTH in bone by measuring BMD at several sites. These studies found that PTH demonstrated the strongest effect on BMD loss at cortical sites, while sites with predominately cancellous bone were either less affected or preserved. In these studies, PTH caused the most bone loss at the femur rather than the spine, due to the femur’s higher proportion of cortical bone relative to cancellous bone.104, 105

Table 2.1.

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<td>Northern Spain 43°N winter and summer</td>
<td>7-10 yr old white males and females n=43 (summer) n=51 (winter)</td>
<td>Summer 12% &lt; 50 nmol/l (≈ 35 nmol/l by RIA) Winter 80% &lt; 50 nmol/l (≈ 35 nmol/l by RIA) 31% &lt; 30 nmol/l (≈ 20 nmol/l by RIA) 25(OH)D was inversely correlated with PTH.</td>
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<tr>
<td>Gordon et al. (2004)7</td>
<td>Boston, MA 42°N male and female adolescents n=307</td>
<td>42% &lt; 50 nmol/l (≈ 35 nmol/l by RIA) 4.6% &lt; 20 nmol/l (≈ 14 nmol/l by RIA) * 25(OH)D correlated with PTH (r= -0.29) *25(OH)D was 40% lower in blacks than in whites.</td>
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<td>Guillemant et al. (1995)90</td>
<td>Paris, France 49°N spring and summer</td>
<td>13-16 yr old white males n=28</td>
<td>25(OH)D strongly correlated with PTH (r=0.493, p=0.0001).</td>
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<tr>
<td>Harkness et al. (2005)100</td>
<td>Cleveland, Ohio 41°N Year-round</td>
<td>post-menarcheal females aged 12-18 of all races n=400</td>
<td>Mean ± SD: 55.0 nmol/l ± 30.4 (40 ± 20 by RIA) * 25(OH)D inversely correlated with PTH, and this relationship was stronger in whites (r=-0.28) than in blacks (r=-0.19). *This inverse relationship between 25(OH)D and PTH turned positive above 25(OH)D &gt; 90 nmol/l (≈ 63 by RIA)</td>
</tr>
</tbody>
</table>

Although less studied in adolescents and children, some research has found similar adverse effects of PTH on bone mineral accrual in adolescent females.92, 97, 106 In a cross-sectional study of Finnish prepubertal and pubertal females, Cheng et al.92 measured PTH and found no association with two dimensional measures of BMC or aBMD. However, when analyzing volumetric BMD
(vBMD) using pQCT, girls with higher PTH showed lower measures of cortical vBMD. The relation between PTH and cortical vBMD found in this study supports the earlier observation that PTH displays the strongest effect on cortical bone. The effect of elevated PTH in adolescent and prepubertal females warrants further research due to the potential lasting health problems caused by impairing the attainment of peak bone mass.45

**Non-calcemic functions of vitamin D**

Scientists have long-established the importance of 1,25(OH)$_2$D$_3$ in bone metabolism, but they are only beginning to clarify the hormone’s other major biochemical functions. Recent research has identified over 30 different tissues with vitamin D- receptors; in addition, scientists have identified 1,25(OH)$_2$D$_3$-dependent genes revealing 1,25(OH)$_2$D$_3$’s role in gene regulation.3 Observational studies have linked vitamin D status to diseases such as multiple sclerosis, cancer, diabetes, and schizophrenia. Because scientists have no means of reliably predicting these diseases, no research to date has identified vitamin D as a direct cause. Nevertheless, the possibility that poor vitamin D status could be a risk factor for various debilitating and life-threatening diseases suggests a critical need for vitamin D status assessment in multiple populations. The early onset of some of these diseases results in a demand for more vitamin D studies in young populations, in particular.

Research from both animal and human studies investigating the relation between vitamin D deficiency and various diseases demonstrates the implications of vitamin D’s wide-ranging functions. Finnish researchers found that infants supplemented with vitamin D in the first year of life reduced their risk for the later development of schizophrenia and type I diabetes.108, 109 Animal studies strengthen support for these findings, as vitamin D depletion in rats and mice resulted in severely impaired brain development and increased risk for developing diabetes.110, 111
Vitamin D experts link vitamin D’s regulation of cell differentiation and proliferation to diseases such as cancer and diabetes. This cellular involvement has profound implications for cancer research, and, already, vitamin D deficiency has been linked to the development of common cancers such as breast and prostate cancer. Also, vitamin D’s function in cell differentiation and proliferation suggests a crucial need for vitamin D sufficiency during human growth years. The mechanisms behind vitamin D’s non-calcemic functions are not fully understood since most of the studies are observational. More basic science research is needed to clarify vitamin D’s association with various diseases.

**Defining Optimal Vitamin D Status**

The Food and Nutrition Board (FNB) determines categories of vitamin D status by assessing the mean serum 25(OH)D in a group of healthy individuals, with the modification that different status categories must be used for different locales, since 25(OH)D greatly varies depending on UV environment. This method of status determination is based on assumption and may be unreliable, since the subclinical effects of vitamin D insufficiency, such as compromised BMD and increased risk for developing chronic diseases, are not apparent in seemingly healthy individuals. In 1997, the FNB published a report on vitamin D recognizing that data were insufficient to determine a normal range for 25(OH)D. Instead, they promoted the reliability of other status determinants such as elevated serum PTH (indicating an increase in calcium mobilization from bone). This report also states that too few studies exist measuring 25(OH)D and PTH in children past infancy, citing only one study that found secondary hyperparathyroidism (serum iPTH > 85 nmol/l) in eight year olds with 25(OH)D levels < 20 nmol/l. However, this was not enough to establish formal vitamin D status ranges for this age group. In addition, PTH concentration below the level of formal hyperparathyroidism could still have harmful effects on bone health. Since the FNB report was
published, several studies (discussed in Effect of PTH section) have found that 25(OH)D suppresses PTH at 25(OH)D levels up to 40-65 nmol/l, lower than the cutoff found in adult studies (50-80 nmol/l) but much higher than the FNB’s recommended cutoff for normal status (20 nmol/l). In addition to the 25(OH)D-PTH association, other physiological data affect vitamin D experts’ discussion of appropriate vitamin D status ranges.

**Calcium Absorption**

Several recent studies of adults showed that calcium absorption increased with 25(OH)D up to a threshold level of 80 nmol/l, providing further evidence that the cutoff level for optimal vitamin D status should be higher than the laboratory reference ranges for normal (usually characterized as 25(OH)D > 37.5 – 50 nmol/l). This evidence suggests that the currently assumed “normal” range between 50 – 80 nmol/l does not maximize calcium absorption and that a person consuming adequate amounts of calcium with serum 25(OH)D < 80 nmol/l would not maintain calcium balance.

![Figure 2.3. Calcium absorption fraction as a function of serum 25(OH)D concentrations in postmenopausal women (n=34)](image-url)
Risk Factors For Compromised Vitamin D Status

An abundance of literature addresses the relation between poor vitamin D status and possible risk of multiple diseases. Table 2.3 summarizes the results of studies estimating 25(OH)D concentrations in at-risk individuals. These studies, discussed below, showed that environment, skin type, and obesity may compromise vitamin D status in certain populations.

Modern Lifestyle

Because 80-100% of a person’s available vitamin D is derived from cutaneous synthesis, exposure to UV radiation plays a particularly influential role in determining vitamin D status. For example, in a study of veiled Arab women living in Denmark, 96% had vitamin D deficiency. Even people wearing common clothing (i.e., unveiled) ran the risk for compromised cutaneous vitamin D synthesis. In addition, Holick found that sunscreen reduces vitamin D synthesis by 95%. Because of the skin’s reliance on outdoor sun exposure for vitamin D synthesis, changing patterns in modern societies, such as increased sunscreen use and more time spent indoors, place people at risk for vitamin D deficiency regardless of their environment. A study of internal medicine residents demonstrated the effect of an indoor lifestyle on vitamin D status. In this study, Haney et al. measured the residents’ serum 25(OH)D before and after winter, finding hypovitaminosis D (25(OH)D < 50 nmol/l) in 51.4% of the residents at one or both time points. Recent epidemiologic research further supported the speculation that modern lifestyle places people at risk for lower vitamin D status, with evidence of high insufficiency rates in adolescent and adult subjects living in high UV environments. For instance, wintertime data taken in the southeastern United States (latitude range 25- 34.9°N) found hypovitaminosis D (< 50 nmol/l) in 8% of white 12-29 year old females and 53% of black females.
Sullivan et al.\textsuperscript{13} conducted a study in 9-11 year old girls living in Maine and found vitamin D insufficiency (\(<\ 50\ \text{nmol/l}\)) in 48\% of the subjects. Although Maine receives considerably less sunlight than southern regions of the United States, other environmental factors place children living in the southern United States at risk. American children may spend so much time indoors playing video games and watching television that even an outdoor environment with adequate UV light year-round may not protect them from vitamin D deficiency. In addition, fear of skin cancer has resulted in the daily application of sunscreen in some families.\textsuperscript{126-128} Therefore, there is a demand for research investigating vitamin D levels in children living in all climates within the United States.

\textit{Ethnicity}

The hormone melanin can impair cutaneous production of vitamin D because it acts as a natural sun block. For this reason, darker skin pigmentation places some individuals at increased risk for vitamin D deficiency.\textsuperscript{129} Evidence from epidemiological studies supports this concept. For example, Nesby-O’Dell et al.\textsuperscript{120} found that the prevalence of hypovitaminosis D (\(<\ 50\ \text{nmol/l}\)) in the black females was 42\%, compared to a rate of only 4.2\% in white subjects living in the same environment. Insufficiency was found in the black women living in both northern and southern regions of the United States and among subjects consuming the recommended dietary intake of vitamin D. Meulmeester et al.\textsuperscript{99} observed the same trend in dark-skinned immigrant children living in Northern Europe, but few studies have investigated the prevalence of deficiency in dark-skinned children living in the southern United States. The resurgence of rickets appearing in black children living in areas with abundant sunshine suggests that these case studies may only represent the tip of the iceberg, and assessment of vitamin D levels in healthy children of all races may reveal surprisingly high rates of insufficiency.\textsuperscript{86}
The finding that the blacks have low 25(OH)D levels led scientists to investigate the PTH status of these populations. As expected, studies determined that PTH levels in blacks are higher than in whites. However, in blacks, low vitamin D caused a less severe PTH elevation than it did in whites. Despite higher PTH, black populations have lower rates of osteoporosis and fracture than whites. Vitamin D experts attribute this phenomenon, among other physiological variations, to differences in vitamin D metabolism.

This broaches the question of whether blacks may have lower cutoffs of insufficiency than whites. Several bone studies suggest that blacks have lower vitamin D requirements. These studies showed that despite lower 25(OH)D concentrations, blacks had higher concentrations of the vitamin D hormone 1,25(OH)₂D₃ than whites. Thus, blacks may require a lower concentration of sun-synthesized and ingested 25(OH)D to produce the same amount of active vitamin D hormone. Also, the finding that blacks have more efficient calcium absorption and retention suggest that less 1,25(OH)₂D₃ may be required in the first place. Despite these suggestions, experts are hesitant to conclude that blacks require less circulating 25(OH)D due to more efficient bone metabolism. This would be rash given emerging evidence of vitamin D’s role in preventing non-osseous diseases. In a recent review of vitamin D studies in blacks, Dawson-Hughes concluded that many studies support the rationale for setting the optimal 25(OH)D level at > 80 nmol/l in whites; however, not enough evidence exists to apply the same ranges to black individuals.

**Obesity**

As obesity rates in America continue to rise, scientists strive to recognize risk factors and problems associated with high body weight. Recent evidence revealed an inverse relationship between adiposity and vitamin D status. Wortsman et al. suggested that the lower levels of vitamin D in overweight people result from a decrease in bioavailable vitamin D, since fat cells tend
to sequester the fat-soluble vitamin. In addition, Looker et al.\textsuperscript{118} analyzed participants from NHANES III (n=6,402) and found the same association between body-fat percentage and 25(OH)D; however, they concluded the association was stronger in whites than in blacks. In addition, the association was stronger in younger vs. older participants of both races.

Thus far, the adiposity-hypovitamosis D relation has been a focus in primarily adult populations. Because the prevalence of childhood overweight has tripled in the last 20 years,\textsuperscript{133} it is an important research question to investigate how an overweight state may alter the vitamin D status of this growing population of heavy children. Although suspecting under-nutrition in seemingly over-nourished children seems counterintuitive, the biochemistry of vitamin D and evidence from adult studies raise concern for vitamin D insufficiency among America’s overweight youth. Studies of vitamin D status in adolescents and children have shown mixed results regarding obesity and increased risk for poor vitamin D status. Gordon et al.\textsuperscript{7} reported a significant, negative correlation between 25(OH)D and BMI in male and female adolescents, attributing BMI to 1.4\% decrease in 25(OH)D (p=0.03). On the other hand, in a Finnish study with younger participants (10-12 yrs old), BMI did not differ between vitamin D deficient, insufficient, and sufficient groups.\textsuperscript{92}

\textit{Studies Estimating Vitamin D Status}

Northern European, Australian, and Chinese researchers in particular have found children living at high southern or northern latitudes have high rates of vitamin D insufficiency.\textsuperscript{2, 4, 92, 97, 109} In addition, studies conducted in varied North American climates discovered a high prevalence of vitamin D insufficiency in dark-skinned adults, older adults, and obese adults.\textsuperscript{13} While researchers have recognized the risk factors for low vitamin D levels (e.g. environment, race, and obesity) in adults and adolescents, no studies to date have investigated the significance of these predictors in
American children living at lower latitudes. Table 2.2 displays the results of the aforementioned studies.

**Table 2.2.**

<table>
<thead>
<tr>
<th>Author</th>
<th>Location Season</th>
<th>Participants</th>
<th>25(OH)D Status and associated findings</th>
</tr>
</thead>
</table>
| Arya et al. (2004)      | Northern India 37°N | Indian male and female adults n= 92 | 78% < 50 nmol/l  
20% < 12.5 nmol/l |
| Carnevale et al. (2001) | Southern Italy 40°N Winter and summer | white male and female adults n=90 | Males  
winter: 51.2 nmol/l ± 13.2 (mean + SD)  
summer: 97.5 nmol/L ± 21.0  
Females  
winter: 38.0 nmol/l ± 14.2  
summer: 76.7 nmol/l ± 20.0  
* 70% of total sample < 50 nmol/l in winter. |
| Gordon et al. (2004)    | Boston, MA 42°N Fall and spring | male and female adolescents n=307 | 42% < 50 nmol/l (35 nmol/l by RIA)  
4.6% < 20 nmol/l (14 nmol/l by RIA)  
* 25(OH)D correlated with PTH (r=- 0.29)  
*25(OH)D was 40% lower in blacks than in whites. |
| Haney et al. (2005)     | Portland, Oregon 45°N | male and female medical residents n=35 | 26% < 50 nmol/l in fall  
47% < 50 nmol/l in spring |
| Looker et al. (2002)    | Southeast US (winter) and northeast US (summer) Nov-Mar (25-41°N) Apr-Oct (25-47°N) | 12 + females and males of all races from NHANES III n= 18,875 | Lower latitude/winter (mean + SD):  
12-29 yr old white females: 83.4 nmol/l (8% < 50 nmol/l)  
12-29 yr old black females: 50 nmol/l (53% < 50 nmol/l) |
| Looker et al. (2005)    | Southeast US (winter) and northeast US (summer) Nov-Mar (25-41°N) Apr-Oct (25-47°N) | 12 + yr old black and white females from NHANES III n=6402 | *25(OH)D was negatively associated with %body fat, but this association was stronger in whites (β=0.22) than in blacks (β=0.10).  
* This association was also strong among younger participants than older. |
| Meddeb et al. (2005)    | Tunis, Tunisia 36°N Winter | 20-60 yr old male and female adults of Arab and African descent | 47.6% < 37.5 nmol/l  
mean in veiled women: 35.07 nmol/l  
mean in unveiled women: 42.5 nmol/l |
42% < 37.5 nmol/l  
Whites:  
4.2% < 37.5 nmol/l |
**Vitamin D and Bone Health in Children**

In many studies of adult populations, 25(OH)D was positively correlated with aBMD and negatively correlated with fracture rate. However, studies of 25(OH)D and bone health in children have shown conflicting results. Some have found that vitamin D status has no significant effect on measures of bone status, while others have found that children with lower vitamin D status have lower measures of BMD or BMC.\(^{36, 78, 134-137}\) Table 2.3 summarizes studies of vitamin D in pediatric bone. Differences in participant age, study length, bone measurement tool, site of measurement, and statistical methodology seem to play a role in this discrepancy.

**Table 2.3.**

<table>
<thead>
<tr>
<th>Author et al. (Year)</th>
<th>Location and season</th>
<th>Participants</th>
<th>25(OH)D Status and Bone Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng et al. (2003)(^{92})</td>
<td>Finland, 62°N winter</td>
<td>10-12 yr old white females n= 193</td>
<td>78% &lt; 40 nmol/l&lt;br&gt;32% ≥ 25 nmol/l&lt;br&gt;* Girls with 25 nmol/l had significantly lower cortical vBMD of the distal radius and tibia shaft and higher PTH.</td>
</tr>
<tr>
<td>Outila et al. (2001)(^{97})</td>
<td>Helsinki, Finland, 60°N winter</td>
<td>14-16 yr old white females n=178</td>
<td>13.5% &lt; 25 nmol/l&lt;br&gt;No association between 25(OH)D and radial or ulnar aBMD by linear regression. In girls with 25(OH)D &lt; 40 nmol/l, aBMD of the radius (p=0.04) and the ulna (p=0.08) was lower than in girls with 25(OH)D &gt; 40 nmol/l.</td>
</tr>
<tr>
<td>Kristinsson et al. (1998)(^{138})</td>
<td>Reykjavik, Iceland, 64°N winter</td>
<td>16, 18, and 20 yr old white females n=246</td>
<td>18.5% &lt; 25 nmol/l&lt;br&gt;* 25(OH)D was positively correlated with total forearm BMC and BMD (r=0.3 and 0.27, p&lt;0.05)&lt;br&gt;* 25(OH)D was not significantly different in females with lower 25(OH)D at any other sites.</td>
</tr>
<tr>
<td>Lehtonen-Veromaa et al. (2002)(^{2})</td>
<td>Finland, 60°N winter</td>
<td>9-15 yr old white females n=171</td>
<td>34.0 ± 13.2 nmol/l (mean + DS)&lt;br&gt;• Over 3 years, 25(OH)D was positively correlated with aBMD of the lumbar spine and femoral neck (r=0.35 and r=0.32, p=0.001), but only in girls in advanced stages of puberty.</td>
</tr>
</tbody>
</table>

**Role of Participant Age**

A study by Lehtonen-Veromaa et al.\(^{2}\) in Finland best demonstrated the importance of participant age in detecting an effect of vitamin D on bone mass. This three-year prospective study followed nine-15 year old girls (n=171), measuring the difference in aBMD at baseline and after
three years, along with winter 25(OH)D at both time points. After adjusting for pubertal stage, height, weight, calcium intake, and physical activity, the researchers found that aBMD increased most among peripubertal participants in the upper tertile group for 25(OH)D (n=44, mean=45.1 nmol/l) compared with peripubertal participants in the middle tertile (n=38, mean=30.2) or lower tertile (n=46, mean=19.2 nmol/l). In the 42 participants who were prepubertal at baseline, the BMD gains were not significantly different between 25(OH)D tertile. The authors concluded that hypovitaminosis D is more harmful to girls during mid-puberty than girls in early puberty, citing research showing that most BMD gain occurs between pubertal Stages 3 and 4 as described by Tanner.\textsuperscript{49} The higher mean 25(OH)D of the prepubertal subgroup (40.8 nmol/l ± 3.6) relative to the peripubertal girls (30.2 nmol/l ± 2.5) is also of note, as higher serum 25(OH)D may not have the same level of detriment to BMD.

**Cross-Sectional Vs. Longitudinal Studies**

Study length also affects the results of bone mass and 25(OH)D status in children. Because so many variables determine BMD in children, controlling for these variables to isolate the effect of vitamin D complicates vitamin D research. In the absence of a severe deficiency state, genetics, body composition, and physical activity have a stronger effect on skeletal health than vitamin D,\textsuperscript{35, 57} making it difficult to make cross-sectional comparisons between participants. Taken at one time point, the study by Lehtonen-Veromaa et al.\textsuperscript{2} would have shown a weak association between vitamin D status and aBMD. However, because they tested for aBMD changes in each participant using a prospective design, the results showed a stronger effect of vitamin D on aBMD accrual over three years.
**Method of bone assessment**

Determining effect of vitamin D on child bone health also depends on how researchers assess bone in children. A cross-sectional study conducted in Finland by Cheng et al.\(^9^2\) showed the importance of assessment tool by measuring both BMC with DXA and vBMD with pQCT in 10 to 12 year old girls (n=193). To analyze the results, the authors classified the girls into three groups based on 25(OH)D status: deficient (≤ 25 nmol/l), insufficient (26-40 nmol/l), and sufficient (> 40 nmol/l). The study population had an extremely high prevalence of vitamin D deficiency (32%) and insufficiency (46%). After adjusting for pubertal stage (all girls were at stage 1 or 2) and BMI, they found that total femur BMC was actually lower in the vitamin D sufficient group (BMC x=18.9 g ± 0.5) than in the deficient group (x=19.7 g ± 0.4), with femoral BMC being highest in the insufficient group (x=20.4 g ± 0.3). The difference between femoral BMC in the sufficient and the insufficient group reached statistical significance (p=0.04). However, the researchers yielded contradictory results when comparing the groups’ mean vBMD (measured by pQCT). These volumetric measures showed a positive correlation between vitamin D status and cortical vBMD of the distal radius (r= 0.261, p < 0.001). The researchers concluded that vitamin D may have a stronger anabolic effect on cortical bone than trabecular bone, a difference detectable by volumetric measures but not by areal measures of bone.

**Site of Measurement**

Because trabecular bone has more metabolic activity than cortical bone, factors such as diet and physical activity may have a stronger effect on sites containing higher proportions of trabecular bone (i.e. spine, forearm) than sites made of predominately cortical bone (i.e. femoral neck).\(^{22}\) The results of the study by Lehtonen-Veromaa et al.\(^2\) support this, finding significant association between 25(OH)D status and lumbar spine aBMD, but not femoral neck aBMD.
Similarly, Outila et al.\textsuperscript{97} found that 25(OH)D status affected radial aBMD more than ulnar aBMD (the ulna contains less trabecular bone than the radius).

\textbf{Statistical Methodology}

Lastly, the outcome of the studies by Cheng et al.\textsuperscript{92}, Outila et al.\textsuperscript{97}, and Lehtonen-Veromaa et al.\textsuperscript{2} differed depending on the model used. In all three studies, linear regression models often failed to detect an association between bone mineral and 25(OH)D in children. However, when the authors employed logistic regression and compared the participants by 25(OH)D tertile or vitamin D status group, they found significant differences between the groups mean aBMD, BMC, or vBMD.\textsuperscript{2, 92, 97} For instance, Cheng et al. found that the cortical vBMD of the tibia was significantly lower in the 25(OH)D deficient group than in the insufficient group (p=0.002), but linear regression showed no significant association between the two variables. This could be explained by the lower tibia vBMD of the sufficient group relative to the insufficient group. When the middle 25(OH)D status group showed higher BMD than the lower and upper groups, linear regression failed to detect a relationship.

\textbf{Gaps in Vitamin D Research}

Researchers have assessed vitamin D status of adolescent and adult population spanning the globe, reporting vitamin D insufficiency at all latitudes and among various ethnicities, with the highest risk noted in people with dark skin with less exposure to direct sunlight.\textsuperscript{4, 5, 100, 120, 121, 139, 140} Fewer reports of vitamin D status in younger children exist, but the studies conducted in Lebanon, Tasmania, and Europe showed 25(OH)D means comparable to the low values found in older populations.\textsuperscript{55, 99, 107, 141, 142} Still, no studies have investigated vitamin D status in young children in the United States, despite building evidence of a high prevalence of hypovitaminosis D in American adolescents and adults.
Reports of poor vitamin D status in adolescents and adults parallel breaking discoveries of the role of vitamin D in disease prevention. Scientists and health professionals no longer view vitamin D as a nutrient necessary to prevent bone loss in older adults; rather, they regard vitamin D as a hormone with multi-faceted functions in human health at all stages of development. Children require optimal levels of nutrients to ensure healthy tissue growth. Because multiple cell types include vitamin D-receptors, deficiency of the hormone could compromise healthy cell function and impair the health of children. Thus, vitamin D research has a growing demand for reports of status in pediatric populations. This thesis research contributes to this demand by measuring 25(OH)D in four to eight year old girls and determining variables that may increase risk for vitamin D insufficiency. In addition, it investigates associations between 25(OH)D and BA, BMC, and aBMD in order to assess the relations between vitamin D status and bone mass.
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CHAPTER 3

SERUM 25(OH)D CONCENTRATIONS IN GIRLS AGED 4-8 LIVING IN THE
SOUTHEAST UNITED STATES

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ABSTRACT

Increasing evidence suggests that adults and adolescents throughout the United States are at risk for poor vitamin D status, but vitamin D levels of young children in the United States have not been assessed. Serum 25(OH)D was assessed in prepubertal females, aged four to eight years (n=168) living in southeast United States and relationships were examined with bone and body composition. Serum 25(OH)D was assessed using radioimmunoassay (DiaSorin Laboratories), and bone area (BA), bone mineral content (BMC), and areal bone mineral density (aBMD) was measured using dual-energy X-ray absorptiometry (Hologic QDR-1000W). Data were analyzed using ANOVA, ANCOVA, stepwise multiple regression, and partial correlations. Mean serum 25(OH)D was 93.8 nmol/l (SD 28.1, range 31.1-181.4). In multiple regression analysis, race and season were the strongest predictors of vitamin D status. Black girls had significantly lower mean 25(OH)D values than white girls (p <0.01), and mean values were significantly different between seasons in the total sample (F=11.9, p<0.001), ranging from 74.4 nmol/l in participants tested in the winter months to 107 nmol/l in those tested in the summer. Furthermore, after adjusting for season, age, race, and BMI, 25(OH)D values had no significant associations with bone variables, except for a negative partial correlation with forearm BMC (r=-0.18; p=0.02). In black girls only, age, calcium intake, and household income were significantly correlated with 25(OH)D (r=-0.41, 0.36, 0.31, respectively). Unlike some prior reports of adults and adolescents living in the southeast United States, vitamin D status was adequate among the prepubertal girls in this study. Moreover, 25(OH)D levels were not positively associated with higher bone mineral in
these children. This project was funded by National Institute on Child Health and Human Development grant 1 RO1 HD 35592-01A1.

INDEX WORDS: BONE MINERAL DENSITY, GEORGIA, PREPUBERTAL GIRLS, RACE, SEASON, SERUM 25-HYDROXYVITAMIN D, VITAMIN D
INTRODUCTION

Throughout most of the 20th century, scientists in the health field viewed vitamin D as a nutrient with the sole function of preventing acute bone diseases such as rickets in children and osteomalacia in adults. Because ultra-violet band (UVB) light stimulates the skin to synthesize vitamin D, vitamin D deficiency was not considered to be a danger in areas with adequate sunlight where few people developed acute bone diseases (1). In the past two decades, however, researchers have found that adults may require a higher amount of vitamin D for the prevention of the chronic bone disease osteoporosis. In addition, the discovery of vitamin D receptors in a multitude of tissues has led to studies linking vitamin D to other chronic, non-osseous diseases such as cancer, multiple sclerosis, diabetes, and schizophrenia (2, 3). The long-term latency of these diseases complicates the prospect of identifying a definitive optimal range for vitamin D. Using functional indices such as secondary hyperparathyroidism, calcium absorption, and glucose tolerance, many vitamin D experts estimate that serum 25-hydroxyvitamin D [25(OH)D] ≥ 80 nmol/l indicates vitamin D sufficiency in adults (4). Due to predominately indoor lifestyles and low dietary vitamin D intake, over half of American adults have wintertime hypovitaminosis D by this criterion, with older adults and people with dark skin at greatest risk (5). Reflecting these findings, the Dietary Guidelines for Americans (6) now recommend that older adults over age 70 years and persons of dark skin consume 1000 international units (IU), which is a substantial increase from the recommendations put forth by the Food and Nutrition Board in 1997.

Because older adults have decreased capacity for cutaneously-derived 25(OH)D and a demand for optimal calcium absorption to minimize bone loss, much research has investigated vitamin D insufficiency in older adult populations. However, recent research shed light on the
role of vitamin D in preventing diseases that could develop throughout the human lifespan, thereby generating interest in the 25(OH)D status of younger populations (5, 7, 8). For instance, evidence from NHANES III showed that 47% of 12 to 19 year old girls living in sunny locations (mean latitude 32°N) had wintertime vitamin D insufficiency (25(OH)D < 62.5 nmol/l) (5). Moreover, reports that adolescents having dark skin pigmentation or high body mass are at higher risk for hypovitaminosis D raises concern for the vitamin D status of certain youth populations (5, 7, 8). Poor vitamin D status in youth could have consequences that compromise adult bone health (9); in fact, studies of adolescent girls in Finland suggest that lower vitamin D status correlates with smaller gains in areal bone mineral density (aBMD) and lower measures of volumetric BMD (10-12).

Three key studies in Northern Spain, Brazil, and Tasmania have investigated vitamin D status in prepubertal populations (13-15). The researchers reported vitamin D insufficiency (25(OH)D < 50 nmol/l) in 10 to 80% of the children in these populations. To date, no studies have assessed vitamin D status in young children in the United States. The purpose of this study was to assess serum 25(OH)D concentration of four to eight year old prepubertal girls living in northeast Georgia (34°N), an area in the southeast United States with sunlight available year-round. Secondly, the study aimed to identify predictors of vitamin D status and investigate its association with bone area (BA), bone mineral content (BMC), and aBMD.
METHODS

Study Participants

A cross-sectional, retrospective study was conducted to investigate 25(OH)D status in black and white girls aged four to eight years from the Athens, Georgia area (34°N) in the southeast United States. The study used baseline data from the University of Georgia Childhood Bone Study, a prospective study investigating the influence of artistic gymnastic training on bone (16). At baseline, all children had participated in limited or no organized physical activity (< 12 weeks). A physician assessed the pubertal stage of each participant using criteria described by Tanner (17) and determined that all participants were prepubertal (Stage I). Participant race/ethnicity was classified by parent identification of the participant as non-Hispanic white, non-Hispanic black, Hispanic, Asian, Asian-Indian, Native American, or any combination of the above. Because a primary focus of this study was to observe racial differences, participants from less represented ethnic groups (n < 7) were excluded, leaving 120 non-Hispanic white girls and 48 non-Hispanic black girls of those originally recruited (n=203). None of the participants had serious medical conditions or used medication known to alter bone metabolism. Parents indicated the total household income on a questionnaire by checking the appropriate salary range (< $10,000 to > $100,000).

Testing Protocol

The Institutional Review Board for Human Subjects at The University of Georgia approved the study protocol. Data collection took place between October 1997 and October 2000 at the University of Georgia. Prior to participation, each participant and her guardian completed informed assent and consent forms, respectively. After the participants fasted overnight, blood was collected between 0730 and 1000 h. Within one week of the blood draw, the subjects returned to the
laboratory for bone scans, anthropometric measures, and questionnaires assessing demographic information, dietary intake, and physical activity.

**Anthropometry**

For anthropometric measurements, participants wore light clothing and no shoes. Participant height and weight were measured to the nearest 0.1 cm and 0.25 kg using a wall-mounted stadiometer and a calibrated double-beam balance scale (Novel Products, Rockton, IL and Fairbanks Scales, Kansas City, MO, respectively). Body mass index (BMI) values were calculated as weight (kg)/height² (m). To determine BMI percentiles for each child, BMI values were plotted on the BMI-for-ages charts ((18).

**Serum 25(OH)D Measurement**

Serum samples were stored at −70 °C until analysis. Serum 25(OH)D was assayed by radioimmunoassay (RIA) and run in duplicate (DiaSorin Laboratories, Stillwater, MN). The inter-assay and intra-assay coefficients of variation were 9.7 to 10.9% and 4.3 to 8.0%, respectively.

**Bone Mineral Density**

Bone area (cm²), BMC (g), and aBMD (g/cm²) of the total body, lumbar spine, total proximal femur, and non-dominant radius were measured by dual-energy x-ray absorptiometry (DXA; QDR-1000W, Hologic, Inc., Waltham, MA). Lumbar spine was analyzed using DXA Low Density Spine Software Version 4.74, whereas body fat composition was analyzed using DXA Pediatric Whole Body Analysis Software Version 5.73. Daily calibration was performed with a calcium hydroxyapatite and epoxy lumbar spine phantom embedded in a Lucite cube (Hologic c-caliber anthropometric spine phantom, model DPA/QDR-1). The laboratory CV was 0.27% from 365 scans over five years (19). In our laboratory, test-retest measurements using
DXA in 6 to 10 year-old girls demonstrated the following CVs for aBMD: total body, 1.2%; lumbar spine, 1.3%; total proximal femur, 1.6%; and forearm, 2.1%; and percent fat, 2.0%.

**Dietary Intake**

To assess energy (kcal), calcium (mg), and vitamin D (μg) intake, participants and their parents completed three-day diet records, a method found to be valid and reliable for estimating energy and nutrient intakes in children (20-22). To ensure accuracy of the records, a trained lab technician gave parents training with food models and a 24-hour recall questionnaire and provided pictures of serving sizes. Diet records included time of eating, type of food and amount, and preparation method. The form specifically inquired about the consumption of calcium-fortified foods and nutritional supplements, and these values were subsequently integrated into the dietary intake data. A trained lab technician analyzed diet records using Food Processor II (version 7.5, ESHA Research, Salem, OR). Using a one-way random effects model, the intra-class correlation for the average measure of 3-days of dietary intake (n=10) completed twice in a two-week period was calculated to be R=0.47, 0.71, and 0.94 for energy, calcium, and vitamin D, respectively.

**Physical Activity**

Physical activity was assessed using accelerometers that measure frequency and intensity of activity in counts/minute (model 7164; Computer Science Applications [CSA]; Fort Walton Beach, FL, USA). Studies have found a strong correlation between accelerometry measures and energy expenditure in young children (23). Participants wore accelerometers for three days, two weekdays and one weekend day. Accelerometers were worn above the iliac crest of the right hip, a placement considered most effective for accelerometry assessment in children (24). The
CSA monitor recorded data for each participant during one-minute epochs, generating three-day averages of activity in counts per minute.

**Statistical Analyses**

Data were analyzed using Statistical Analysis Software (SAS, Cary, NC, Version 9). Descriptive statistics were run for all variables. For all data, a p-value less than or equal to 0.05 was considered statistically significant. Two-tailed independent t-tests were used to compare means between black and white girls for all independent variables. To analyze differences in 25(OH)D concentrations between races, an ANCOVA test was employed, controlling for age and season (identified as significant by stepwise linear regression). Partial correlation coefficients between 25(OH)D and independent variables were computed by correlation procedures, controlling for necessary covariates. Non-constant variance was observed in BA, BMC, and aBMD variables; thus, these variables were weighted by the inverse of its square.

Because season of testing was not evenly distributed across the total sample, it was considered a potential confounding variable in all statistical models that adjusted for covariates. Means for most variables were significantly different between black and white girls; thus, participant characteristics and mean 25(OH)D concentration by season were displayed separately for blacks and whites. In addition, partial correlation coefficients are reported separately for blacks and whites when significantly different associations were found.
RESULTS

Participant Characteristics

Participant characteristics are presented in Table 3.1. Black and white girls did not differ significantly in age, BMI, or physical activity; however, significant differences were observed in 25(OH)D concentration, parent income, dietary intake, body fat composition, and BA, BMC, and aBMD at all sites (Table 3.1).

Mean serum 25(OH)D concentration in the total sample was 93.8 nmol/l, with the mean being 19% lower in black than in white girls (Table 3.1). None of the participants had a 25(OH)D concentration under the reference point for severe vitamin D deficiency (<27.5 nmol/l). Using 50 nmol/l as the upper limit for vitamin D insufficiency or hypovitaminosis D, few participants tested in winter (n=2) or spring (n=2) and none tested in summer or fall were considered vitamin D insufficient. Mean 25(OH)D of every season was significantly lower in blacks than in whites (Figure 3.1). In addition, mean serum 25(OH)D levels of participants tested in winter was 30.5% lower than those tested in summer, and this significant seasonal difference was observed in both races (Figure 3.1). The multiple regression model identified race and season as significant predictors of vitamin D status.

Mean vitamin D intake, calcium intake, and multivitamin use were higher among white versus black girls (Table 3.1). Mean vitamin D intake was higher in winter versus the summer (10.6 vs. 8.3; p=0.09). Both races had mean dietary vitamin D intakes above the adequate intake (AI) level (5 μg/d, although median intake among black girls (median= 4.0 μg) was lower than the AI. In white girls, median daily intake of vitamin D was above the AI at 11.7 μg. Both mean and median calcium intakes (median= 697 mg/d) in black girls were slightly below the Dietary
Reference Intake (DRI) for calcium (800 mg/d), while mean and median calcium intake (median= 856 mg/day) in white girls were sufficient by this criterion.

**Partial Correlations with 25(OH)D Concentrations**

**Bone Variables**

Serum 25(OH)D concentration was negatively correlated with BA, BMC, and aBMD at all sites (Table 3.2: Model 1). However, after adjusting for season, race, age, and BMI, no significant associations remained except for forearm BMC (p= 0.021; Table 3.2: Model 2). This weak but significant inverse association remained after additional adjustment for BMI, physical activity, and calcium intake (p=0.069; Table 3.2: Model 3).

**Age, Household Income, and Dietary Intake**
After adjusting for season, no significant associations were found between 25(OH)D levels and BMI percentile, body fat percentage, or physical activity in the total sample. Significant associations were found between 25(OH)D and age, household income, dietary vitamin D, and dietary calcium (Table 3.3). Age displayed a weak inverse association with 25(OH)D concentration in the total sample. However, comparing partial correlation coefficients between blacks and whites showed that this association was only significant among black girls (Table 3.3). Moreover, the difference in mean 25(OH)D concentration between black and white girls grew stronger with increasing participant age. Like age, household income had a positive association with 25(OH)D concentration in the total sample and among black participants. No significant association was observed between income and 25(OH)D in white participants. Both vitamin D and calcium intake were positively associated with 25(OH)D concentration in the total sample, but these associations were only statistically significant among black participants (Table 3.3).
**Table 3.1. Participant Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Whites</th>
<th></th>
<th>Blacks</th>
<th></th>
<th>P value for differencea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>6.1 (1.6)</td>
<td>120</td>
<td>6.5 (1.6)</td>
<td>48</td>
<td>0.20</td>
</tr>
<tr>
<td>Parent Income Levelb</td>
<td>4.8 (2.1)</td>
<td>118</td>
<td>3.3 (2.3)</td>
<td>47</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anthropometrics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>16.9 (2.3)</td>
<td>119</td>
<td>17.5 (3.1)</td>
<td>48</td>
<td>0.112</td>
</tr>
<tr>
<td>% body fat</td>
<td>27.2 (7.8)</td>
<td>118</td>
<td>24.3 (9.5)</td>
<td>47</td>
<td>0.042</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td>99.2 (28.2)</td>
<td>120</td>
<td>80.4 (23.1)</td>
<td>48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>aBMD (g/cm²)c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>0.647 (0.051)</td>
<td>118</td>
<td>0.705 (0.076)</td>
<td>47</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>0.534 (0.055)</td>
<td>118</td>
<td>0.583 (0.075)</td>
<td>47</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>0.582 (0.077)</td>
<td>86</td>
<td>0.651 (0.076)</td>
<td>37</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Forearm</td>
<td>0.349 (0.034)</td>
<td>119</td>
<td>0.380 (0.047)</td>
<td>47</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Dietary Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>9.7 (5.7)</td>
<td>114</td>
<td>6.8 (5.5)</td>
<td>42</td>
<td>0.005</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>920 (410)</td>
<td>114</td>
<td>726 (294)</td>
<td>42</td>
<td>0.002</td>
</tr>
<tr>
<td>Multivitamin users</td>
<td>62%</td>
<td>117</td>
<td>32%</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Physical Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(counts/min)</td>
<td>736 (229)</td>
<td>113</td>
<td>704 (235)</td>
<td>44</td>
<td>0.433</td>
</tr>
</tbody>
</table>

Values are means (±SD).

a Tests of significance between racial groups are based on two-tailed independent t tests.

b Ordinal numbers represent range of income in US dollars (0= < 10,000, 1= 10,000-20,000… 10= 100,000 +).

c areal bone mineral density
Figure 3.1. A boxplot graph shows serum 25(OH)D concentrations by race and season of testing. Shaded areas represent values within the 25th to 75th percentile, and lines represent the range.

* Means between season are significantly different for each race, controlling for age (p < 0.05).

b Means between races are significantly different for each season (p < 0.05).

N = 31, 27, 40, and 22 for fall, winter, spring, and summer, respectively.

d N = 5, 7, 22, and 14 for fall, winter, spring, and summer, respectively.
Table 3.2. Partial Correlation Coefficients of serum 25(OH)D concentration with bone area (BA), bone mineral content (BMC), and areal bone mineral density (aBMD) in the total sample

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th>Model 3&lt;sup&gt;c&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>BA (cm&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Body</td>
<td>-0.204</td>
<td>0.009</td>
<td>-0.003</td>
<td>0.969</td>
<td>-0.025</td>
<td>0.773</td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>-0.226</td>
<td>0.004</td>
<td>-0.088</td>
<td>0.271</td>
<td>-0.093</td>
<td>0.277</td>
</tr>
<tr>
<td>Forearm</td>
<td>-0.240</td>
<td>0.002</td>
<td>-0.125</td>
<td>0.114</td>
<td>-0.132</td>
<td>0.120</td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>-0.251</td>
<td>0.005</td>
<td>-0.007</td>
<td>0.943</td>
<td>-0.006</td>
<td>0.949</td>
</tr>
<tr>
<td>BMC (g)&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Body</td>
<td>-0.224</td>
<td>0.004</td>
<td>-0.0245</td>
<td>0.759</td>
<td>-0.019</td>
<td>0.825</td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>-0.235</td>
<td>0.002</td>
<td>-0.031</td>
<td>0.699</td>
<td>-0.024</td>
<td>0.780</td>
</tr>
<tr>
<td>Forearm</td>
<td>-0.263</td>
<td>0.001</td>
<td>-0.182</td>
<td>0.021</td>
<td>-0.154</td>
<td>0.069</td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>-0.249</td>
<td>0.005</td>
<td>0.048</td>
<td>0.605</td>
<td>0.070</td>
<td>0.467</td>
</tr>
<tr>
<td>aBMD (g/cm&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Body</td>
<td>-0.213</td>
<td>0.006</td>
<td>-0.021</td>
<td>0.793</td>
<td>-0.012</td>
<td>0.886</td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>-0.207</td>
<td>0.008</td>
<td>0.005</td>
<td>0.951</td>
<td>-0.001</td>
<td>0.987</td>
</tr>
<tr>
<td>Forearm</td>
<td>-0.218</td>
<td>0.005</td>
<td>-0.087</td>
<td>0.279</td>
<td>-0.035</td>
<td>0.681</td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>-0.170</td>
<td>0.061</td>
<td>0.129</td>
<td>0.166</td>
<td>0.135</td>
<td>0.159</td>
</tr>
</tbody>
</table>

<sup>a</sup>Univariate correlations.

<sup>b</sup>Adjusted for season, race, age, and BMI

<sup>c</sup>Adjusted for season, race, age, BMI, calcium intake, and physical activity

<sup>d</sup>Bone area

<sup>e</sup>Bone mineral content

<sup>f</sup>Areal bone mineral density
Table 3.3. Partial Correlation Coefficients of serum 25(OH)D concentration with multiple variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Sample</th>
<th></th>
<th>Whites</th>
<th></th>
<th>Blacks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.15</td>
<td>0.056</td>
<td>-0.02</td>
<td>0.810</td>
<td>-0.41</td>
<td>0.005</td>
</tr>
<tr>
<td>Household Income</td>
<td>0.25</td>
<td>0.001</td>
<td>0.09</td>
<td>0.323</td>
<td>0.31</td>
<td>0.040</td>
</tr>
<tr>
<td>BMI Percentile</td>
<td>-0.03</td>
<td>0.734</td>
<td>0.03</td>
<td>0.714</td>
<td>-0.06</td>
<td>0.674</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>0.01</td>
<td>0.954</td>
<td>-0.03</td>
<td>0.712</td>
<td>-0.09</td>
<td>0.543</td>
</tr>
<tr>
<td>Vitamin D intake (μg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21</td>
<td>0.010</td>
<td>0.10</td>
<td>0.310</td>
<td>0.27</td>
<td>0.101</td>
</tr>
<tr>
<td>Calcium intake (mg)</td>
<td>0.25</td>
<td>0.002</td>
<td>0.12</td>
<td>0.207</td>
<td>0.36</td>
<td>0.028</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>0.08</td>
<td>0.306</td>
<td>0.05</td>
<td>0.623</td>
<td>0.08</td>
<td>0.610</td>
</tr>
</tbody>
</table>

<sup>a</sup>All partial correlation coefficients adjusted for season.

<sup>b</sup>Vitamin D and calcium intake adjusted for season and kcal intake.
DISCUSSION

This is the first report of vitamin D status in young children living in the southeast Unites States. In this population, no participants were vitamin D deficient by current standards for children (< 27.5 nmol/l) (25); moreover, few girls (n=4) had vitamin D insufficiency (< 50 nmol/L) as defined by a previous study in prepubertal children (15). However, if 80 nmol/l and above is considered to be cutoff for optimal vitamin D status, then 28% of white girls and 52% of black girls would have vitamin D insufficiency. Mean serum serum 25(OH)D values were lower among black girls versus white girls in every season and were lowest in winter and highest in summer in the total population. Furthermore, BA, BMC, and aBMD were not positively associated with 25(OH)D in the total population.

The current study of four to eight year old girls bears similarities to an NHANES III report of vitamin D status in 12 to 29 year old females by Looker et al (5). Although participants were older, researchers tested them at comparable latitudes (25-41°N) in the southeastern United States and measured 25(OH)D by RIA from the same manufacturer used in the current study. In the winter months, mean 25(OH)D values were 42.3 nmol/l and 74.9 nmol/ in black females (n=447) and white females (n=181), respectively. In the current study, the 25(OH)D mean of the white girls (mean=76.7 nmol/l) was similar to the white females in previous report, while black girls had slighter higher 25(OH)D values (mean=65.7 nmol/l) than the black females in the older study population. The NHANES III population had a higher prevalence of vitamin D insufficiency than the current study, with 70% black females and 15% of white females having 25(OH)D concentrations < 50 nmol/L. In contrast, the present study found that none of white girls had serum 25(OH)D values under 50 nmol/l, and only two of seven black girls tested in winter qualified as vitamin D insufficient by this criterion.
The younger age range of our participants may account for the higher serum 25(OH)D concentrations observed in this study. Previous studies that assessed vitamin D status in different age ranges noted lower vitamin D status among the older age groups (5, 26). Age-related decline in cutaneous synthesis of vitamin D may partly account for this, although the age when this decline occurs is unknown (27). In the current study, the negative correlation between age and 25(OH)D may be associated with an increase in time spent indoors between childhood and adolescence, a behavior change supported by longitudinal studies of children reporting an increase in sedentary activity with age, especially among black adolescent females (28, 29). We found a strong negative association between age and 25(OH)D in black girls only. Because dietary vitamin D intake was constant despite participant age, the lower mean 25(OH)D among older black girls suggests that they engaged in more indoor activity than younger black girls. Thus, we speculate that if sun exposure continues to decrease with age in these prepubertal black girls, they have the potential to develop hypovitaminosis D in adolescence and adulthood, consistent with reports in 12 to 29 year old black females (5).

To our knowledge, only three studies have reported the vitamin D status of prepubertal children. These studies, conducted in Brazil (34°S) (13), Spain (43°N) (15), and Tasmania (42°S) (14), provide evidence that girls in the current study have higher serum 25(OH)D than children of similar age living outside the United States. In children living in Brazil—the latitudinal equivalent of our study locale—the wintertime mean 25(OH)D values (mean=53 nmol/l) were 28% lower than wintertime mean values in our population (13). Also, while only 2% of our girls had vitamin D insufficiency, the prevalence of vitamin D insufficiency was 12% in summer and 80% in winter among 7 to 10 year old children in Spain (15) and 10% among children tested in spring and winter in Tasmania (14). Unlike the present study, none of these existing reports
compared mean 25(OH)D between participants of different race or ethnicity. Higher latitudes, clouder weather, or difference in vitamin D-fortification in food may account for the lower mean 25(OH)D values in Spain, Tasmania, and Brazil.

In agreement with earlier studies, we found that season was the strongest predictor of vitamin D status. Despite the availability of year-round sunlight in the southeast United States, mean 25(OH)D concentration fluctuated with season of testing. In girls tested in winter, mean serum 25(OH)D concentration was 31% lower than in summer. Colder winter climates may have reduced outdoor activity, explaining the nadir of mean 25(OH)D concentration during this time. As in other studies, season showed a stronger association with 25(OH)D than did dietary vitamin D intake (8, 11, 30). Even girls with vitamin D intake below the AI for vitamin D did not have significantly lower serum 25(OH)D than girls with vitamin D intake above 5 μg, adding support to the idea that adequate sun exposure ensures optimal vitamin D status regardless of diet (31).

After season, race had the strongest association with vitamin D status, consistent with population studies that found people with more melanin content have lower vitamin D status compared to people with less skin pigmentation (5, 7, 8, 32). In the current study, the significant racial difference in mean 25(OH)D existed throughout all seasons of testing, with the total mean being 19% lower in black versus white girls. Partial correlation coefficients with 25(OH)D also differed between blacks and whites. For instance, 25(OH)D concentration was significantly associated with age, calcium intake, and household income in black girls, but not in white girls. The positive association between household income and 25(OH)D may relate to increased outdoor activity in children living in neighborhoods of higher socioeconomic status (SES). El-Hajj Fuleihan et al. (30) reported similar findings in Lebanon, where children of low SES had
higher risk for poor vitamin D status. The positive association between calcium intake and 25(OH)D concentration in black girls is also consistent with earlier studies in adults and children that found a connection between low calcium intake and poor vitamin D status (33-35). Drinking vitamin D-fortified milk may account for this, a product that would increase the intake of calcium as well as serum 25(OH)D concentrations.

Like earlier cross-sectional studies using two-dimensional measurements of bone mineral, we did not find a positive association between 25(OH)D concentration and bone measures. Furthermore, we found a significant inverse association between 25(OH)D concentration and forearm BMC. Cross-sectional design, lack of volumetric BMD measures, young age and maturational status of the participants, and high calcium intake coupled with sufficient vitamin D status may account for the lack of positive association. For example, Lehtonen-Veromaa et al. (36) found that vitamin D status related to differences in aBMD accrual over three years rather than at one time point, and this effect was only found in girls in pubertal stages 3 to 4 (when the rate of bone growth accelerates). The researchers noted that with only baseline measurements or prepubertal participants, they would have failed to find an association between aBMD and vitamin D status. On the other hand, Cheng et al. (10) used a cross-sectional design to study prepubertal girls and found an association between bone measures and vitamin D status, but only in cortical forearm volumetric BMD, not in aBMD measured by DXA. In fact, they found that vitamin D deficient girls had higher femoral aBMD than vitamin D sufficient girls, even after adjusting for confounding factors. These results demonstrate that bone mineral assessment method can influence the direction of the relation between bone variables and vitamin D, suggesting that vitamin D has an effect on structural properties of bone.
Unlike the Finnish studies, most girls in our sample had what most experts consider optimal vitamin D status, along with high calcium intake. Because vitamin D benefits bone by increasing the absorption of calcium, high calcium intake could mask the effects of suboptimal 25(OH)D concentration on bone health (34, 35, 37). In addition, studies have found a threshold effect of calcium in growing children, with no effect on bone observed beyond this point (38, 39). Because girls in our study had near optimal calcium intakes and enough vitamin D for proper absorption, the threshold effect may account for the lack of association between vitamin D and bone measures. Similarly, other studies failed to find a positive association between BMC and vitamin D, proposing a protective effect from the high calcium intake of the sample (26, 40).

One limitation of this study was that we did not measure parathyroid hormone (PTH). Researchers in the vitamin D field agree that the level at which vitamin D suppresses PTH can be used as a functional index of sufficient vitamin D status (41). Unfortunately, evidence of the threshold for optimal vitamin D status is conflicting, especially in children (7). Previous studies have observed suppression of PTH at 25(OH)D concentrations between 30 and 50 nmol/l in children (aged seven to 10 years) and 40 to 60 nmol/l in adolescents (7, 12, 15, 42). If we extrapolate that 50 nmol/l indicates optimal PTH suppression in this age group, only 2% of our sample was at risk for vitamin D insufficiency.

In this temperate climate, children in the present study seem to have year-round sufficiency of vitamin D, though 25(OH)D means still differed depending on season of testing. Direct measurement of UV exposure may help determine the amount of UV exposure that is needed to ensure optimal vitamin D status in different population groups. The negative association between 25(OH)D and age in black girls may imply future risk of hypovitaminosis D, possibly during the more critical period of bone growth during puberty. Prospective studies are
needed to address the effect of lower vitamin D status on bone mineral accrual in black adolescents.

ACKNOWLEDGEMENTS

This research was supported by the National Institute on Child Health and Human Development grant 1 RO1 HD 35592-01A1. Additional financial support was provided to Elizabeth M. Stein through The University of Georgia College of Family and Consumer Sciences.
REFERENCES


CHAPTER 4
SUMMARY AND CONCLUSIONS

The present study measured vitamin D status in young girls living at a location with sunlight available year-round and identified variables associated with their status. Serum 25(OH)D concentrations were assessed once per participant from blood drawn at different times of year for each participant, and, during the same week, anthropomorphic data, bone measures, dietary assessment, and physical activity data were collected.

The primary finding was that most girls in this population had optimal vitamin D status by currently accepted standards. No participants were vitamin D deficient by standards for children (< 27.5 nmol/l); moreover, few girls (n=4) had vitamin D insufficiency by commonly used adult standards (< 50 nmol/l). However, if 80 nmol/l and above is considered to be cutoff for optimal vitamin D status, then 28% of white girls and 52% of black girls would have vitamin D insufficiency. Mean serum 25(OH)D fluctuated between season, with 25(OH)D means lowest in winter and highest in spring. Black girls had significantly lower 25(OH)D values than white girls. Also, black girls had different variables associated with 25(OH)D concentration, including household income, age, and calcium intake. Furthermore, BA, BMC, and aBMD were not positively associated with 25(OH)D in the total population.

Although the present study is the first to assess vitamin D status in young children in the United States, other studies have measured 25(OH)D in children of similar age in other locations. These studies, conducted in Lebanon, Spain, Brazil, Finland, and Tasmania, reported a higher prevalence of vitamin D insufficiency compared to overall optimal vitamin D status of
the present study population. For instance, in Lebanon (33.5°N), mean 25(OH)D was 55 + 15 nmol/l among 10 to 16 year old females wearing nontraditional clothing (exposed arms and legs), despite blood draw in the spring and fall. In the present study, which took place at a comparable latitude (34°N), the combined spring and fall mean was considerably higher (95.7 ± 27.2 nmol/l). Extreme latitudes may explain the difference between the vitamin D status of our population and studies conducted in Finland and Tasmania, but reasons for the differences observed between our population and populations in Spain, Brazil, and Lebanon are less clear. We speculate that more sun exposure and higher dietary vitamin D intake in our participants results in more optimal vitamin D status than children in other reports.

Season was the strongest predictor of vitamin D status, followed by participant race. Despite the year-round availability of sunlight in northeast Georgia, mean 25(OH)D differed significantly between season of test, with the mean being 31% lower in winter versus summer months. Because dietary vitamin D intake was only weakly correlated with serum 25(OH)D concentration at a level of borderline significance, it is likely that more skin pigmentation of the black girls accounted for their lower vitamin D status, a finding consistent with both experimental studies and population studies of individuals with varying skin pigmentation.

Like earlier studies measuring bone mass by two-dimensional measurements at one time-point, we did not find a positive association between 25(OH)D concentration and bone measures. Furthermore, we found a significant inverse association between 25(OH)D concentration and forearm BMC. High calcium intake coupled with sufficient vitamin D status may account for the lack of positive association. Because vitamin D benefits bone by increasing the absorption of calcium, high calcium intake could mask the effects of suboptimal 25(OH)D concentration on bone health. In addition, studies have found a threshold effect of calcium in
growing children, with no effect on bone observed beyond this point.\textsuperscript{20, 21} Because girls in our study had near optimal calcium intakes and enough vitamin D for proper absorption, the threshold effect may account for the lack of association between vitamin D and bone measures. Similarly, other studies failed to find a positive association between BMC and vitamin D, proposing a protective effect from the high calcium intake of the sample.\textsuperscript{13, 16}

In this temperate climate, children in the present study seem to have year-round sufficiency of vitamin D, though 25(OH)D means still differed depending on season of testing. Direct measurement of UV exposure may help determine the amount of UV exposure that is needed to ensure optimal vitamin D status in different population groups. Serum 25(OH)D was negatively associated with age in black females, implying a future risk of hypovitaminosis D, possibly during the more critical period of bone growth during puberty. We did not observe a positive association between 25(OH)D and BA, BMC, or aBMD; however, we did not measure structural properties of bone, and few of the girls had serum 25(OH)D concentrations low enough to compromise bone health. Prospective studies are needed to determine the effect of poor 25(OH)D status on volumetric bone mineral accrual.
REFERENCES

1. Intakes SCotSEoDR. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride: Food and Nutrition Board, Institute of Medicine, 1997:250-287.


APPENDIX A

Assent and Consent Forms
Assent Form

I ___________________ agree to take part in a study about bone health and growth.

I do not have to be in this study if I do not want to be. I have the right to leave the study at any time without giving any reason, and without penalty.

I will have pictures taken of my bones. During one set of pictures I will lie on a table for approximately one hour. I will take short breaks between the different pictures that are taken. During another set of pictures I will place my arm on a box for about 5 minutes.

I will have a blood sample taken from my arm. I will also have my height measured against a wall and my weight measured on a scale.

I will answer questions about the activities that I participate in, the foods that I eat and how I perceive the shape of my body. Some of the questions may cause me to be uncomfortable. I may skip any question that I do not wish to answer.

I will wear a little pouch during two weekdays and one weekend day. The pouch will measure how much I move around.

My parent and I will write down what I eat during two weekdays and one weekend day.

My answers and any information about me will be kept confidential. This means that the researchers will not use my name. It also means that my responses to questions and any information about me will not be shared with anyone else. If the researchers feel that my health may be in danger, some of my answers will be shared with my parent.

If you have any questions or concerns you can always ask me or call my teacher, Dr. Richard Lewis at the following number: 542-4901.

Sincerely,

Emma Laing, Ph.D.
Department of Foods and Nutrition
University of Georgia
279 Dawson Hall

I understand the project described above. My questions have been answered and I agree to participate in this project. I have received a copy of this form.

____________________________
Signature of the Participant/Date

Please sign both copies, keep one and return one to the researcher.

For additional questions or problems about your rights as a research participant please call or write: Chris A. Joseph, Ph.D., Human Subjects Office, University of Georgia, 606A Boyd Graduate Studies Research Center, Athens, GA 30602-7411; Telephone (706) 542-3199; E-mail Address: IRB@uga.edu
CONSENT FORM (PARENT)

I _______________ agree to give consent for my child, _______________, to participate in the research study titled “Bone Response to Gymnastics Training in Young Girls,” which is being conducted by Dr. Richard D. Lewis and Dr. Emma Laing of the Department of Foods and Nutrition at the University of Georgia. Dr. Lewis and Dr. Laing may be reached in room 279 Dawson Hall at 542-4901 or 542-4918. I understand that the participation of my daughter is completely voluntary. I can withdraw consent at any time without penalty and have the results of the participation, to the extent that which it can be identified as my child’s, removed from the research records, or destroyed.

The following points have been explained to me:
1) The reason for the research is to study the impact of physical activity on bone and growth in children. The benefits that my daughter can expect from participation are the assessment of bone health (bone mineral density), body composition (percentage of body fat and nonfat tissue), diet and growth. In addition, my daughter’s gymnastics or other activity classes will be paid for the duration of her participation in the study (up to $65/quarter during the first two years of the study and $100/year thereafter). Payments will be distributed only if all testing sessions are completed for a given time point. A brief summary of the testing and payment schedule is provided in the table below. If my daughter does not complete a testing session, she will be removed from the study. All measurements are being used for research purposes only, not medical purposes. However, if abnormalities are found in any measure, I will be notified and my daughter will be referred to an appropriate health care professional.

2) The procedures are as follows:
   a) Testing will be conducted at nine different time points (the first five, each 6 months apart: months 0, 6, 12, 18 and 24) and (the last four, each 12 months apart: 36, 48, 60 and 72). At 0, 12, 24, 36, 48, 60 and 72 months three different testing sessions (Session 1, Session 2, Session 3 and Session 4) will be required, whereas, only Session 3 will be required at 6 and 18 months (See table).

<table>
<thead>
<tr>
<th>Time point (months)</th>
<th>Sessions to be Completed</th>
<th>Payment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,2,3,4</td>
<td>Up to $65/quarter</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>Up to $65/quarter</td>
</tr>
<tr>
<td>12</td>
<td>1,2,3,4</td>
<td>Up to $65/quarter</td>
</tr>
<tr>
<td>18</td>
<td>3</td>
<td>Up to $65/quarter</td>
</tr>
<tr>
<td>24</td>
<td>1,2,3,4</td>
<td>$520 minus payments at 0, 6, 12, and 18 mont</td>
</tr>
<tr>
<td>36</td>
<td>1,2,3,4</td>
<td>$100</td>
</tr>
<tr>
<td>48</td>
<td>1,2,3,4</td>
<td>$100</td>
</tr>
<tr>
<td>60</td>
<td>1,2,3,4</td>
<td>$100</td>
</tr>
<tr>
<td>72</td>
<td>1,2,3,4</td>
<td>$100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total = $920</td>
</tr>
</tbody>
</table>

b) My daughter will fast the night before Session 1. On the day of testing for Session 1, my daughter and I will arrive in the Sports Nutrition Lab in Dawson Hall at the scheduled time. Prior to any testing or participation, a consent form will be read to me and an assent form will be read to my daughter. After which, the researcher and I will sign the consent form and the
researcher and my daughter will sign the assent form. During the reading of the consent and assent forms, my daughter and I will be briefed and familiarized with the testing procedures that will be used during the study (15 minutes). My daughter and I will be given the opportunity to reread the consent and assent forms and ask any questions that we may have about the study. Each phase of the study will be explained to my daughter and me throughout testing, and my daughter can withdraw from the study at any time. Prior to any testing, my daughter and I will be walked through all procedures. My daughter, a female chaperone and I will walk to a private room where a Gynecologist/Obstetrician will assess my daughter’s pubic hair and breast development, to determine her level of sexual maturation.

My daughter will be walked to the female restroom and will collect a urine specimen in private. A trained pediatric phlebotomist will then draw approximately 20 mL of blood from my daughter, after which she will be given a snack (15-20 minutes). If a blood sample cannot be obtained after two attempts, no further attempts will be made. A Research Assistant will then familiarize my daughter and me with the use of an accelerometer (an instrument used to assess physical activity) and completion of physical activity diaries. My daughter will wear the accelerometer at each time point for three days. Session 1 will require approximately 90 minutes. Upon completion of Session 1, my daughter and I will be scheduled for Session 2, Session 3 and Session 4.

c) For Session 2, my daughter and I will arrive at University Health Services. To assess bone age, an X-ray of the hand/wrist will be conducted by a trained radiologist (10 minutes including waiting time).

d) For Session 3, my daughter and I will arrive at the Sports Nutrition Lab. I will answer questions regarding demographic information and medical history and my daughter will complete questionnaires dealing with her body shape perception, diet and physical activity (approximately 30 minutes). I understand that while information about diet and eating habits are being obtained, none of the researchers are clinical psychologists and the information alone cannot be used to accurately diagnose an eating disorder. Dr. Patrick O’Connor in the Department of Exercise Science has experience to provide an accurate interpretation of my daughter’s answers on the body image questionnaires. If there are any concerns with my daughter’s responses, I will be informed and my daughter will be referred to a mental health professional in the Athens area.

e) After completion of the questionnaires, my daughter’s height, sitting height, leg length and weight, and my height will be measured. She will also have her bone mineral density and body composition measured using a bone/body composition analyzer (DXA). These measurements will require approximately 60 minutes, which includes a small break in between each scan (four scans total). I understand that a trained laboratory technician or graduate assistant under the supervision of Dr. Richard D. Lewis will conduct all measurements.

3) The discomforts or stresses that may be faced during this research are minor discomfort from blood draws, urine collection and sexual maturation ratings. If undue discomfort or stress occurs, my daughter may decide to discontinue the testing at any time.
4) I understand that the only foreseen risk to my daughter is exposure to a small amount of radiation when assessing body composition and bone mineral density with DXA and bone age with X-rays. The scans will give a total maximum radiation dose of 7.5 mR per testing session. This dose is very small, as radiation doses from a dental bite-wing film are 334 mR, environmental background is 3.5 mR/week, and chest x-ray films are about 25-40 mR for 2 standard films. Thus, the exposure per session is 19-30% of standard chest x-rays. In the event that information from any scan is lost or unusable, no additional scans will be performed.

5) The results of my daughter’s participation will be confidential and will not be released in any identifiable form without my daughter’s prior consent and mine unless required by law. While the body image questionnaires will be administered to my daughter in private, I will be informed if there are any concerns with my daughter’s responses. My signature on this form authorizes the use of my daughter’s data in group analyses, which may be prepared for public dissemination, without breaching my own or my daughter’s confidentiality. To accomplish this, my daughter will be assigned a four digit subject participation code which will be used on all data collected during my participation and my daughter’s participation in this research. A master list with my name, my daughter’s name and corresponding code number will be kept separate from testing data and locked at all times.

6) The investigator will answer any further questions that my daughter or I may have about this research, either now or during the course of the project.

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

Richard Lewis/Emma Laing ___________________________  ___________________________  
Name of Researcher  Signature  Date

Telephone: _542-4901_____   __
Email: _rlewis@fcs.uga.edu____

Name of Parent or Guardian  Signature  Date

Please sign both copies, keep one and return one to the researcher.

Additional questions or problems regarding your child’s rights as a research participant should be addressed to Chris A. Joseph, Ph.D. Human Subjects Office, University of Georgia, 606A Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu
APPENDIX B

24-Hour Recall Questionnaire
# 24 HOUR RECALL

Date of Record ___________________________ Subject Code No. ___________________________

DAY OF WEEK TAKE: M T W TH F S SUN (CIRCLE)

<table>
<thead>
<tr>
<th>Food and Beverage Consumed</th>
<th>WHAT DID YOU EAT?</th>
<th>AMOUNT</th>
<th>COOKING METHOD</th>
<th>TIME OF DAY</th>
<th>ACTIVITY WHILE EATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example</td>
<td>EGGS</td>
<td>2 med.</td>
<td>fried</td>
<td>7:30 a.m.</td>
<td>talking with family</td>
</tr>
</tbody>
</table>

**BREAKFAST**

**SNACK**

**LUNCH**

**SNACK**

**DINNER**

**SNACK**

**ANY OTHER TIME**
APPENDIX C

Three-Day Diet Record
DIRECTIONS FOR KEEPING A 3-DAY DIET DIARY

Please write down everything you eat (meals, snacks, beverages) for three days on these forms. Please select **TWO WEEKDAYS AND ONE WEEKEND DAY.** Use as much space as you need.

5. Write down the date and day at the top of the form.

6. Write down the first foods you ate for that day. Write down:

3) The time of day you ate the food(s).

4) Each food that you ate.

5) How the food was prepared (baked, boiled, fried, microwaved).

6) How much you ate (cup, 1/2 cup, pieces, tablespoons, teaspoons).

7. **It is important to describe each food you eat in detail.**
   
   For example:

   Write down brand names for each food you ate if you know them.

   Write down the type of milk (whole, 2%, or skim) and bread (white, wheat, etc).

   Write down if the food was fresh, frozen, or canned.

   If you ate a casserole or a salad, write down the foods there were in it and amounts.

   If you add things like butter, jelly, sugar, honey, or cream to foods or beverages, please write them down with the amounts used.

4. Do you drink whole ______, 2% ______, 1% ______, or skim ___ milk?

5. Do you use white ______ or whole-wheat ______ bread?

4. What is the complete name and brand name of bread that you eat most often?

   __________________________________________________________________________

5. About how many glasses of water do you drink each day? ________________

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DAY 1 OF THE DIET DIARY

ID: ___________________________ CHECKED BY: ________________________

DATE: ________________________ DAY OF THE WEEK: ________________

Did you drink a calcium-fortified beverage today (e.g. Calcium-fortified orange juice) or eat a calcium-fortified food (e.g. Total breakfast cereal)? Yes No

If yes, list all the calcium-fortified beverages/foods, with the BRAND name, and how much:

Write down everything you eat, beginning with the first thing you have for breakfast. Be sure to include very detailed information such as how the food was prepared, how much you ate, and the brand names.

<table>
<thead>
<tr>
<th>Time Eaten</th>
<th>Foods Eaten</th>
<th>Preparation Methods</th>
<th>Amount (cup, 1/2 cup, piece, etc)</th>
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<tbody>
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</tbody>
</table>
ACTIVITY MONITOR INSTRUCTIONS

✓ Attach monitor to your daughter's waist
✓ Wear for THREE (3) days: 2 weekdays and 1 weekend day
✓ Monitor should be worn at all times, from wake-up time, until bedtime EXCEPT: during baths and/or swimming
✓ Please complete the 3-day period by the date at the top of the page
✓ Record days, dates and time the monitor was worn in the spaces below:

☆ ~AND RETURN THIS SHEET WITH THE MONITOR~ ☆

<table>
<thead>
<tr>
<th>DAY ONE:</th>
<th>day:</th>
<th>date:</th>
<th>time on:</th>
<th>time off:</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAY TWO:</td>
<td>day:</td>
<td>date:</td>
<td>time on:</td>
<td>time off:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAY THREE:</td>
<td>day:</td>
<td>date:</td>
<td>time on:</td>
<td>time off:</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DAY FOUR if applicable):</td>
<td>day:</td>
<td>date:</td>
<td>time on:</td>
<td>time off:</td>
</tr>
</tbody>
</table>

CAUTIONS

✓ Never get the monitor wet
✓ Please check clothing before washing to avoid laundering
✓ Tape is placed around the monitor case so it cannot be opened

If you have questions about the monitor, please call:

Dr. Richard Lewis- (706) 542-4901
Dr. Pat O'Connor- (706) 542-4382
Emma Laing- (706) 542-4918

THANK YOU FOR YOUR PARTICIPATION IN OUR STUDY!
APPENDIX E

Demographic Questionnaire
GENERAL INFORMATION

QUESTIONNAIRE

Subject ID#: __________________________

Interviewer: __________________________

Date of Interview: ______________________

Demographic Data:

I am going to ask you some questions about your age, family, and education. Your mother or father can help you answer.

1. What is your date of birth? Month ________ Day ________ Year ________
2. What is your age? Years ___________ Months __________
3. Gender: (Circle One) Female Male
4. What is your race? (Circle One) Caucasian Black Asian Hispanic American Indian Other __________________________

5. Do you live with your parents? (Circle One) YES NO
   5a. If no, with whom do you live? __________________________

6. Do you have any brothers or sisters? (Circle One) YES NO
   6a. If yes, list ages of: _______ Years (Brother) _______ Years (Sister)
          _______ Years (Brother) _______ Years (Sister)
          _______ Years (Brother) _______ Years (Sister)

   6b. If yes, do they participate in sports? (Circle One) YES NO

   6c. If yes, list the sport and gender of sibling.

          Sport ____________ (Brother or Sister)
          Sport ____________ (Brother or Sister)
          Sport ____________ (Brother or Sister)

7. Do you have a twin brother or sister? (Circle One) YES NO

8. At what age did you start gymnastics? ____________ Years ________ Months

9. Was your mother a gymnast? (Circle One) YES NO
10. What is your parents’ income? (Circle One)  
   Less than $9,999
   $10,000 - $19,999
   $20,000 - $29,999
   $30,000 - $39,999
   $40,000 - $49,999
   $50,000 - $59,999
   $60,000 - $69,999
   $70,000 - $79,999
   $80,000 - $89,999
   $90,000 - $99,999
   More than $100,000

11. What grade are you in school? __________

12. What is your mother’s occupation?__________________________

13. What is your father’s occupation?__________________________
Health Data:

Now, I am going to ask you to respond to a few questions about your health. I am the only one that will know how you answer these questions, so please be honest with your answers.

1. How much do you weigh? ________ Pounds

2. How tall are you? ________ Feet ________ inches

3. Have you gained or lost any weight (≥ 10 pounds) in the past 3 months? (Circle One) YES NO

3a. If yes, how much? +_______ pounds OR -_______ pounds

4. Have you had any height changes in the past 3 months? (Circle One) YES NO

4a. If yes, how much? ________ feet ________ inches

5. How much would you like to weigh? ________ pounds

6. How tall would you like to be? ________ Feet ________ inches

7. How would you rate your present health? (Circle One)

   Poor  Fair  Good  Excellent

8. Do you have any diseases or illnesses? (Circle One) YES NO

   10a. If yes, what diseases? ________________

9. Are you taking any medications either prescribed by a doctor or over-the-counter (self-prescribed)? (Circle One) YES NO

   9a. If yes, what medications? ________ Amount per day ________

   ________ Amount per day ________

   ________ Amount per day ________

Those were some difficult questions to answer because the questions were so private. I want to assure you again that I am the only person who knows how you answered these questions. Thank you for being so honest with your answers.
Nutrition Data:
These next questions are about your diet and eating habits. Try to think about how you eat.

1. Do you eat three meals per day? (Circle One) YES NO
   1a. If no, why not? ________________________________

2. Do you eat snacks during the day? (Circle One) YES NO
   2a. If yes, how many snacks per day do you eat? _______ snacks per day

3. Are you following a special kind of diet? (Circle One) YES NO
   3a. If yes, what kind of diet? ________________________________

4. Do you take any vitamin or mineral supplements or any “nutrition pills”? (Circle One) YES NO
   4a. If yes, what kind? ______________________ Amount per day ______________________
       ______________________ Amount per day __________
       ______________________ Amount per day __________

5. Have you ever been on a diet to lose or gain weight? (Circle One) YES NO
   5a. If yes, what kind of a diet was it? ________________________________
   5b. How old were you when you were on this diet?
       ___________ years _______ months

Thank you for answering all of those questions. You did really well, and I appreciate your being so truthful with your answers. Next, I am going to ask you about your physical activity during the past 7 days. Try to think back on last week and the activities that you may have done.
Physical Activity:

The next questions that I will ask you are about your physical activity such as P.E and exercise outside of school. There are no right or wrong answers, so please answer these questions the best that you can.

1. How would you rate your physical activity level? (Circle One)
   - Inactive
   - Average
   - Very high
   - Below average
   - Above average

2. Do you have any health problems that limit your activity? (Circle One)
   - YES
   - NO

   2a. If yes, what health problem? __________________________

3. Do you exercise regularly (not including P.E. class)? (Circle One)
   - YES
   - NO

   3a. If yes, how often? ____________ hours per day/week/month

4. Do you participate in P.E. at school? (Circle One)
   - YES
   - NO

   4a. If yes, how often? ____________ hours per day/week/month

5. Do you play games during recess? (Circle One)
   - YES
   - NO

   5a. If yes, what games or activities do you play?

6. Do you play games/sports after school? (Circle One)
   - YES
   - NO

   6a. If yes, what games or activities do you play?

   6b. If yes, how many hours/minutes do you play after school? _______
Bone Health Data:
The next questions have to do with your bones and your family’s bones.
1. Does anyone in your family (including your parent’s, grandparents, aunts, uncles, cousins) have osteoporosis or “humpback”? (Circle One) YES NO
   1a. If yes, who is it? _____________________________
2. Has anyone in your family (including your parents, grandparents, aunts, uncles, cousins) had a hip or wrist fracture? (Circle One) YES NO
   2a. If yes, who is it? _____________________________
3. Have you ever had a bone fracture or broken bone? (Circle One) YES NO
   3a. If yes, what bone(s)? _____________________________
   3b. If yes, how old were you? _________years _________months
4. Have you ever been told by a doctor that you have bone disease?
   (Circle One) YES NO
   4a. If yes, what disease? _____________________________
   4b. If yes, how old were you? _________years _________months
APPENDIX F

Tanner Stages of Sexual Maturation
APPENDIX G

Anthropometric Data Recording Sheet
ANTHROPOMETRIC DATA SHEET

ID NUMBER: ____________trial____

HEIGHT _______________ (TO NEAREST 0.1 CM)
WEIGHT _______________ (TO NEAREST 0.25 KG)
LEG LENGTH ______________ (TO NEAREST 0.1 CM)
SITTING HEIGHT _______________ (TO NEAREST 0.1 CM)
MOM’S HEIGHT _______________ (TO NEAREST 0.1 CM)
    SELF-REPORT? YES    NO
DAD’S HEIGHT _______________ (TO NEAREST 0.1 CM)
    SELF-REPORT? YES    NO
LENGTH OF RADIUS IN CENTIMETERS _____________  R or L?

______________________________

TO BE COMPLETED BY INVESTIGATOR:

GROWTH VELOCITY

    HEIGHT _________________
    LEG LENGTH _______________
    SITTING HEIGHT ____________
% HEIGHT FOR AGE _____________
% WEIGHT FOR AGE _____________
% WEIGHT FOR HEIGHT ___________
BMI_____________________________
APPENDIX H

Growth and Body Mass Index Chart