25-HYDROXYVITAMIN D, INSULIN-LIKE GROWTH FACTOR-I AND BONE MINERAL ACCRUAL DURING GROWTH

by

MARIA ELIZABETH BREEN

(Under the Direction of Richard D. Lewis)

ABSTRACT

This project sought to determine relationships among changes in plasma 25(OH)D, plasma IGF-I, and BMC in prepubertal females (N = 76; aged 4 to 8 years), over a period of up to 9 years. BMC was measured using DXA. Plasma 25(OH)D and plasma IGF-I were assessed using ELISA and RIA, respectively. Linear mixed modeling that was employed to analyze the proportion of variance each explained on the four bone outcomes. IGF-I was more strongly associated with BMC accrual than 25(OH)D at the total proximal femur ($R^2 = 0.847$ vs. 0.771), radius ($R^2 = 0.812$ vs. 0.759), and lumbar spine ($R^2 = 0.759$ vs. 0.698). At each skeletal site, the rate of BMC accrual was negatively associated with changes in 25(OH)D, but positively associated with changes in IGF-I. These longitudinal data in early adolescent females indicate that both 25(OH)D and IGF-I have a significant impact on bone mineral accrual.

INDEX WORDS: Vitamin D, Insulin-like growth factor-I, Pediatrics, Bone mineral accrual

INSULIN-LIKE GROWTH FACTOR-I, 25-HYDROXYVITAMIN D, AND BONE MINERAL ACCRUAL DURING GROWTH IN FEMALES

by

MARIA ELIZABETH BREEN

B.S.F.C.S., The University of Georgia, 2007

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

MASTER OF SCIENCE

ATHENS, GEORGIA

2009

INSULIN-LIKE GROWTH FACTOR-I, 25-HYDROXYVITAMIN D, AND BONE MINERAL ACCRUAL DURING GROWTH IN FEMALES

by

MARIA ELIZABETH BREEN

Major Professor: Richard D. Lewis

Committee: Clifton A. Baile
Dorothy B. Hausman

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2009

DEDICATION

This thesis is dedicated to my parents, John and Lorraine Breen, for their unwavering support and unconditional love. Your passion for furthering your knowledge has been my main source of inspiration throughout all of my educational endeavors.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the entire Lewis Labs for their guidance, support, and assistance throughout my graduate studies. I do not even know how to begin to thank Dr. Lewis, he has been such a wonderful mentor and advisor. I am continuously awed and inspired by his intelligence, and his ability to teach and pass along his knowledge to others is unparalleled. I feel so blessed to have had Emma supporting; not only is she an incredibly nice and compassionate person, she so scholarly, intelligent, and a master of all things Microsoft office related. Norman, thank you for always being there to help with anything I needed, and I absolutely love all the lectures on life lessons that I have yet to discover. Ruth, it was so nice have someone to talk to that completed a thesis and encouraged me in the most stressful times. To my fellow graduate students in the lab, Ashley, Claire, and Katherine, I truly enjoyed working with you everyday. The friendship and laughter you all provided was my therapy over these past two years. I have made so many wonderful memories in the years I spent as a member of this lab that I will hold with me for the rest of my life. Go Team Lewis!!!

To my family and friends, I love you all more than these words can express. You have supported me in everything I have ever sought to accomplish, and this thesis is no exception. My mother and father's knowledge and passion for academics has been a constant source of inspiration. They have supported my academics from the first day of kindergarten throughout graduate school. They always knew just what to say to reassure me or they simply allowed me to vent my fears, frustrations, and stresses. You both have held my hand, and I know I would not be where I am today without you. I can honestly say you have been perfect, and I cannot think of

one thing I could have wished to be different. I also thank my sister and closest friend, Emily. Your gracious willingness to listen to me talk about my thesis when your passion is far removed from the scientific realm did not go unnoticed, and was greatly appreciated. I could not have asked for better roommates supporting me and helping me. Emily, I loved every lunch and dinner you made me. Rachel, surprising me by cleaning my room and folding my clothes was truly above and beyond and wonderful. All three of my roommates were a source of entertainment during times of stress including Samira's singing of songs and witty puns, Rachel's outrageous gifts, the Christy Lane 1980s VHS instructional dance video performances in our living room, and the humorous acknowledgement that my devotion of to my studies lead to an unfortunate downward turn in appearance. To my friends, Olivia and Randi, while you were not in Athens, you always made sure to check in and encourage me, and it meant so much to know you thought of me and supported me.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Osteoporosis is a degenerative disease characterized by low bone mass and structural deterioration of bone tissue leading to fragile bones that are vulnerable to fracture. Healthy People 2010 identified osteoporosis as a public health focal area in the United States.[1] An estimated 10 million Americans are diagnosed with osteoporosis, and an additional 34 million individuals have low bone mass placing them at an increased risk of developing the disease.[2] Together these individuals comprise 55% of the population aged 50 years and older. One in two women and one in four men over the age of 50 years will suffer an osteoporosis-related fracture. In 2005, the projected healthcare expenditure associated with osteoporosis related fractures was 19 billion dollars. These costs are expected to rise to 25.3 billion dollars by year 2025.[2]

One of the aims of osteoporosis prevention is to optimize bone strength during childhood.[3] The majority of bone acquisition occurs between 10 to 18 years. At least 90% of peak bone mass (PBM) is acquired by age 18.[4] Peak bone mass and the subsequent loss of bone are contingent on the interaction between genetic, hormone, nutrition, and environmental factors, yet it remains unclear to what extent each of these key factors modulates bone growth.[5] Much of the research in children and adolescents has focused on exogenous lifestyle and environmental factors governing bone mass accrual. In order to efficaciously address osteoporosis prevention efforts, it is necessary

to better delineate the endocrine factors that influence bone mineral accrual during growth.

Recently, there has been heightened interest in vitamin D and the active role it plays in bone metabolism. An increasing number of older adults are at increased risk for vitamin D deficiency. In adults, poor vitamin D status results in decreased calcium absorption and elevated levels of parathyroid (PTH) hormone and is related to low bone mass and increased risk of fracture.[6,7] While the relationships between serum 25(OH)D and bone in adults are more clearly documented, major gaps still exist with regard to our understanding of the vitamin D status of growing children.

Few studies have been conducted in children to examine vitamin D status and bone mineral accrual over time. In a 12-month double blind, placebo-controlled vitamin D supplementation trial in 10 to 17 year-old Lebanese females conducted by El-Hajj Fuleihan et al[8] significant negative correlations between baseline 25(OH)D and the 1-year percent change in spine bone mineral content [BMC (r= -0.20)], femoral neck BMC (r= -0.16), and radius BMC (r= -0.17) were reported ($P<0.05$ for all) indicating that those girls with low baseline 25(OH)D values responded to supplementation to a greater extent than girls with higher baseline 25(OH)D values.

Preliminary data from our laboratory in pre- and early- pubertal females over a time frame of 1 to 7 years showed a significant effect of 25(OH)D on BMC gains, with the greatest rates of accrual occurring for participants with lower levels of plasma 25(OH)D.[9] There is a need to elucidate the relationship of vitamin D and bone in the pediatric population; it is not currently known if increased vitamin D favorably alters accrual of bone mass during adolescence. Based on our laboratory's preliminary findings,

an NIH reviewer suggested vitamin D may not be essential to mineral accrual in growing children. The reviewer further posed the question, which hormones are preeminent in bone development? It may be that during childhood other hormones, particularly insulin-like growth factor I (IGF-I), may exert a greater influence on bone mineral accrual.

IGF-I regulates cells involved in the development and homeostatic maintenance of bone and cartilage.[10,11,12] *In vitro* and *in vivo* studies provide substantive evidence to indicate IGF-I involvement in osteoblast and osteoclast cell proliferation. The pubertal period is marked by a rapid increase of serum concentrations of IGF-I.[13] Secretion of IGF-I and bone mineral accrual follow analogous curves, increasing during pubertal maturation, peaking in the years surrounding peak height velocity (PHV), and declining thereafter. [12,14,15,16] A cross-sectional study by Libanati et al[17] observed a significant positive correlation between serum IGF-I and lumbar BMC, adjusted for BMI, in Tanner stages II and III (r=0.67 and r=0.60, respectively; $P<0.05$). In a prospective analysis of 1,119 healthy adolescents, Léger and colleagues reported serum IGF-I to be positively correlated with both bone alkaline phosphatase (BAP) and CrossLaps before puberty (Tanner 1: $\rho= 0.17$, $P\leq0.01$ and $\rho= 0.30$, $P\leq0.0001$; respectively) and after puberty (Tanner 5: $\rho=0.15$ and $\rho=0.13$, respectively; $P\leq0.01$).

The purpose of this project was to gain a better understanding of the hormonal influences on bone growth through prospective investigation of the relationships between BMC accrual and plasma concentrations of both IGF-I and 25(OH)D in pre- and early-pubertal females, ages 4 to 15. We hypothesize that changes in plasma concentrations of IGF-I are more strongly associated with BMC accrual than changes in plasma concentration of 25(OH)D.

References

1. 2005 Healthy People 2010, vol. 2008. U.S. Department of Health and Human Services.

2. National Osteoporosis Foundation, 2008, Washington DC accessed from http://www.nof.org/ on November 6, 2008.

3. 2004 Bone Health and Osteoporosis: A Report of the Surgeon General. U.S. Department of Health and Human Services, Rockville, MD.

4. Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, Andon MB, Smith KT, Heaney RP 1994 Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest **93**(2)**:**799-808.

5. Cusack S, Cashman KD 2003 Impact of genetic variation on metabolic response of bone to diet, pp 901-912.

6. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ 1992 Vitamin D_3 and calcium to prevent hip fractures in elderly women. New England Journal of Medicine **327:**1637-1642.

7. Dawson-Hughes B, Bischoff-Ferrari HA 2007 Therapy of osteoporosis with calcium and vitamin D, pp V59-V63.

8. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R 2006 Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab **91**(2)**:**405-12.

9. Laing E, Voorhees C, Hall D, Hausman D, Lewis R 2006 A prospective analysis of plasma 25-hydroxyvitamin D and bone mass in white and black prepubertal females. Journal of Bone and Mineral Research **21**(Suppl 1)**:**S205.

10. Zhang M, Xuan, S., Bouxsein, M., Stechow, D., Akeno, N., Faugere, M., Malluche, H., Zhao, G., Rosen, C., Efstratiadis, A., Clemens, T. 2002 Osteoblast-specific Knockout of Insulin-like Growth Factor (IGF) Receptor Gene Reveals an Essential Role of IGF Signaling in Bone Matrix Mineralization. The Journal of Biological Chemistry **277**(46)**:**44005-44012.

11. Croucher P, Russell, G. 1999 Growth Factors. In: Seibel M, Robins, S., Bilezikian, J. (ed.) Dynamics of Bone and Cartilage Metabolism. Academic Press, San Diego, pp 83-95.

12. Rosen CJ 1999 Serum Insulin-like growth factors and insulin-like growth factor-binding proteins: Clinical Implications. Clinical Chemistry **45**(8(B))**:**1384-1390.

13. Juul A, Holm K, Kastrup KW, Pedersen SA, Michaelsen KF, Scheike T, Rasmussen S, Muller J, Skakkebaek NE 1997 Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. Journal of Clinical Endocrinology and Metabolism **82**(8)**:**2497-2502.

14. Cadogan J, Blumsohn A, Barker ME, Eastell R 1998 A longitudinal study of bone gain in pubertal girls: Anthropometric and biochemical correlates. Journal of Bone and Mineral Research **13(10):**1602-1612.

15. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, Muller J, Hall K, Skakkebaek NE 1994 Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab **78**(3)**:**744-52.

16. Lofqvist C, Andersson E, Gelander L, Rosberg S, Blum WF, Albertsson Wikland K 2001 Reference values for IGF-I throughout childhood and adolescence: a model that accounts simultaneously for the effect of gender, age, and puberty. J Clin Endocrinol Metab **86**(12)**:**5870-6.

17. Libanati C, Baylink DJ, Lois-Wenzel E, Srinvasan N, Mohan S 1999 Studies on the potential mediators of skeletal changes occurring during puberty in girls. J Clin Endocrinol Metab **84**(8)**:**2807-14.

CHAPTER 2

REVIEW OF THE LITERATURE

In order to gain a better understanding of hormones and bone mineral accrual, this chapter provides a compendium of the current empirical literature that discusses: biology of bone, skeletal development in children, and the determinants of peak bone mass. In particular, hormonal influences on bone will be addressed, with a primary focus being on insulin-like growth factor I (IGF-I) and vitamin D.

Bone Biology

Anatomy

Bones are vital to the body for functioning in movement, mechanical support, and protection of body structures. Additionally, they are responsible for blood cell production and serve as a mineral reservoir in the body.[1] Bone consists of three components: an organic matrix, an inorganic matrix, and water. The structural, mechanical, and biochemical properties of bone are partially determined by the organic matrix, which is comprised of Type I collagen, bone cells, and noncollagen proteins. The inorganic component is crystalline hydroxyapatite formed from calcium and phosphorous. Bone accounts for 98% of calcium in the body; the remaining 2% resides in the blood.[3]

Types of bone

There are two types of bone: cortical bone and cancellous bone. Cortical bone, accounting for 80% of skeletal mass, is the dense calcified tissue that makes up the outer

portion of most bones and the shaft of long bones.[4] The hard, rigid cortical bone tissue provides the skeleton with compression strength. Cortical bone is organized into functional units called osteons, which are composed of a Haversian canal surrounded by collagen (see figure 1). The collagen is organized into concentric rings. Cavities called lacunae, embedded within these rings, house osteocytes. Through channels called canaliculi, osteocytes residing in the lacunae communicate with the central Haversian canal that surrounds blood vessels, nerve fibers, and lymphatics. Cancellous bone is predominately located in the axial skeleton, flat bones, and the ends of long bones. Cancellous bone is also known as trabecular bone because it is arranged into thin calcified honeycomb-like structures called trabeculae. This configuration places the trabecular bone in contact with blood vessels, connective tissue, and bone marrow, which allows the network to function metabolically.[5,6]

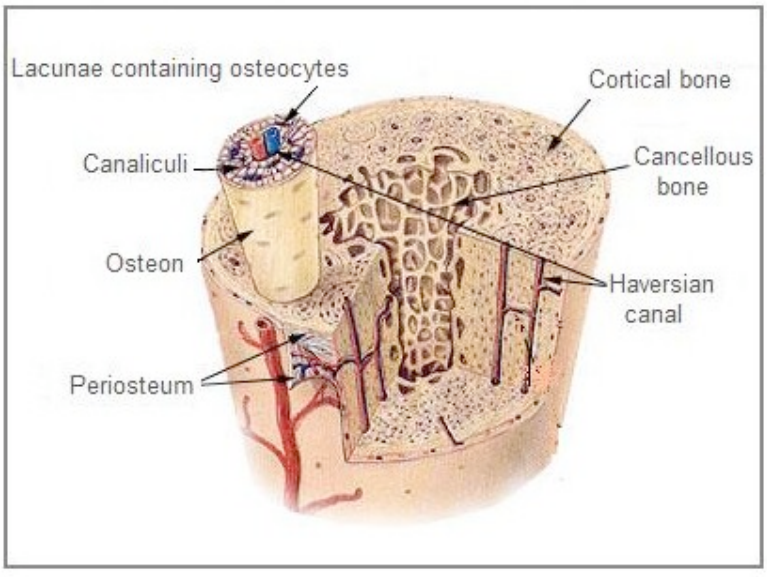


Figure 1. The microstructure of bone. Adapted from SEER training module.[7]

Bone cells

Bone cells function in regulating bone metabolism. There are three types of bone cells: osteoblasts, osteoclasts, and osteocytes. Osteoclast cells are responsible for bone resorption, the removal of damaged bone tissue. Osteoclasts are sizeable multinucleated cells that have an ephemeral lifespan of approximately 2 weeks.[8] These cells contain a ruffled border surrounded by contractile protein with which they use to attach themselves to bone. Once attached, the osteoclasts form a resorption cavity known as a Howship lacuna. Lysosomal enzymes secreted from the osteoclast's ruffled border breakdown the inorganic hydroxyapatite, and the remaining collagen is removed by cathepsin or collagenase.[9] Osteoblasts are cuboidal single nucleated cells that are responsible for the synthesis and deposition of bone matrix. The number of osteoblasts at the lacunae sites determines the rate of bone formation. Osteoblast cells either evolve into osteocytes or become inactive cells that line bone surfaces. Osteocytes are flattened mature cells that comprise 90 to 95% percent of bone cells.[10]

Bone remodeling and bone modeling

Throughout life, bones are constantly undergoing the process of bone remodeling. Bone remodeling is the process by which the microstructure of bones is repaired through replacement of fatigued bone with newly formed tissue (see figure 2). The process is mediated by osteoclasts and does not result in a net gain of bone mass, rather it is a coupled process by which the removal of bone initiates bone formation. This coupling of bone formation to resorption is likely directed by the insulin-like growth factor system.[11] Remodeling occurs at discrete loci of the bone. Bone that is going through remodeling is called a basic structural unit, and the cells involved in this process make up the basic

multicellular unit. Approximately 20% of cancellous bone undergoes remodeling at a given time, and in a period of about 2 years every surface of bone is remodeled.[12] At the end of the remodeling process, the osteoblasts become osteocytes (see figure 2). The process of bone remodeling is affected by various environmental factors including dietary intake and physical activity. Hormones, in particular, exert a great influence over the osteoblast and osteoclast activity. When homeostatic mechanisms of bone remodeling become off balance uncoupling can occur, resulting in a net loss or a net gain of bone. The increased resorption activity can lead to bone loss.

During periods of growth and bone healing, new bone is formed on existing bone through the process of bone modeling. During modeling, bone resorption and formation occur at different sites, and therefore, the alterations in structure are not dependent on the coupled activity of osteoblasts and osteoclasts. Modeling changes the shape of bone through apposition of bone on the outer or periosteal surface and resorption on the inner endosteal surface. Bone modeling is directed by both genetic determinants and the responses to load.

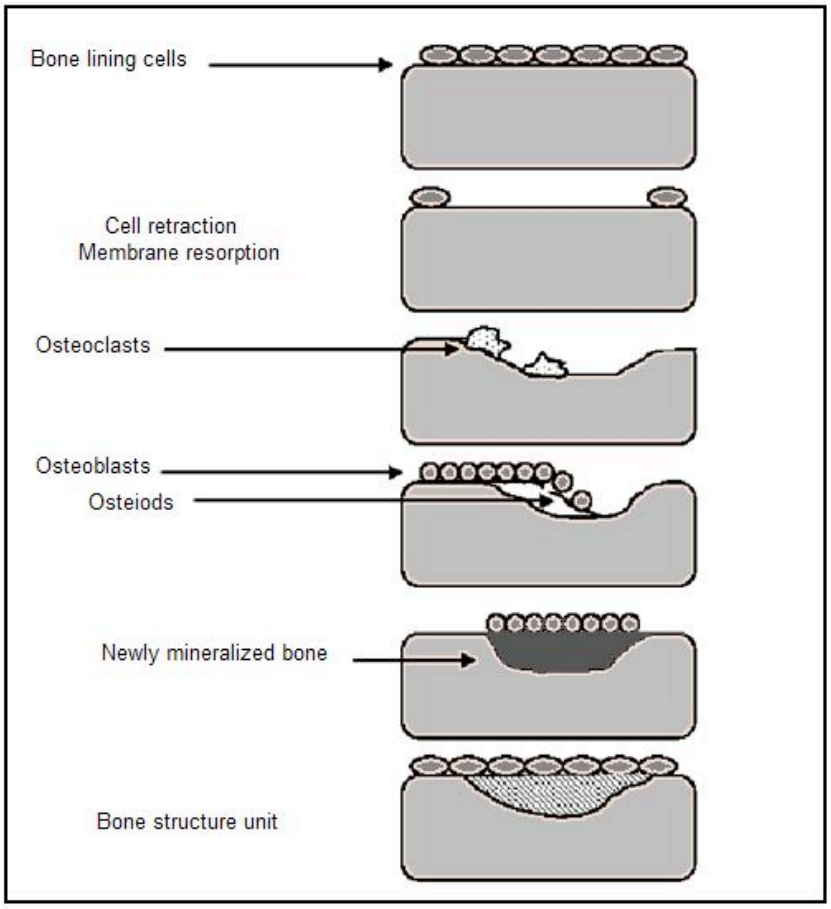


Figure 2. Bone remodeling process. Adapted from Compston et al[13]

Bone Strength

Bone strength is influenced by of both the material and structural properties of bone.[14,15] Material properties such as bone mineral density (BMD) are often the focal area of bone research. As a result, there is a propensity for bone mass and bone strength to be regarded as one and the same. Bone mass, bone architecture, bone geometry, and bone size are all components of bone strength. During growth, bones increase in both length and width. Bone is added to the outer periosteal surface, and resorbed from the inner medullary cavity leading to an expansion of the cavity which results in increased bone strength. While increases in both length and width are known to occur during

growth, the geometrical changes that occur during this time period are unknown. Concern about the accuracy of reporting areal bone mineral density (aBMD) by dual energy x-ray absorptiometry (DXA) in adolescent populations has emerged because aBMD does not account for the changes that occur in bone size during growth.[16] The only densitometric parameter of bone that is consistent with bone geometry in children is bone mineral content (BMC). BMC is recognized as the primary contributing factor of bone strength, and has been proposed as a more appropriate measure for the assessment than the BMD of bone in growing individuals.[17]

Skeletal Development in Children

The growth phase of childhood and adolescence is deemed a critical time for potentially optimizing bone strength given that the majority of bone mineral accrual occurs between 10 to 18 years of age. Moreover, during this life phase there is an interaction between genetic, hormonal, and environmental influences that enhances skeletal mineralization, bone expansion, and linear growth.[18] Any disorder or condition that reduces bone formation or enhances bone resorption during the maturational period may lead to suboptimal skeletal development and, presumably, a greater risk of osteoporotic fractures later in life.[19,20]

Prospective studies of total body BMC measurements indicate that approximately 25% of adult bone is accrued during the two-year period surrounding peak height velocity.[21] Peak height velocity (PHV) occurs at 11.8 and 13.4 years in females and males, respectively. The maximal rate of BMC accrual occurs 6 to 12 months after PHV (see figure 3).[2] Boys and girls reach 90% of their adult stature, but only 57% of their BMC at PHV.[2] At least 90% of peak bone mass (PBM), defined as the highest amount

of bone mass present at the end of skeletal maturation, is acquired by age 18 years.[22] It is reported that half of PBM is gained during the pubertal years, with 37% of the gain occurring between pubertal stages 2 and 5.[23] While the precise time period of PBM attained is debated, the majority of bone mass is achieved by the second decade of life.[22,24,25]

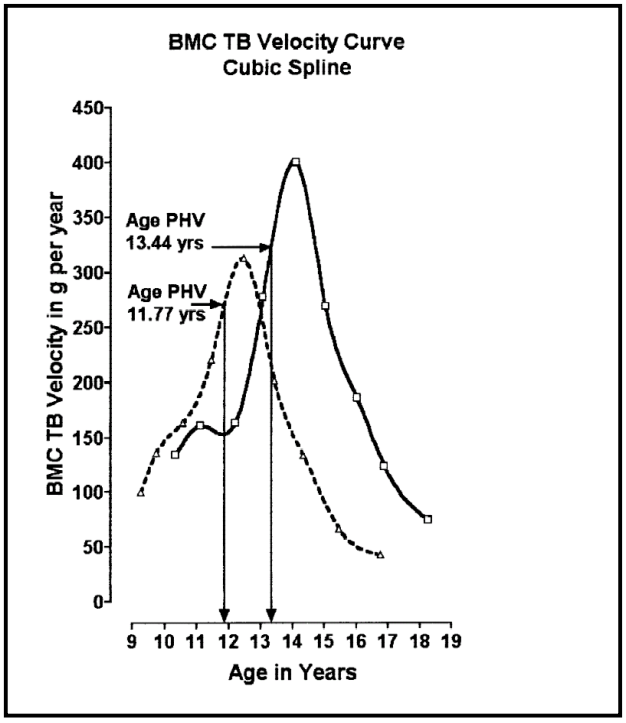


Figure 3. Ages at total body (TB) peak bone mineral content (BMC) and peak height velocities (PHV) by chronological age for boys and girls. Adapted from Bailey at al.[2]

A cross-sectional study of 265 females conducted by Matkovic et al,[24] reported that total body BMD and BMC (measured using DXA) leveled off at age 18 years, and remained unchanged at age 50 years. The peak BMD of the spine and of the wrist were attained at ages 23 and 20 years, respectively, with no significant losses prior to menopause. The hip peak BMD was attained at an earlier age of 17 years, and a gradual decline in bone mass ensued immediately following PBM. While cross-sectional data

indicate it is conceivable that the time occurrence of PBM is marginally variable between individuals, it is implicit that attainment of the greatest possible bone mass functions in the protection against bone loss related fractures. Moreover, with the major gains in bone mineralization occurring during pubertal years, this life phase may be particularly critical in the prevention of bone loss.[22]

Determinants of bone mineral accrual

Genetics

There is a strong genetic component in the pathogenesis of osteoporosis.[26] Genetic factors play a role in bone mineral development and are resultantly related to fracture risk.[27] It has been estimated from twin studies that 50 to 85% of interindividual variability in bone mass may be attributed to genetic factors.[28,29,30] Over the past few decades numerous candidate genes for osteoporosis have been identified, some of theses include vitamin D receptor (VDR), estrogen receptor, apolipoprotein E, collagen I α 1, and transforming growth factor β 1.[26,31] Findings from studies linking osteoporosis candidate genes with various bone indices are inconsistent. Normal genetic variation in bone phenotypes is likely due to polymorphisms. Polymorphisms can lead to changes in gene-gene interactions and gene-environment interactions that further perplex the relationship.[32] Heredity and environment are not entirely separable. Manipulation of an environmental factor, such as nutrition, can influence the expression of a genetic influence.[26] The identification of environment factors is particularly important because, unlike genotype, they are modifiable. Genetic determinants on bone will have little impact on the prevention and treatment of osteoporosis without the identification of both the specific genes involved and the environmental factors they interact with.

Body Composition

DXA technology allows for the measurement of total body bone and soft-tissue, compartmentalizing the body into bone mineral, fat tissue, and lean tissue. The relative contributions of lean mass and fat mass to bone mass vary throughout the lifespan. Of the two forms of soft-tissue, lean (fat-free) mass has proved to be the best correlate with bone mass and/or density in children.[5] In a cross-sectional study of 115 healthy adolescents, 5 to 18 years of age, Manzoni et al[33] reported lean mass was a strong correlate with total BMC in both normal weight and obese children (r=0.94 and r=0.91, respectively). Prospective data from University of Saskatchewan Pediatric Bone Mineral[34] Study (n=138) showed peak lean mass velocity was independently associated with peak BMC velocity (r=0.50, $P<0.001$), which suggests the development of bone is driven by muscle. Young et al[35] observed females, 10 to 26 years of age, and reported an approximate 1% greater proximal femoral aBMD for each kilogram of lean mass, holding all other factors constant. Empirical evidence supports a link between lean mass and bone growth and development. Fat mass appears to be less influential during childhood, but has been shown to contribute to bone mass during infancy and in the years following menopause.[5]

Physical Activity

Studies predominately demonstrate that participation in physical activity for individuals of all ages results in higher aBMD and BMC than those who are less active.[21,36,37,38,39] The degree to which physical activity influences bone is dependent on the mode and intensity of the activity, the age at which activity began, and the number of years spent training.[40] Prospective data from the Saskatchewan Bone Mineral Accrual Study showed that in the year following peak BMC velocity, active boys and girls

showed 9% and 17% greater bone mineral accrual, respectively, compared to inactive peers.[21] Weight-bearing exercise may contribute to the prevention of bone loss by increasing the amount of bone accrued during growth.[41,42,43,44] Furthermore, it has been proposed that during growth, weight-bearing exercise produces its most beneficial effects because it is at this time that peak bone mass is attained.[45,46,47] The mechanostat theory proposes an explanation for the positive effect of weight-bearing exercise on bone, stating the relationship between the intensity of bend or strain on bone and the adaptation of bone to these forces.[5] Bone resorption will exceed formation when activity falls below the physiological minimal effective strain threshold. Bone is maintained within the threshold, and net gains will occur only when the intensity of loading is increased.

New bone can be produced in response to extremely high loads. While physical activity clearly increases bone accrual in adolescence it remains unclear whether these benefits persist into adulthood. Retrospective assessment in retired athletes report conflicting results of both sustained gains and loss of bone mass after cessation or reduction of activity.[48,49] An 8 year follow-up study of children who participated in a 7-month jumping intervention conducted by Gunter et al[50] showed a 1.4% ($P<0.05$) increase in BMC at the hip in participants who completed the high-impact jumping exercises compared to the nonimpact stretch group control participants. Baxter-Jones et al[51] reported young adults who were active in adolescence had 8-10% ($P<0.05$) greater hip BMC compared to peers who were less active in their early years. It is important to note, however, that the participants who were active as adolescents remained significantly more active in early adulthood ($P<0.05$).

Dietary Intake

Adequate dietary intake during the growth may be critical to the attainment bone growth potential. A number of micronutrient factors have been linked with skeletal growth and development either independently or through the modification of calcium metabolism. These nutrients include minerals: calcium, phosphorous, magnesium, copper, manganese, and zinc; and vitamins: D, C, and K.[22,52] A clearly established association between calcium and bone health exists, however, calcium metabolism is a complex process in children and adolescents, and the intake necessary to maintain a positive calcium balance that promotes maximal acquisition of bone mass is not known. Calcium supplementation trials in children have shown an augmentation of bone mass (aBMD) in the total body and spine with food-based and nonfood supplements.[53,54] In addition to the micronutrient influences, adequate macronutrient consumption is essential to bone matrix synthesis. Protein energy malnutrition in childhood can lead to growth retardation and decreased formation of cortical bone.[22,55] Adequate protein is required for the production of IFG-I, and the impact of malnutrition on bone likely results from inadequate levels of IGF-I factor amongst other factors.[53] Fruit and vegetable intake is positively associated with BMC in adolescent girls, but not boys.[56] However, the positive relationship may be because they are a marker of an overall healthy diet rather than by cause of actual consumption of the foods. While individual nutritional related factors influence bone, consumption of an overall diet that is adequate in energy and balanced nutrients, and promotes a healthy body weight, appears to be the paramount factor in maximizing bone growth and development through dietary intake.

Hormones

Hormones are chemical substances released from glands that send messages to cells throughout the body. An array of hormones function in the regulation and regeneration of bone throughout life. The ability of hormones to function properly is dependent on a variety of factors, including body weight, age, dietary intake, and health status.[57] Moreover, the inability of hormones to function properly may lead to imbalances that can be detrimental to bone. There are a number of hormones involved in regulation of bone development. Several of these pivotal hormones are listed in table 1.

Table 1. Key hormones in bone development.

Hormones	**Functions on bone**
Estrogen	▪regulates the rate of bone formation and bone resorption ▪prevents calcium loss and maintains levels of vitamin D
Androgens	▪stimulate bone growth
Parathyroid hormone	▪stimulates osteoclastic bone resorption indirectly to release calcium ▪stimulates bone formation that is coupled to bone resorption ▪increases renal tubular reabsorption of calcium ▪stimulates the renal production of 1,25 dihydroxyvitamin D
Calcitonin	▪inhibits osteoclast resorption ▪delays calcium absorption from the intestine
25 hydroxyvitamin D	▪promotes gastrointestinal absorption of calcium and phosphorus
Insulin-like growth factor-I	▪increases rate of protein synthesis for bone formation ▪increases rate of mitosis of osteoblasts
Thyroid hormone	▪increases the rate of protein synthesis ▪controls energy production rate
Insulin	▪decreases calcium absorption from the intestines
Glucocorticoid	▪inhibits bone formation ▪increases bone resorption ▪decreases sex steroid production

Adapted from Hurley et al (2004).[58]

Some hormones influence bone by controlling serum calcium levels.[5] These hormones that directly respond to changes in the plasma–ionized calcium concentrations include parathyroid hormone, vitamin D and calcitonin. Other hormones released by factors other than changes in calcium levels can directly and indirectly influence bone through modification of calcium metabolism. These hormones include estrogen, testosterone, growth hormone, insulin-like growth factor I, corticosteroids, and thyroid hormone.

Currently, there is an overall lack of research in hormonal influences on childhood bone. Published research inconsistently reports hormone bone relationships, and it not known which hormones exert the greatest influences on BMC accrual during bone growth. To our knowledge, the hormonal factors of vitamin D and IGF-I have never been prospectively analyzed together regarding their relationship with bone mineral accrual in pre- and early-pubertal females.

Insulin-like Growth Factor-I and Bone

IGF-I is a 70 amino acid residue polypeptide ligand.[59] It is a constituent of the IGF system, which also includes two structurally homogenous peptides, IGF-II and insulin, as well as three cell surface receptors, six insulin-like growth factor binding proteins (IGFBPs), and proteases. Synthesis of IGF occurs in both the liver, functioning as an endocrine hormone, and in the periphery tissue, operating as a paracrine/autocrine factor.[60] Hepatic synthesis, accounting for 80% of the IGF-I peptide in the blood, is regulated by growth hormone (GH), nutritional status, and insulin. IGF-I receptors (IGF-IR) are present in nearly all tissues. Most cell types in the body are, therefore, affected by the hormone.

IGF-I has the highest affinity for an IGF-I receptor (IGF-IR), but can bind to an insulin receptor (IR) as well.[59] The binding of IGF-I to its receptor is moderated by the IGFBPs. IGF-I has a higher affinity for each of the six binding proteins than to its receptor. More than 99% of circulating IGF-I is bound in complex with IGFBP. More than 75% of the bound IGF-I forms a tertiary complex with IGFBP-3 and an acid-labile subunit.[61] IGFBP-3 is the largest molecular weight IGFBP. Binding of IGF-I with IGFBPs prolongs its half-life and forms a circulating pool of the hormone.[61] IGFBPs also function as carrier proteins transporting IGF-I from the circulation system to its target cells.

Insulin-like growth factors function in the regulation of cells involved in the development and homeostatic maintenance of bone and cartilage in children and adults.[62,63,64] IGF-I is available to bone through exocrine delivery via the circulation, paracrine and autocrine synthesis, and liberation from stores in the bone matrix.[60,65] During growth, IGF-I secretion and bone mineral accrual follow analogous patterns, increasing during pubertal maturation reaching zenith in the years surrounding PHV, and declining thereafter. [64,66,67,68] Moreover, serum IGF-I declines with age, with a slope similar to age-related bone loss.[69] The influence of IGF-I on the bone remodeling process may consequently affect bone mass and fracture risk.[70,71]

IGF is involved in both the bone formation and the bone resorption aspects of bone remodeling. *In vitro* and *in vivo* studies provide substantive evidence to indicate IGF-I involvement in osteoblast and osteoclast cell proliferation, but the process is immensely complex, and the precise role of the hormone has yet to be defined.[72,73,74] There is recent evidence to suggest that local secretion of IGF-I further influences bone

formation by recruiting osteoblasts to fracture and bone resorbing sites.[75] Studies of human marrow stromal cells (hMS), a cell line that differentiates into either osteoblasts or adipocytes, failed to show a direct relationship between IGF-I and recruitment/commitment into osteoblasts.[76] IGF-I was shown to increase hMS proliferation, but it did not induce expression of the osteoblast commitment transcription factor. IGF-I did, however, stimulate osteoblastic cell proliferation, through the stimulation of type I IGF collagen expression, in hMS cells induced to differentiate into the osteoblast lineage. In fully differentiated osteoblasts, IGF-I has been shown to promote bone matrix formation by binding to IGF-type I receptors on osteoblast cells, and subsequently stimulating collagen synthesis.[77,78] IGF-I has also been shown to inhibit the activity of collagen synthesis and, therefore, reduce osteoblast degradation.[78] Moreover, there is recent evidence to suggest that local secretion of IGF-I further influences bone formation by recruiting osteoblasts to fracture and bone resorbing sites.[75] Less is known about the action of IGF-I on osteoclast cells and bone resorption. Murine studies show IGF-I can stimulate osteoclast activation,[79] but the process is mediated through and dependent on the presence of osteoblast cells.[80,81] Additionally, mature human osteoclast cells express type I IGF receptors which may directly impact osteoclast function. The role of IGF-I in bone remodeling has also been supported physiologically through correlational studies link low circulating levels of IGF-I with low bone mineral density.

Cross-sectional studies in healthy children and adolescents reveal conflicting reports of hormonal influences of IGF-I on bone growth.[82,83,84,85,86] These inconsistencies may be due to between-study variability in the form of IGF-I measured

[i.e. serum IGF-I, IGFBP-(1-6), IGF-I/IGFBP-3 ratio, etc.], the bone variable observed (aBMD, BMC, cortical thickness, bone biomarkers etc.), the bone site measured (lumbar, total body, metacarpal, etc.), and the maturational stage of participants. A cross-sectional study conducted by Libanati et al[85] investigating the potential mediators of skeletal changes occurring during puberty, in 65 girls aged 9-14 years, showed a pubertal stage dependent positive correlation between serum IGF-I and lumbar bone mass as measured by DXA. During maturation from Tanner stage II to IV the girls exhibited a 40% increase in lumbar aBMD, and an 87% increase in lumbar BMC. Tanner stages II and III are associated with a 50% increase ($P<0.001$) in serum levels of IGF-I. However, the maturation advancement from stage III to IV did not significantly correlate with an increase in serum IGF-I. Serum IGF-I was positively correlated with lumbar BMC, adjusted for BMI, in Tanner stages II and III (r=0.67 and r=0.60, respectively; $P<0.05$). For all subjects, ranging from Tanner stage II through IV, serum IGF-I was positively correlated with increased cortical metacarpal thickness, (r=0.60, $P<0.001$) and metacarpal total thickness (r=0.66, $P<0.001$), with the strongest association occurring during Tanner stage II of development[82,87].

Léger and colleagues[84] conducted a prospective analysis of 1,119 healthy adolescents 10-16 years, of age reporting that IGF-I is independently associated with bone turnover markers, bone-specific alkaline phosphatase (BAP; bone formation), and CrossLaps (bone resorption). Serum IGF-I was positively correlated with both BAP and CrossLaps before puberty (Tanner 1: $\rho= 0.17$, $P\leq0.01$ and $\rho= 0.30$, $P\leq0.0001$; respectively) and after puberty (Tanner 5: $\rho=0.15$ and $\rho=0.13$, respectively; $P\leq0.01$), but during puberty only the CrossLaps was weakly correlated to serum IGF-I. Conversely,

Kanubur[83] et al reported no significant association between IGF-I and bone formation markers, BAP, and osteocalcin in girls. The study, investigating 205 adolescents aged 9 to 17, found the bone formation markers were only significantly associated to serum IGF-I in boys. A study utilizing computed tomography conducted by Mora et al[70] reported a direct relationship between levels of IGF-I and femur cross-sectional area (CSA; r=0.49) and cortical bone area (r=0.50; P<0.01) in healthy adolescents 7 to 18 years of age (n=197). However, the study reported no significant association of IGF-I and aBMD.

Studies comparing IGF-I with diseases known to affect bone metabolism indicate that diseased individuals have lower IGF-I concentrations than healthy individuals. Chlebna-Sokol et al[82] investigated IGF-I concentrations in individuals, 7 to 18 years of age, with idiopathic osteoporosis. They exhibited a lower mean value serum IGF-I than the control group (583 and 850 ng/ml, respectively; $P<0.05$). Additionally, IGF-I positively correlated with total body and spinal aBMD (R=0.85 and R=0.80, respectively; $P<0.00001$). In a study comparing adolescent girls diagnosed with anorexia nervosa(AN) to bone age matched controls (n=38), Soyka and colleagues[82] reported significantly lower lateral spine aBMD (0.68 ± 0.02 and 0.81 ± 0.02 g/cm^2, respectively; P<0.0001). Sixty-three percent of the AN participants' lumbar spine aBMD was more than one SD below normal mean, and 26% fell more than 2 SD below normal mean. Compared to the control group individuals, the AN subjects also displayed significantly lower LBMD, LBMC, and total body BMC, but a significant reduction in total aBMD between the groups was not observed. Serum IGF-I was significantly lower in the AN subjects (219 ± 41 and 511 ± 35 ng/mL, respectively; $P<0.0001$). Additionally, bone formation markers BAP and osteocalcin were significantly reduced ($P=0.02$) in the AN group versus

controls. Through stepwise regression analysis, it was determined that the majority of the decreased bone formation was explained by decreased IGF-I concentrations (osteocalcin: r^2=0.72, *P*= 0.002 and BSAP: r^2=0.53, *P*=0.01).

Longitudinal studies investigating IGF-I and bone mass are very limited in number. A study conducted by Cadogan and colleagues[66] that followed 31 12-year old girls for a period of 24 months, failed to detect significant association between serum IGF-I and change in total BMC (g/yr) and total aBMD (g/cm^2/yr). Additionally, the researchers reported a negative relationship between IGF-I and change in height (cm/yr), attributing the negative correlation to the large percentage of participants reaching PHV velocity and suggesting IGF-I no longer had a stimulatory effect on the growth plate. The study findings are not consistent with the literature on the action of IGF-I on bone accrual and longitudinal growth.

Most of the literature favors a positive association between IGF-I and bone accrual, however, some cross-sectional reports contravene the relationship, and therefore it remains to be elucidated. There is a need for more longitudinal research on the IGF-I/ bone accrual relationship during pubertal years.

Vitamin D and Bone

Vitamin D is essential to the growth and regulation of bone. Poor vitamin D in the elderly is related to low bone mass. Moreover, in this age group, vitamin D supplementation is shown to increase calcium absorption, suppress PTH, and favorably alter functional outcomes of bone metabolism.[88,89,90] While much is known regarding serum 25(OH)D and bone in adults, major gaps still exist with regard to our understanding of the status of vitamin D in growing children. The years leading up to

menarche may be particularly crucial for maximizing bone accrual, however, the vitamin D level that optimizes bone mass in this age group is not known.

Few studies have investigated the relationship between serum 25(OH)D levels and bone indices in children and adolescents, and those that do report inconsistent findings, with some studies showing no relationships,[91,92] some positive,[93,94,95] and others negative.[96] The variability between studies may be attributed to the varying ages, maturation status, and vitamin D status of the participants. Racial differences between participants may have an additional impact on the studies' outcomes. In spite of having lower levels 25(OH)D, black females have higher total body BMC, [96,97,98,99] and they are at a lower risk for osteoporotic fractures compared to white females.[100,101,102]

Few studies have been conducted in child and adolescent populations observing vitamin D status and bone mineral accrual over time. Prospective data from our laboratory showed a significant negative relationship between 25(OH)D on BMC gains in adolescent females followed throughout pre- and early- puberty.[99] Over a seven-year period, subjects with lower levels of 25(OH)D showed greater rates of gain than those with higher levels of 25(OH)D.[99] Likewise, Tylavsky et al[92] showed 25(OH)D was inversely related to gain in total body BA, BMC, and BMD in a two year longitudinal study of 69 adolescents, ages 8 to 13. In a 3-year prospective study in Finnish girls, ages 9 to 15, wintertime baseline serum 25(OH)D levels were significantly associated with the change in lumbar spine and femoral aBMD ($r=0.35$, $P<0.001$ and $r=0.32$, $P<0.001$, respectively).[103] For the participants who reached sexual maturity, mean change from baseline in lumbar spine aBMD was 26% higher in the girls with the highest baseline serum 25(OH)D compared to the girls with the lowest circulating levels at baseline.

BMC, the most appropriate outcome variable for the assessment of bone in growing individuals was, unfortunately, not reported. [104]

It is unclear as to whether increased vitamin D intakes or supplementation favorably alters bone metabolism during adolescence. El-Hajj Fuleihan et al[95] conducted a 12-month double blind, placebo-controlled vitamin D supplementation trial in 10 to 17 year-old Lebanese females with mean 25(OH)D concentrations of 35 nmol/L. The girls were randomized into 3 groups: 1,400 IU/week (~200 IU/day), 14,000 IU/week (~2,000 IU/day), or placebo. There was a significant association between baseline serum 25(OH)D and radius BMC (r=0.16, $P<0.05$). The 1-year percent change in spine BMC (r= -0.20), femoral neck BMC (r= -0.16), and radius BMC (r= -0.17) significantly negatively correlated with baseline 25(OH)D. ($P<0.05$ for all). The negative correlations suggest girls with low baseline 25(OH)D values responded to supplementation to a greater degree than girls with higher baseline 25(OH)D values. There was a significant dose-dependent increase in both trochanteric BMC and lumbar spine aBMD in premenarcheal girls, but there were no significant differences in changes in lean mass, aBMD, or BMC in the participants who were postmenarcheal.

While it is clearly understood that poor vitamin D status is related to low bone mass in adults, less is known about the potentiating effect of this hormone on skeletal growth in children. It is imperative that the role vitamin D plays in bone mineral accrual is further investigated to determine the optimal levels for maximizing skeletal gains.

IGF-I and Vitamin D

An endogenous interrelationship between IGF-I and vitamin D may have an added effect on bone metabolism (see figure 4). Cell culture studies indicate that IGF-I

and 1,25$(OH)_2$D up-regulate each other. 1,25$(OH)_2$D promotes the action of IGF-I by increasing IGF-I receptors. In turn IGF-I stimulates the hydroxylation of the circulating 25(OH)D to the active 1,25$(OH)_2$D form in the kidneys through the stimulation of the 1α-hydroxylase enzyme.[105,106]

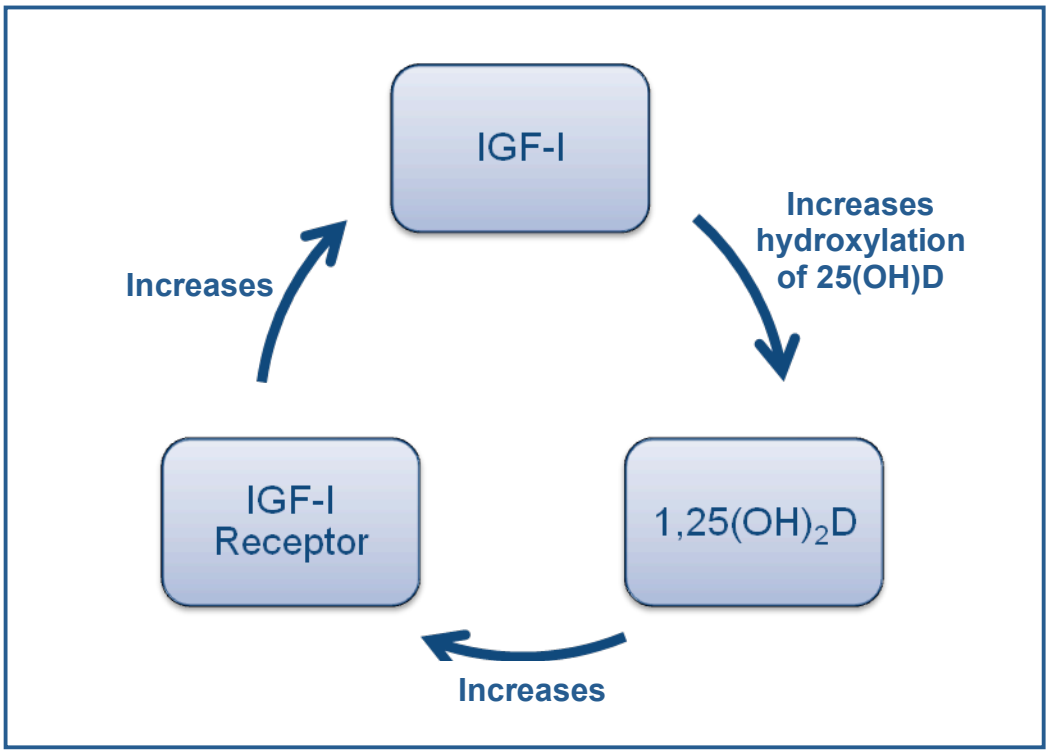


Figure 4. The interrelationship between IGF-I and 1,25(OH)D

Osteoblast- like cells exposed to recombinant human IGF-I increased bone formation markers, alkaline phosphatase and osteocalcin in the presence of 1,25$(OH)_2$D. Cells cultured in the absence of active vitamin D metabolite did not exhibit an increase in bone formation.[107] Studies conducted with IGF-I knockout mice further support this relationship. Kasukawa el al[108] discovered there is a significant decrease in serum1,25$(OH)_2$D concentration in IGF-I knockout mice (24%, $P<0.05$) compared to

wild type (WT) controls. Additionally, it was reported that the IGF-I knockout mice exhibited a 70% decrease in kidney vitamin D receptor expression. Very few human studies have investigated the relationship between IGF-I and the vitamin D endocrine system. A cross-sectional study in 61 middle aged men conducted by Rucker[106] and colleagues ascertained a positive relationship between 25(OH)D and IGF-I. Correcting for age and BMI, the two hormones were correlated (β= 0.39, P<0.01). Bianda et al[109] treated healthy men (n=7) with a subcutaneous infusion of IGF-I (8μg/kg/hr) for 5 days followed by a subcutaneous injection of GH (6 U twice daily on days 3,4, and 5 of IGF-I infusion). Free $1,25(OH)_2D$ rose significantly following IGF-I treatment ($2.2 \pm 5 \times 10^{-5}$ to $2.81 \pm 0.25 \times 10^{-5}$) but was not significantly altered with the addition of the GH injections. Serum bone formation markers osteocalcin and carboxyterminal propeptide of type I collagen were also significantly increased following IGF-I infusion (P<0.02). Bianda again investigated the relationship in GH-deficient adults (n=8).[110] IGF-I infusion was positively associated with bone formation and $1,25(OH)_2D$ in this population as well. The PTH, calcium, and phosphate levels in the GH-deficient adults were not significantly affected by the IGF-I/GH treatment. In a study of children suffering from nutritional rickets who were treated with a megadose of vitamin D_3 (300,000 IU), Soliman et al[111] reported both a significant increase in vitamin 25(OH)D levels and IGF-I concentrations. The two hormones were significantly positively correlated both before and after vitamin D_3 treatment (r=0.325 and r=0.314, respectively; P<0.001). Moreover, the children's growth velocity standard deviation score following treatment correlated with the increase of IGF-I and 25(OH)D (r=0.325 and r=314, respectively; P<0.01) indicating growth is mediated through the insulin-like growth

factor system. In vitro and in vivo studies provide a substantial amount of evidence indicating the interrelationship of IGF-I and $1,25(OH)_2D$, however, there is a need for further investigation through human research into their synergy and resulting potential influences on bone metabolism.

Summary

While peak bone mass achieved in adolescence is primarily determined by genetic predisposition, hormonal and environmental influences can enhance skeletal mineralization, bone expansion, and linear growth.[18] The extent to which hormones contribute to variation in PBM is unclear. Moreover, it is unknown which of the hormones involved in bone metabolism exerts the greatest influence on bone mineral accrual. Empirical evidence supports a relationship with the hormones vitamin D and IGF-I and bone mineral accrual, but their relative contribution to the attainment of peak bone mass is currently unknown. To date, vitamin D and IGF-I have never been prospectively analyzed together in a pediatric population.

References

1. Rodan GA 1992 Introduction to bone biology. Bone **13:**s3-s6.

2. Bailey AJ, Sims TJ, Ebbesen EN, Mansell JP, Thomsen JS, Mosekilde L 1999 Age-related changes in the biochemical properties of human cancellous bone collagen: relationship to bone strength. Calcif Tissue Int **65**(3)**:**203-10.

3. Heaney RP 1999 Bone Biology in Health and Disease: A Tutorial. In: Shils ME, Olson, J.A., Shike, M., Ross, A.C. (ed.) Modern Nutrition in Health and Disease, 9th ed., vol. 83. Williams and Wilkins, Baltimore, pp 1327-1338.

4. Mundy GR 1996 Bone remodeling and disorders. Martin Dunitz Ltd, London, Great Britain.

5. Khan KM, McKay HA, Kannus P, Bailey D, Wark J, Bennell K 2001 Physical Activity and Bone Health. Human Kinetics, Champaign, IL, pp 275.

6. U.S. National Institutes of Health NCI SEER Training Modules. In: Tissue B (ed.).

7. SEER Training Modules Bone Tissue. NIH, National Cancer Institute Assessed on April 28, 2009 from: http://training.seer.cancer.gov/.

8. Rodan GA 1998 Control of bone formation and resorption: biological and clinical perspective. Journal of Cell Biochemistry Supplement **30/31:**55-61.

9. Rosen CJ, Donahue, L.R., and Hunter, S.J. 1994 Insulin-like growth factors and bone: the osteoporsis connection. Proc Soc Exp Bio Med **206:**83-102.

10. Bonewald LF 2008 Osteocytes Primer on metabolic bone diseases and disorders of mineral metabolism, 7th ed. American Society for Bone Mineral Research, Washington D.C., pp 22-27.

11. Johansson AG, Eriksen EF, Lindh E, Langdahl B, Blum WF, Lindahl A, Ljunggren O, Ljunghall S 1997 Reduced serum levels of the growth hormone-dependent insulin-like growth factor binding protein and a negative bone balance at the level of individual remodeling units in idiopathic osteoporosis in men. J Clin Endocrinol Metab **82**(9)**:**2795-8.

12. AM Parfitt MD, FH Glorieux, JA Kanis, H Malluche, PJ Meunier, SM Ott, RR Recker 1987 Bone Histomorphometry: Standardization of Nomenclature, Symbols, and Units. Journal of Bone Mineral Research **2:**595-610.

13. Compston JE 2001 Sex steroids and bone. Physiological Reviews **81**(1)**:**419-447.

14. van der Meulen MC, Jepsen KJ, Mikic B 2001 Understanding bone strength: size isn't everything. Bone **29**(2)**:**101-4.

15. Turner CH, Burr DB 1993 Basic biomechanical measurements of bone: a tutorial. Bone **14**(4)**:**595-608.

16. Schonau E 1998 Problems of bone analysis in childhood and adolescence. Pediatric Nephrology **12**(5)**:**420-429.

17. Petit MA 2005 Examining the developing bone: what do we measure and how do we do it. Journal of Musculoskeletal Neuronal Interaction **5**(3)**:**213-224.

18. Recker RR, Heaney RP 1993 Peak bone mineral density in young women. Jama **270**(24)**:**2926-7.

19. Hui SL, Slemenda CW, Johnston CC, Jr. 1988 Age and bone mass as predictors of fracture in a prospective study. J Clin Invest **81**(6)**:**1804-9.

20. 2004 Bone Health and Osteoporosis: A Report of the Surgeon General, Rockville: US Department of Health and Human Services.

21. Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA 1999 A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. J Bone Miner Res **14**(10)**:**1672-9.

22. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C 2000 Peak bone mass. Osteoporos Int **11**(12)**:**985-1009.

23. Ilich JZ, Badenhop NE, Jelic T, Clairmont AC, Nagode LA, Matkovic V 1997 Calcitriol and bone mass accumulation in females during puberty. Calcif Tissue Int **61**(2)**:**104-9.

24. Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, Andon MB, Smith KT, Heaney RP 1994 Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest **93**(2)**:**799-808.

25. Saggese G, Baroncelli GI 1996 Bone mineral density and biochemical parameters of bone turnover in children with growth hormone deficiency. Horm Res **45**(Suppl 1)**:**67-8.

26. Cusack S, Cashman KD 2003 Impact of genetic variation on metabolic response of bone to diet, pp 901-912.

27. Zapalowski C 2004 Genetics of Osteoporosis. In: McDermott M, Zapalowski, C., Miller, P. (ed.) Osteoporosis. Hanley and Belfus, Philadelphia, PA, pp 7-14.

28. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D, Vogt TM 1995 Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med **332**(12)**:**767-73.

29. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S 1987 Genetic determinants of bone mass in adults. A twin study. J Clin Invest **80**(3)**:**706-10.

30. Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston Jr. CC 1991 Role of physical activity in the development of skeletal mass in children. Journal of Bone and Mineral Research **6:**1227-1233.

31. Ferrari S 2008 Human genetics of osteoporosis. Best Practice & Research Clinical Endocrinology & Metabolism **22**(5)**:**723-735.

32. Peacock M, Turner CH, Econs MJ, Foroud T 2002 Genetics of osteoporosis. Endocr Rev **23**(3)**:**303-26.

33. Manzoni P, Brambilla P, Pietrobelli A, Beccaria L, Bianchessi A, Mora S, Chiumello G 1996 Influence of body composition on bone mineral content in children and adolescents. American Journal of Clinical Nutrition **64**(4)**:**603-607.

34. Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R 2004 The 'muscle-bone unit' during the pubertal growth spurt. Bone **34**(5)**:**771-775.

35. Young D, Hopper JL, Macinnis RJ, Nowson CA, Hoang NH, Wark JD 2001 Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. Osteoporos Int **12**(6)**:**506-15.

36. Teegarden D, Proulx WR, Kern M, Sedlock D, Weaver CM, Johnston CC, Lyle RM 1996 Previous physical activity relates to bone mineral measures in young women. Med Sci Sports Exerc **28**(1)**:**105-13.

37. Bakker I, Twisk JW, Van Mechelen W, Roos JC, Kemper HC 2003 Ten-year longitudinal relationship between physical activity and lumbar bone mass in (young) adults. J Bone Miner Res **18**(2)**:**325-32.

38. Kirchner EM, Lewis RD, O'Connor PJ 1996 Effect of past gymnastics participation on adult bone mass. J Appl Physiol **80**(1)**:**226-32.

39. Laing EM, Massoni JA, Nickols-Richardson SM, Modlesky CM, O'Connor PJ, Lewis RD 2002 A prospective study of bone mass and body composition in female adolescent gymnasts. J Pediatr **141**(2)**:**211-6.

40. Beck BR, Snow CM 2003 Bone health across the lifespan--exercising our options. Exerc Sport Sci Rev **31**(3)**:**117-22.

41. Cooper C, Cawley M, Bhalla A, Egger P, Ring F, Morton L, Barker D 1995 Childhood growth, physical activity, and peak bone mass in women. J Bone Miner Res **10**(6)**:**940-7.

42. Magnusson H, Linden C, Karlsson C, Obrant KJ, Karlsson MK 2001 Exercise may induce reversible low bone mass in unloaded and high bone mass in weight-loaded skeletal regions. Osteoporos Int **12**(11)**:**950-5.

43. Teegarden D, Proulx WR, Martin BR, Zhao J, McCabe GP, Lyle RM, Peacock M, Slemenda C, Johnston CC, Weaver CM 1995 Peak bone mass in young women. J Bone Miner Res **10**(5)**:**711-5.

44. Ho SC, Wong E, Chan SG, Lau J, Chan C, Leung PC 1997 Determinants of peak bone mass in Chinese women aged 21-40 years. III. Physical activity and bone mineral density. J Bone Miner Res **12**(8)**:**1262-71.

45. Bass S, Pearce G, Bradney M, Hendrich E, Delmas PD, Harding A, Seeman E 1998 Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. J Bone Miner Res **13**(3)**:**500-7.

46. Uusi-Rasi K, Sievanen H, Vuori I, Heinonen A, Kannus P, Pasanen M, Rinne M, Oja P 1999 Long-term recreational gymnastics, estrogen use, and selected risk factors for osteoporotic fractures. J Bone Miner Res **14**(7)**:**1231-8.

47. McGuigan FE, Murray L, Gallagher A, Davey-Smith G, Neville CE, Van't Hof R, Boreham C, Ralston SH 2002 Genetic and environmental determinants of peak bone mass in young men and women. J Bone Miner Res **17**(7)**:**1273-9.

48. Kontulainen S, Kannus P, Haapasalo H, Sievanen H, Pasanen M, Heinonen A, Oja P, Vuori I 2001 Good maintenance of exercise-induced bone gain with decreased training of female tennis and squash players: a prospective 5-year follow-up study of young and old starters and controls. J Bone Miner Res **16**(2)**:**195-201.

49. Rautava E, Lehtonen-Veromaa M, Kautiainen H, Kajander S, Heinonen OJ, Viikari J, Mottonen T 2007 The reduction of physical activity reflects on the bone mass among young females: a follow-up study of 142 adolescent girls. Osteoporosis International **18**(7)**:**915-922.

50. Gunter K, Baxter-Jones AD, Mirwald RL, Almstedt H, Fuchs RK, Durski S, Snow C 2008 Impact Exercise Increases BMC During Growth: An 8-Year Longitudinal Study. **14**(2)**:**54-54.

51. Baxter-Jones ADG, Kontulainen SA, Faulkner RA, Bailey DA 2008 A longitudinal study of the relationship of physical activity to bone mineral accrual from adolescence to young adulthood. Bone **43**(6)**:**1101-1107.

52. 2003 Nutritional Aspects of Bone Health. In: New SaB, Jean-Philippe (ed.). The Royal Society of Chemistry, Cambridge, UK.

53. Cromer B, Harel Z 2000 Adolescents: At increased risk for osteoporosis? Clinical Pediatrics **39**(10)**:**565-574.

54. French SA, Fulkerson JA, Story M 2000 Increasing weight-bearing physical activity and calcium intake for bone mass growth in children and adolescents: A review of intervention trials. Preventive Medicine **31**(6)**:**722-731.

55. Ortner DJ 1972 Earlier gain and later loss of cortical bone - garn,sm. American Journal of Physical Anthropology **36**(2)**:**304-305.

56. Whiting SJ, Vatanparast H, Baxter-Jones A, Faulkner RA, Mirwald R, Bailey DA 2004 Factors that affect bone mineral accrual in the adolescent growth spurt, pp 696S-700S.

57. Soyka LA, Fairfield WP, Klibanski A 2000 Clinical review 117: Hormonal determinants and disorders of peak bone mass in children. J Clin Endocrinol Metab **85**(11)**:**3951-63.

58. Hurley MaL, J. 2004 Systemic and Local Regulators of Bone Remodeling. In: Bronner FaF-C, M. (ed.) Bone Formation. Springer-Verlag, London, pp 44-70.

59. Clemmons DR 2000 Insulin-like Growth Factors: Their Binding Proteins and Growth Regulation. In: Canalis E (ed.) Skeletal Growth Factors. Lippincott Williams & Wilkins, Philadelphia, pp 79-100.

60. Conover CA 2000 In vitro studies of insulin-like growth factor I and bone. Growth Hormone & Igf Research **10:**107-110.

61. Zofkova I 2003 Pathophysiological and clinical importance of insulin-like growth factor-I with respect to bone metabolism. Physiological Research **52**(6)**:**657-679.

62. Zhang M, Xuan, S., Bouxsein, M., Stechow, D., Akeno, N., Faugere, M., Malluche, H., Zhao, G., Rosen, C., Efstratiadis, A., Clemens, T. 2002 Osteoblast-specific Knockout of Insulin-like Growth Factor (IGF) Receptor Gene Reveals an

Essential Role of IGF Signaling in Bone Matrix Mineralization. The Journal of Biological Chemistry **277**(46)**:**44005-44012.

63. Croucher P, Russell, G. 1999 Growth Factors. In: Seibel M, Robins, S., Bilezikian, J. (ed.) Dynamics of Bone and Cartilage Metabolism. Academic Press, San Diego, pp 83-95.

64. Rosen CJ 1999 Serum Insulin-like growth factors and insulin-like growth factor-binding proteins: Clinical Implications. Clinical Chemistry **45**(8(B))**:**1384-1390.

65. Howard GA SM 1991 Modern concepts of insulin-like growth factors. In: M.E. S (ed.) IGFpparathyroid hormone interactions in bone in vitro in vivo. Elsevier, New York.

66. Cadogan J, Blumsohn A, Barker ME, Eastell R 1998 A longitudinal study of bone gain in pubertal girls: Anthropometric and biochemical correlates. Journal of Bone and Mineral Research **13(10):**1602-1612.

67. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, Muller J, Hall K, Skakkebaek NE 1994 Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab **78**(3)**:**744-52.

68. Lofqvist C, Andersson E, Gelander L, Rosberg S, Blum WF, Albertsson Wikland K 2001 Reference values for IGF-I throughout childhood and adolescence: a model that accounts simultaneously for the effect of gender, age, and puberty. J Clin Endocrinol Metab **86**(12)**:**5870-6.

69. Rosen CJ, Conover, C 1997 Growth hormone/insulin-like growth factor-I axis in aging: a summary of a National Institutes of Health aging-sponsored symposium. J Clin Endocrinol Metab **82:**3919-3922.

70. Mora S, Pitukcheewanont P, Nelson JC, Gilsanz V 1999 Serum levels of insulin-like growth factor I and the density, volume, and cross-sectional area of cortical bone in children. J Clin Endocrinol Metab **84**(8)**:**2780-3.

71. Canalis E, McCarthy TL, Centrella M 1991 Growth factors and cytokines in bone cell metabolism. Annu Rev Med **42:**17-24.

72. Hayden JM, Mohan S, Baylink DJ 1995 The insulin-like growth factor system and the coupling of formation to resorption. Bone **17:**93S-98S.

73. Canalis E 1993 Insulin like growth factors and the local regulation of bone formation. Bone **14:**273-276.

74. Sakata T, Halloran, B.P., Elalieh, H., Munson, S., Rudner, L., Venton, L., Ginsinger, D., Rosen, C., Bikle, D. 2003 Skeletal unloading induces resistance to insulin-like growth factor I on bone formation. Bone **32:**669-680.

75. Nakasaki M, Yoshioka K, Miyamoto Y, Sasaki T, Yoshikawa H, Itoh K 2008 IGF-I secreted by osteoblasts acts as a potent chemotactic factor for osteoblasts. Bone **43**(5)**:**869-879.

76. Thomas T, Gori F, Spelsberg TC, Khosla S, Riggs BL, Conover CA 1999 Response of bipotential human marrow stromal cells to insulin-like growth factors: Effect on binding protein production, proliferation, and commitment to osteoblasts and adipocytes. Endocrinology **140**(11)**:**5036-5044.

77. Tanaka H, Moriwake T, Matsuoka Y, Nakamura T, Seino Y 1998 Potential role of rhIGF-I/IGFBP-3 in maintaining skeletal mass in space. Bone **22**(5 Suppl)**:**145S-147S.

78. Canalis E 1997 Insulin-like growth factors and osteoporosis Bone **21**(3)**:**215-6.

79. Mochizuki H, Hakeda Y, Wakatsuki N, Usui N, Akashi S, Sato T, Tanaka K, Kumegawa M 1992 Insulin-like growth factor-I supports formation and activation of osteoclasts. Endocrinology **131**(3)**:**1075-1080.

80. Conover CA 2000 Insulin-like Growth Factors and the Skeleton. In: Canalis E (ed.) Skeletal Growth Factors. Lippincott Williams & Wilkins, Philadelphia, pp 101-116.

81. Hill PA, Reynolds JJ, Meikle MC 1995 Osteoblasts mediate insulin-like growth-factor-I and growth-factor-II stimulation of osteoclast formation and function. Endocrinology **136**(1)**:**124-131.

82. Chlebna-Sokol D RA 2006 Insulin-like growth factor-I and mineral metabolism markers in children with idiopathic decrease in bone mass. Clinica Chimica Acta **366**(1/2)**:**257-263.

83. Kanbur NO DO, Kinik E 2005 The relationship between pubertal development, IGF-1 axis, and bone formation ion healthy adolescents. Journal of Bone MIneral Metabolism **23:**76-83.

84. Leger J 2007 The relationship between the GH/IGF-I axis and serum markers of bone turnover metabolism in healthy children. European Journal of Endocrinology **157**(5)**:**685-692.

85. Libanati C, Baylink DJ, Lois-Wenzel E, Srinvasan N, Mohan S 1999 Studies on the potential mediators of skeletal changes occurring during puberty in girls. J Clin Endocrinol Metab **84**(8)**:**2807-14.

86. Soyka LA, Grinspoon S, Levitsky LL, Herzog DB, Klibanski A 1999 The effects of anorexia nervosa on bone metabolism in female adolescents. J Clin Endocrinol Metab **84**(12)**:**4489-96.

87. Kurland ES, Rosen CJ, Cosman F, McMahon D, Chan F, Shane E, Lindsay R, Dempster D, Bilezikian JP 1997 Insulin-like growth factor-I in men with idiopathic osteoporosis. Journal of Clinical Endocrinology and Metabolism **82**(9)**:**2799-2805.

88. Dawson-Hughes B, Bischoff-Ferrari HA 2007 Therapy of osteoporosis with calcium and vitamin D, pp V59-V63.

89. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ 1992 Vitamin D_3 and calcium to prevent hip fractures in elderly women. New England Journal of Medicine **327:**1637-1642.

90. Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, Nebiker M, Theiler R, Pfeifer M, Begerow B, Lew RA, Conzelmann M 2003 Effects of vitamin D and calcium supplementation on falls: A randomized controlled trial. Journal of Bone and Mineral Research **18**(2)**:**343-351.

91. Abrams SA, Griffin IJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO 2005 Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. J Clin Endocrinol Metab **90**(10)**:**5576-81.

92. Tylavsky FA, Ryder KA, Lyytikainen A, Cheng S 2005 Vitamin D, parathyroid hormone, and bone mass in adolescents. J Nutr **135**(11)**:**2735S-8S.

93. Outila TA, Karkkainen MU, Lamberg-Allardt CJ 2001 Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: associations with forearm bone mineral density. Am J Clin Nutr **74**(2)**:**206-10.

94. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B 2004 Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med **116**(9)**:**634-9.

95. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R 2006 Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab **91**(2)**:**405-12.

96. Stein EM, Laing EM, Hall DB, Hausman DB, Kimlin MG, Johnson MA, Modlesky CM, Wilson AR, Lewis RD 2006 Serum 25-hydroxyvitamin D concentrations in girls aged 4-8 y living in the southeastern United States. Am J Clin Nutr **83**(1)**:**75-81.

97. Yanovski JA, Sovik KN, Nguyen TT, Sebring NG 2000 Insulin-like growth factors and bone mineral density in African American and White girls. J Pediatr **137**(6)**:**826-32.

98. Hui SL, Dimeglio LA, Longcope C, Peacock M, McClintock R, Perkins AJ, Johnston CC, Jr. 2003 Difference in bone mass between black and white American children: attributable to body build, sex hormone levels, or bone turnover? J Clin Endocrinol Metab **88**(2)**:**642-9.

99. Laing E, Voorhees C, Hall D, Hausman D, Lewis R 2006 A prospective analysis of plasma 25-hydroxyvitamin D and bone mass in white and black prepubertal females. Journal of Bone and Mineral Research **21**(Suppl 1)**:**S205.

100. Farmer ME, White LR, Brody JA, Bailey KR 1984 Race and sex differences in hip fracture incidence. Am J Public Health **74**(12)**:**1374-80.

101. Kellie SE, Brody JA 1990 Sex-specific and race-specific hip fracture rates. Am J Public Health **80**(3)**:**326-8.

102. Barrett-Connor E, Siris ES, Wehren LE, Miller PD, Abbott TA, Berger ML, Santora AC, Sherwood LM 2005 Osteoporosis and fracture risk in women of different ethnic groups. J Bone Miner Res **20**(2)**:**185-94.

103. Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS 2002 Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. Am J Clin Nutr **76**(6)**:**1446-53.

104. Heaney RP 2005 BMD: the problem. Osteoporos Int **16**(9)**:**1013-5.

105. Gomez JM 2006 The role of insulin-like growth factor I components in the regulation of vitamin D. Current Pharmaceutical Biotechnology **7**(2)**:**125-132.

106. Rucker D, Ezzat S, Diamandi A, Khosravi J, Hanley DA 2004 IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. Clinical Endocrinology **60**(4)**:**491-499.

107. Chenu C, Valentinopran A, Chavassieux P, Saez S, Meunier PJ, Delmas PD 1990 Insulin-like growth factor-I hormonal-regulation by growth-hormone and by $1,25(OH)_2D_3$ and activity on human osteoblast-like cells in short-term cultures. Bone **11**(2)**:**81-86.

108. Kasukawa Y, Baylink DJ, Wergedal JE, Amaar Y, Srivastava AK, Guo RQ, Mohan S 2003 Lack of insulin-like growth factor I exaggerates the effect of calcium deficiency on bone accretion in mice. Endocrinology **144**(11)**:**4682-4689.

109. Bianda T, Hussain MA, Glatz Y, Bouillon R, Froesch ER, Schmid C 1997 Effects of short-term insulin-like growth factor-I or growth hormone treatment on bone turnover, renal phosphate reabsorption and 1,25 dihydroxyvitamin D-3 production in healthy man. Journal of Internal Medicine **241**(2)**:**143-150.

110. Bianda T, Glatz Y, Bouillon R, Froesch ER, Schmid C 1998 Effects of short-term insulin-like growth factor-I (IGF-I) or growth hormone (GH) treatment on bone metabolism and on production of 1,25-dihydroxycholecalciferol in GH-deficient adults. Journal of Clinical Endocrinology and Metabolism **83**(1)**:**81-87.

111. Soliman AT, Al Khalaf F, AlHemaidi N, Al Ali M, Al Zyoud M, Yakoot K 2008 Linear growth in relation to the circulating concentrations of insulin-like growth factor I, parathyroid hormone, and 25-hydroxy vitamin D in children with nutritional rickets before and after treatment: endocrine adaptation to vitamin D deficiency. Metabolism **57**(1)**:**95-102.

CHAPTER 3

25-HYDROXYVITAMIN D, INSULIN-LIKE GROWTH FACTOR-I AND BONE MINERAL ACCRUAL DURING GROWTH

Breen, M.E., Laing, E.M., Hall, D.B., Hausman, D.B., Taylor, R.G., Isales, C.M., Ding, K., Pollock N.K., Hamrick M.W., Baile C.A., Lewis R.D. 2009. To be submitted to the Journal of Bone and Mineral Research.

25-Hydroxyvitamin D, Insulin-Like Growth Factor-I and Bone Mineral Accrual During Growth

Breen, M.E.[1], Laing, E.M. [1], Hall, D.B. [2], Hausman, D.B. [1], Taylor, R.G. [1], Isales, C.M. [3], Ding, K. [3], Pollock N.K. [4], Hamrick M.W. [3], Baile C.A., Lewis R.D. [1]

[1]Department of Foods and Nutrition, The University of Georgia, Athens, GA, USA
[2]Department of Statistics, The University of Georgia, Athens, GA, USA
[3]Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA, USA
[4]Department of Pediatrics, Prevention Institute, Medical College of Georgia, Augusta, GA, USA

Running Title: VITAMIN D, IGF-1 AND BMC ACCRUAL

Emails:
mebreen@uga.edu
emonk@uga.edu
danhall@uga.edu
dhausman@uga.edu
rgildea@uga.edu
CISALES@mail.mcg.edu
kding@mail.mcg.edu
NPOLLOCK@mail.mcg.edu
mhamrick@mail.mcg.edu
cbaile@uga.edu
rlewis@fcs.uga.edu

Correspondence and reprint requests should be addressed to: Richard D. Lewis, Ph.D., Room 279 Dawson Hall, Department of Foods and Nutrition, The University of Georgia, Athens, GA 30602. Phone: 706-542-4901. Fax: 706.542.5059. Email: rlewis@fcs.uga.edu

ABSTRACT

The extent to which 25-hydroxyvitamin D [25(OH)D] and insulin-like growth factor-I (IGF-I) influence bone mineral accrual from early to mid-puberty is unclear. This project sought to determine relationships among changes in plasma 25(OH)D, plasma IGF-I, and bone mineral content (BMC) in prepubertal females (N = 76; aged 4 to 8 years at baseline), over a period of up to 7 years. BMC was measured using dual-energy X-ray absorptiometry (Hologic QDR-1000W). Plasma 25(OH)D and plasma IGF-I were assessed using and radioimmunoassay (DiaSorin, Inc.) and quantikine enzyme-linked immunosorbent serologic assay (R&D Systems), respectively. Prior to the primary analyses, 25(OH)D and IGF-1 were log-transformed and further adjusted, using two-way ANOVA, for baseline differences in season and race. Linear mixed modeling that included a random subject-specific intercept and a random subject-specific slope on age was employed to analyze the transformed 25(OH)D and IGF-1 variables for the proportion of variance each explained on the four bone outcomes. IGF-I was more strongly associated with BMC accrual than 25(OH)D at the total body ($R^2 = 0.874$ vs. 0.809), total proximal femur ($R^2 = 0.847$ vs. 0.771), radius ($R^2 = 0.812$ vs. 0.759), and lumbar spine ($R^2 = 0.759$ vs. 0.698). At each skeletal site, the rate of BMC accrual was negatively associated with changes in 25(OH)D, but positively associated with changes in IGF-I. When IGF-I and 25(OH)D were included in the same regression equation, 25(OH)D did not have a significant predictive effect on BMC accrual at any site above and beyond that of IGF-I. These longitudinal data in early adolescent females indicate that both 25(OH)D and IGF-I have a significant impact on bone mineral accrual;

however, the positive effect of IGF-I on BMC is greater than the negative influence from 25(OH)D.

Key words: Vitamin D, Insulin Like Growth Factor-I, Pediatrics, Bone Mineral Accrual

INTRODUCTION

Poor vitamin D status in older adults is related to low bone mass, and supplementation increases calcium absorption, suppresses PTH secretion and favorably alters functional outcomes of bone metabolism.[1,2,3] While much is known regarding vitamin D and bone metabolism in adults, major gaps still exist with regard to our understanding of the status of vitamin D in growing children. At least 90% of adult bone mass is acquired by the end of adolescence with 28% of adult bone mass accrued during the 2 years surrounding peak BMC velocity.[4] Vitamin D status in children and adolescents may have significant health implications, particularly with respect to bone metabolism, however, the 25(OH)D concentration(s) reflecting optimal vitamin D status in children and adolescents is unknown.

Prospective data from our laboratory showed a significant negative relationship between 25(OH)D on BMC gains in adolescent females followed throughout pre- and early- puberty.[5] Over a seven-year year period, subjects with lower levels of 25(OH)D showed greater rates of gain than those with higher levels of 25(OH)D. These preliminary data suggest that vitamin D may not be essential with respect to bone mineral accrual in growing children. It may be that during childhood other hormones, particularly insulin-like growth factor I (IGF-I), may exert a greater influence on bone mineral accrual.

The pubertal period of adolescence is marked by a rapid increase of serum concentrations of IGF-I[6] that later declines with increasing age with a slope similar to

age-related bone loss.[7] IGF-I regulates cells involved in the development and homeostatic maintenance of bone and cartilage. *In vitro* and *in vivo* studies provide substantive evidence to indicate IGF-I involvement in osteoblast and osteoclast cell proliferation, but the process is immensely complex, and the precise role of the hormone has yet to be defined.[8,9,10] Cross-sectional studies investigating correlations between IGF-I and bone mineral accrual in healthy children and adolescents have reported conflicting results.[11,12,13,14,15]

To our knowledge, IGF-I and vitamin D have never been prospectively analyzed together with regard to their relationship to bone mineral accrual during growth. The purpose of the present study was to determine the relationships between BMC accrual and plasma concentrations of both IGF-I and 25(OH)D in pre- and early-pubertal females, ages 4 to 15. We hypothesize that changes in plasma concentrations of IGF-I are more strongly associated with BMC accrual than changes in plasma concentration of 25(OH)D in adolescent females.

MATERIALS AND METHODS

Participants

The study participants were females, 4 to 8 years of age, at baseline who were originally recruited to participate in a prospective study investigating the influence of artistic gymnastics on bone.[16] The present study followed subjects in the nonintervention group (n=96) for a period of up to 9 years. All of the girls were healthy and were not taking any medications known to effect bone metabolism. The participants' ethnicity (Hispanic or Latino, Non-Hispanic or Latino) and race (American Indian or Alaska Native, Asian, black or African American, Native Hawaiian or other Pacific Islander,

white, or any combination of the above) were classified through parent identification according to the National Institutes of Health Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research.[17] Ethnic/racial groups with small sample sizes ($n < 7$) were excluded from the current project, leaving 83 participants (49 white and 34 black girls) of those originally recruited. Sixty-five (34 white, 31 black) subjects provided annual serum samples over 4 years, 12 (11 white, 1 black) over 5 years, 6 (2 white, 2 black) over 6 years, 1 (white) over 7 years, and 1(white) subject provided a serum sample over 9 years. The University of Georgia Institutional Review Board for Human Subjects approved the study protocol. Prior to every testing session, each participant and her guardian completed informed assent and consent forms, respectively.

Data Collection

Data collection took place in the Bone and Body Composition Laboratory between October 1997 and October 2008. The enrollment was staggered throughout the fall (September-November) winter (December-February), spring (March-May), and summer (June-August). Annual testing of each participant fell within the same season as the baseline testing session. Blood was collected from participants between 0730 and 1000 h following a 12-hour fast. The participants returned to the laboratory within l week of their blood draw to provide bone scans and anthropometric measures (i.e., height and weight), and complete questionnaires assessing demographic information, dietary intake, and physical activity.

Anthropometric Measures

Anthropometric measures were conducted according to Anthropometric Standardization Reference protocol.[18] The participants wore light clothing and no shoes

for the height and weight measurements. Using a wall mounted stadiometer (Novel Products, Rockton, IL) height was measured to the nearest 0.1 cm. Weight was measured to the nearest 0.25 kg using a calibrated double-beam balance scale (Fairbanks Scales, Kansas City, MO). Single measure intraclass correlation (ICC) coefficients were calculated in females 6 to 10 years of age (n = 10) measured twice in a 2-week period by the same individual. The height and weight ICC (*R* value) and the test-retest CV (%) values were 0.99 and 0.4% and 0.99 and 1.4% for height and weight, respectively. Body mass index (BMI; in kg/m^2) values were plotted on BMI-for-age percentile charts.[19]

Sexual Maturation

A physician conducted annual sexual maturation assessments using criteria for stages of breast development (stages 1-5) as described by Tanner.[20] Computed results of one-way random effects model and single measure ICCs indicated perfect agreement (R=1.0) for the staging of breast and pubic hair development in females from current study (n=10).

Bone Mineral Content and Body Composition

The participants' bone area (in cm^2) and bone mineral content (BMC; in g) were assessed at total body, lumbar spine, non-dominant proximal femur, and non-dominant distal radius by dual energy X-ray asorptiometry (DXA; QDR-1000W, Hologic Inc, Waltham, MA). All scans were performed on the same instrument. Daily calibration of the DXA was performed using 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 calculated from 365 scans over 5 y was 0.27%. In our laboratory, test-retest measurements with DXA in 6 to 10-year-old girls (n=10)

showed the following CVs for aBMD: total body, 1.2%; lumbar spine, 1.3%; proximal femur, 1.6%: and forearm, 2.1%. The lumbar spine was analyzed with the use of DXA low density spine software version 4.74 (Hologic Inc.). The fat free soft tissue (FFST) mass (in g) and percentage body fat were determined by DXA and analyzed using pediatric whole body analysis version 5.73 (Hologic Inc). An external three step soft tissue wedge composed of aluminum and Lucite, calibrated against Stearic acid (100% fat) and water (8.6% fat), was run in tandem with each whole body scan for quality control. The percentage fat single measure ICC and CV in participants 5 to 8 years of age (n=10) scanned twice in a 1-week period were R=0.99 and 2.0%, respectively.

Biochemical Assays

Fasting blood samples were collected for analysis of plasma 25(OH)D and IGF-I. Samples were stored at –70 °C until analysis. IGF-I and 25(OH)D are stable over the period of up to 9 years in blood samples stored at –70 °C.[21,22] All samples were run in duplicate using a block design, such that all samples from the same subject were assayed at one time and an equal number of black and white subjects were analyzed using the same kit. Plasma 25(OH)D samples were assayed in a duplicate block design using a radioimmunoassay (RIA; DiaSorin Laboratories, Stillwater, MN) and were reported by Willis et al[23] in 2007. The inter- and intra- assay coefficients of variation were 7.3 to 10.5% and 5.9 to 7.0%, respectively. Plasma IGF-I concentrations were determined using recombinant human IGF-I quantitative sandwich immunoassay technique (ELISA; R&D Systems, Minneapolis, MN). The inter- and intra- assay coefficients of variation were 7.5% to 8.3% and 3.5 to 4.3%, respectively.

Statistical Analyses

Statistical analyses were performed using Statistical Analysis Software version 9.1 (SAS, Cary, NC). Descriptive statistics were expressed as mean ± SD and a P value ≤ 0.05 was considered statistically significant. Linear mixed-effects models were used to analyze the effects of IGF-I and 25(OH)D on bone mineral accrual. These models allowed for between subject and within subject variation analyses in participants who are different ages at baseline and/or different ages or different ages at repeat measures. Models were fixed at each of the four scan sites: total body, lumbar spine, non-dominant proximal femur, and non-dominant forearm. The models assumed a random subject-specific intercept and a random subject-specific slope allowing for correlation between random effects. Three models were fit for each of the four sites. Model 1 regressed BMC on: age, age^2, baseline age, baseline age^2, natural log ($\log_e$) of IGF-I, the interaction of $\log_e$ IGF-I and age, the interaction of $\log_e$ IGF-I and age^2, $\log_e$ IGF-I and baseline age, $\log_e$ IGF-I and baseline age^2, $\log_e$ 25(OH)D (adjusted for season and race), the interaction of $\log_e$ 25(OH)D and age, the interaction of $\log_e$ 25(OH)D and age^2, $\log_e$ 25(OH)D and baseline age, $\log_e$ 25(OH)D and baseline age^2, and race. Age and baseline age are centered from the mean age (8.52 yrs) averaged over all subjects at all testing sessions. This model accounts for within-subject correlation between repeated measures on the same subject showing the pattern of change in BMC over time accounting for baseline differences among the girls. This model including both IGF-I and vitamin D functions a standard of comparison. Model 2 is the same as model 1 except model 2 excludes all terms involving 25(OH)D, capturing how bone mineral accrual depends on IGF-I. Model 3, excluding all terms involving IGF-I, captures the impact of 25(OH)D on bone accrual over time.

Predictive curves of BMC accrual were plotted for each bone site based on $\log_e$ IGF-I and $\log_e$ 25(OH)D percentile values. The models were reanalyzed, controlling for lean mass and baseline lean mass, to determine the partial association between BMC accrual and $\log_e$ IGF-I and between BMC accrual and $\log_e$ 25(OH)D. A bivariate mixed-model analysis was used to assess the correlation between variables IGF-I and 25(OH)D (adjusted for season and race) at different means. This model took into consideration random subject effects to account for within-subject correlation over time.

RESULTS

Participant Characteristics

Baseline characteristics of the participants are presented in **Table 1**. Using the staging criteria described by Tanner,[20] all but 4 girls were classified as breast stage 1 (i.e., prepubertal) at baseline. Throughout the 9-year study, 6 girls had reached menarche (and had advanced to breast stages 4 or 5). The majority of participants (n=44) at baseline had BMI-for-age percentiles that were between the 5th and <85th percentile, 3 participants had BMI-for-age percentiles that were less than the 5th percentile, 15 participants had BMI-for-age percentiles between the ≥85th and <95th percentiles and 14 participants had BMI-for-age percentiles that were ≥95th percentile.

Vitamin D and IGF-1

Participant baseline plasma 25(OH)D_3 and IGF-I are presented in table 1. Prospective vitamin D data have been described elsewhere by Willis et al.[23] The majority of participants (n=51) had plasma 25(OH)D values above 80 nmol/L, 20 participants had 25(OH)D values that were between 50 and 80 nmol/L, and 3 participants had 25(OH)D values that fell below 50 nmol/L. A large percentage of the participants

(~75%) had plasma 25(OH)D concentrations that fell <80 nmol/L at least once during the investigation.[23] Statistically significant linear ($P < 0.001$) and quadratic ($P = 0.020$) effects demonstrated a decreasing trend in 25(OH)D that accelerated with age. Baseline plasma IGF-I values ranged from 55 to 470 ng/mL and the spread of these IGF-I values plotted against chronological age are illustrated in **Figure 1**.

Influence of 25(OH)D and IGF-1 on BMC Accrual

Table 2 depicts the R^2 values for change over time in BMC at the total body, lumbar spine, proximal femur, and forearm, and changes in either model 1 [IGF-I + 25(OH)D], model 2 [25(OH)D], or model 3 (IGF-I). For each of the four skeletal sites, IGF-I was more strongly associated with BMC accrual compared to 25(OH)D. When IGF-I and 25(OH)D were included in the same regression equation, 25(OH)D did not have a significant predictive effect on BMC accrual at any site above and beyond that of IGF-I. The addition of fat-free soft tissue to the model testing the strength of the partial association between BMC accrual and changes in IGF-I and 25(OH)D, explained more of the variability in BMC accrual as indicated by higher R^2 values [i.e., IGF-I vs. 25(OH)D, respectively, at the total body ($R^2 = 0.953$ vs. 0.947), lumbar spine ($R^2 = 0.932$ vs. 0.925), proximal femur ($R^2 = 0.928$ vs. 0.921), and forearm ($R^2 = 0.927$ vs. 0.921)].

Figure 2 illustrates the predicted curves of BMC accrual based on either baseline $\log_e$ 25(OH)D or $\log_e$ IGF-I for a subject who entered the study at age 4 years (i.e., the minimum age represented in our sample). Each of the 9 sequential curves (10^{th}, 20^{th}, 30^{th} … up to the 90^{th}) represents a percentile of either $\log_e$ 25(OH)D or $\log_e$ IGF-I in the sample. The respective percentiles of $\log_e$ IGF-I and $\log_e$ 25(OH)D are: 5.04 and -0.45 (10^{th}), 5.30 and -0.32 (20^{th}), 5.46 and -0.23 (30^{th}), 5.54 and -0.15 (40^{th}), 5.69 and -0.07

(50^{th}), 5.78 and -0.01 (60^{th}), 5.89 and 0.07 (70^{th}), 6.07 and 0.14 (80^{th}), as well as 6.24 and 0.26 (90^{th}). The spread among these curves is greater for the $\log_e$ IGF-I vs. the $\log_e$ 25(OH)D plots, showing graphically that IGF-I influences these growth curves more strongly than does 25(OH)D. The plots also demonstrate the greater positive effect of IGF-I on BMC accrual vs. the negative influence from 25(OH)D. Finally, a statistically significant negative correlation was observed between $\log_e$ IGF-I and $\log_e$ 25(OH)D ($r = -0.325$; $P < 0.0001$).

DISCUSSION

This is the first prospective investigation of the influences of vitamin D and IGF-I on bone mineral accrual in children. In adults, higher levels of 25(OH)D are associated with maximal suppression of iPTH, increased calcium absorption, attenuation of bone loss, and reduced risk of fractures.[1,24,25] What is less clear, are the roles of vitamin D in bone metabolism during childhood and adolescence. The primary finding of this study was that IGF-I is more strongly associated with BMC accrual at the total body, total proximal femur, radius, and lumbar spine regions in comparison to 25(OH)D. When IGF-I and 25(OH)D were included in the same regression equation, 25(OH)D did not have a significant predictive effect on BMC accrual at any site above and beyond that of IGF-I. At each skeletal site, the rate of BMC accrual was inversely associated with baseline 25(OH)D, but was positively associated baseline IGF-I.

According to our findings that 25(OH)D concentrations are inversely associated with the rate of BMC accrual, it is not known if higher serum 25(OH)D concentration(s) lead to more favorable changes in bone status. Consistent with our results, in a study following healthy adolescents over a two year period, Tylavsky et al[26] reported

25(OH)D was inversely related to gain in total body BA, BMC, and BMD. It is important to note that, in both studies, the majority of the subject population had sufficient baseline vitamin D status. Two 12-month vitamin D intervention trials in female adolescents reported small dose dependent improvements in femoral[27,28] and vertebral spine[28] BMC. The participants in both studies had low mean baseline 25(OH)D values. One of the trials conducted in Lebanon by El-Hajj Fuleihan et al[27] reported significant negative correlations between baseline 25(OH)D and the 1-year percent change in BMC at the spine, femoral, neck, and radius bone sites. These negative correlations show girls with low baseline 25(OH)D values responded to supplementation to a greater extent than girls with higher baseline 25(OH)D values. These studies and our findings indicate that, unlike adults, low 25(OH)D in children and adolescents maybe reflective of the potential for greater bone mineral gains. Influences of other hormones present during puberty, particularly IGF-I, may contribute to the observed 25(OH)D and BMC relationship. Much of the focus has been on researching PTH and estrogens to explain the observed differences, this study investigated IGF-I and its potential role as a modulator of vitamin D and bone mineral accrual during growth.

Cell culture and animal model studies have provided the bulk of the evidence supporting the role of IGF-I in bone mineral accrual during growth. Few studies have been conducted in children and adolescents to confirm the relationship.[8,9,10,29,30] During growth, both IGF-I secretion and bone mineral accrual increase during pubertal maturation, peak in the years surrounding PHV, and decline thereafter.[31,32,33,34] Evidence supporting a positive correlation between IGF-I and BMD is primarily based on cross-sectional studies. Libanati et al[14] showed a pubertal stage dependent positive correlation

between serum IGF-I and lumbar BMC as measured by DXA. The present study was the first prospective investigation to demonstrate a significant positive correlation between changes in IGF-I and BMC. To our knowledge, only one other study has prospectively analyzed IGF-I and the change in BMC.[32] This study did not show a significant relationship between IGF-I and change in BMC and BMD over a two year period in healthy adolescent females with a baseline age of 12 years. While this study did capture the significant changes in serum IGF-I accompanying puberty, significant changes in the girls' BMC and BMD were only observed during the final 6 months of the study.

The negative correlation between IGF-I and 25(OH)D observed in the present study indicates participants with lower circulating levels of vitamin D had higher levels of IGF-I over the nine-year period compared to the participants with higher levels of vitamin D. These findings lend support to the interrelationships observed between the two hormones. Cell culture studies indicate that IGF-I and $1,25(OH)_2D$ up regulate each other. $1,25(OH)_2D$ promotes the action of IGF-I by increasing IGF-I receptors. In turn, IGF-I stimulates the hydroxylation of the circulating 25(OH)D to the active $1,25(OH)_2D$ form in the kidneys through the stimulation of the 1α-hydroxylase enzyme.[35,36] The high levels of IGF-I may explain the negative correlation between 25(OH)D and BMC. It may be that in the presence of higher circulating levels of IGF-I leads to lower levels of 25(OH)D because IGF-I is stimulating hydroxylation to the active $1,25(OH)_2D$ form which is positively influencing bone. Bianda et al[37] observed a significant increase in $1,25(OH)_2D$ ($P<0.06$) and osteocalcin ($P<0.02$) in young healthy males following a 5 day subcutaneous IGF-I infusion.

Although circulating 25(OH)D is recognized as the best functional indicator of vitamin D status in adults, a limitation of this study was $1,25(OH)_2D$ was not assessed. By focusing only on the 25(OH)D form, information on the functional role of vitamin D during childhood bone growth may not have been captured. In support of this hypothesis, Abrams el al[38] reported that higher $1,25(OH)_2D$ concentrations were significantly correlated with calcium absorption in 93 healthy adolescents, whereas 25(OH)D was not significantly associated with calcium absorption. Another limitation to this study is that the majority of the participants had sufficient vitamin D status. The associations between changes in IGF-I and bone mineral accrual may be different in children and adolescents with insufficient levels of vitamin D. Finally, estradiol was not assessed in the current study and may have impacted the findings. However, reference values indicate that estradiol levels markedly increase around age 14.[39] Only two of the samples in this study were collected from girls 14 years of age. The majority of the girls in the study were prepubertal with only 6 participants having reached menarche.

In summary, changes in IGF-I were the strongest predictor of BMC accrual at all skeletal sites over a 9-year period in prepubertal girls. Vitamin D was also a strong predictor of BMC gain, but in an inverse manner such that subjects with lower 25(OH)D levels showed greatest BMC gain. Further studies are needed to examine the role of vitamin D in bone growth and mineralization, and to delineate the relationships of IGF-I and $1,25(OH)_2D$ and their effects on bone growth in children.

TABLE 1. Baseline descriptive characteristics of participants.

Variable	Mean	SD	n
Age (yrs)	6.35	1.55	76
Income Level[1]	4.42	2.60	73
Anthropometrics			
Weight (kg)	25.8	8.43	73
Height (cm)	120.0	11.8	73
BMI	17.5	3.23	73
BMI-For-Age Percentile	69.1	28.3	73
% Body Fat[2]	27.3	9.78	73
FFST Mass (kg)[2]	16.8	4.25	73
Bone Mineral Content (g) [2]			
Total Body	859.7	262.5	73
Lumbar Spine	17.8	4.84	73
Proximal Femur	10.7	3.97	72
Forearm	2.43	0.77	73
Physical Activity[3]	3.52	0.78	73
Dietary Intake[4]			
Vitamin D (μg)	5.34	3.24	73
Calcium (mg)	522.7	148.2	73
Breast Stage[5]			
Stage 1	N/A	N/A	71
Stage 2	N/A	N/A	3
Stage 3	N/A	N/A	1
25(OH)D (nmol/L)	88.3	24.5	74
IGF-1 (ng/mL)	251.3	94.1	76

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

[2] Body fat %, fat-free soft tissue mass and bone mineral content measured by DXA.

[3] Physical activity level assessed by parent questionnaire developed by Slemenda et al. 1991 [(40)] (1 = inactive, 2 = below average, 3 = average, 4 = above average, and 5 = very high).

[4] Dietary and supplement intake corrected per 1000 kcal energy intake.

[5] Sexual maturation stage (Breast) as described by Tanner 1962 [(20)].

TABLE 2. Correlation (R^2 values) explaining changes in bone mineral content (BMC) relative to plasma 25(OH)D, IGF-1 and plasma 25(OH)D + IGF-1 in prepubertal females (N=76) over a period of up to 9 years.[1]

Skeletal Site	*25(OH)D + IGF-1* [2]	*25(OH)D* [3]	*IGF-1* [4]
Total Body	0.837	0.809	0.874
Lumbar Spine	0.718	0.698	0.759
Proximal Femur	0.807	0.771	0.847
Forearm	0.780	0.759	0.812

[1] Linear mixed models were employed to analyze the proportion of variance that 25(OH)D and IGF-1 explained on BMC accrual at 4 skeletal sites.

[2] The R2 value depicts the pattern of change over time accounting for how growth in BMC might be influenced by both 25(OH)D and IGF-1. This model regressed BMC on: age, age^2, baseline age, baseline age^2, natural log ($\log_e$) of IGF-I, the interaction of $\log_e$ IGF-I and age, the interaction of $\log_e$ IGF-I and age^2, $\log_e$ IGF-I and baseline age, $\log_e$ IGF-I and baseline age^2, $\log_e$ 25(OH)D (adjusted for season and race), the interaction of $\log_e$ 25(OH)D and age, the interaction of $\log_e$ 25(OH)D and age^2, $\log_e$ 25(OH)D and baseline age, $\log_e$ 25(OH)D and baseline age^2, and race.

[3] The R^2 value depicts the pattern of change over time accounting for how growth in

BMC might be influenced by 25(OH)D, but not IGF-1. This model is the same as the 25(OH)D + IGF-1 model [(2)], except it excludes all terms involving IGF-1, capturing how bone mineral accrual depends on 25(OH)D.

[4] The R2 value depicts the pattern of change over time accounting for how growth in BMC might be influenced by IGF-1, but not 25(OH)D. This model is the same as the 25(OH)D + IGF-1 model [(2)], except it excludes all terms involving 25(OH)D, capturing how bone mineral accrual depends on IGF-1.

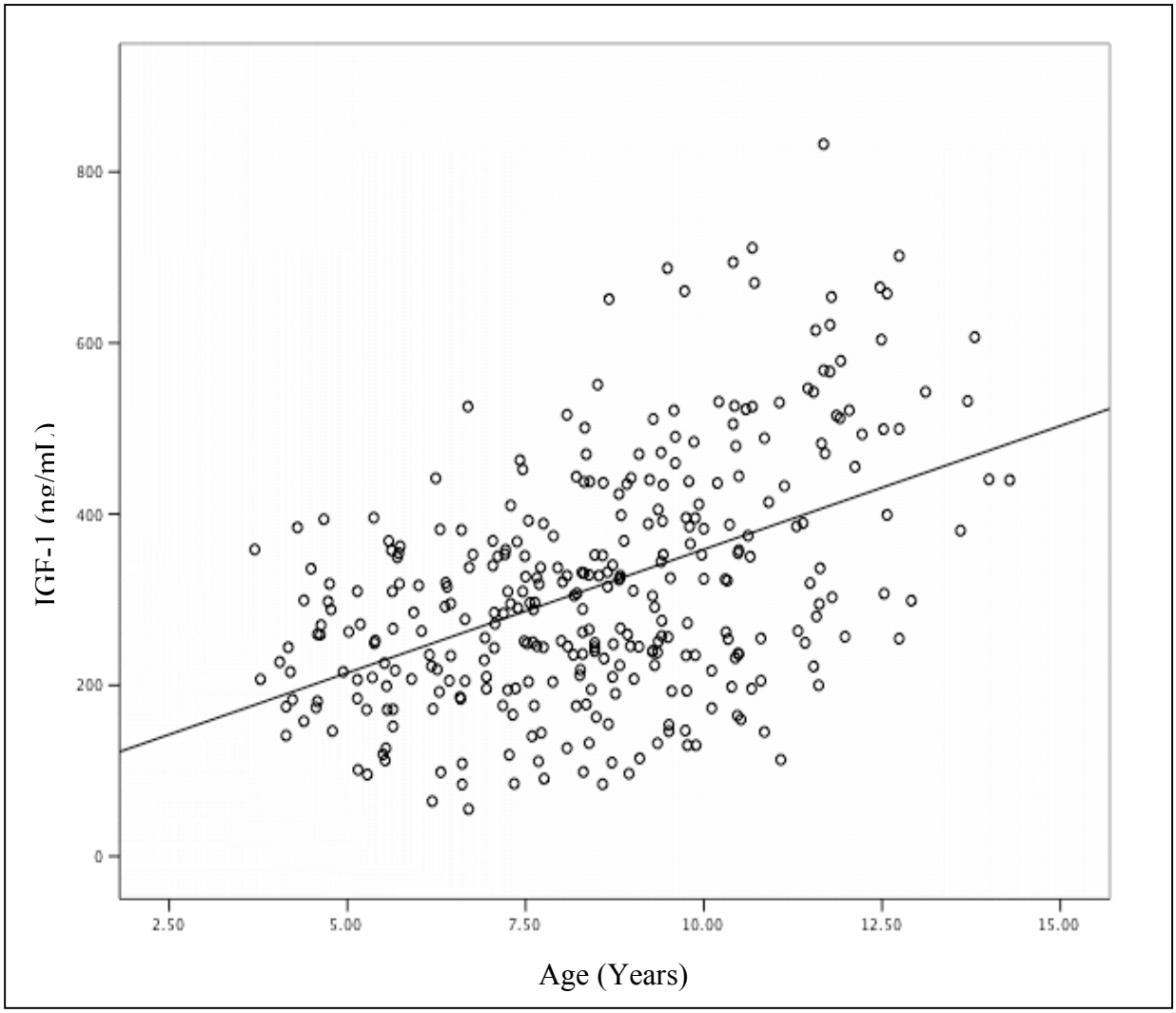
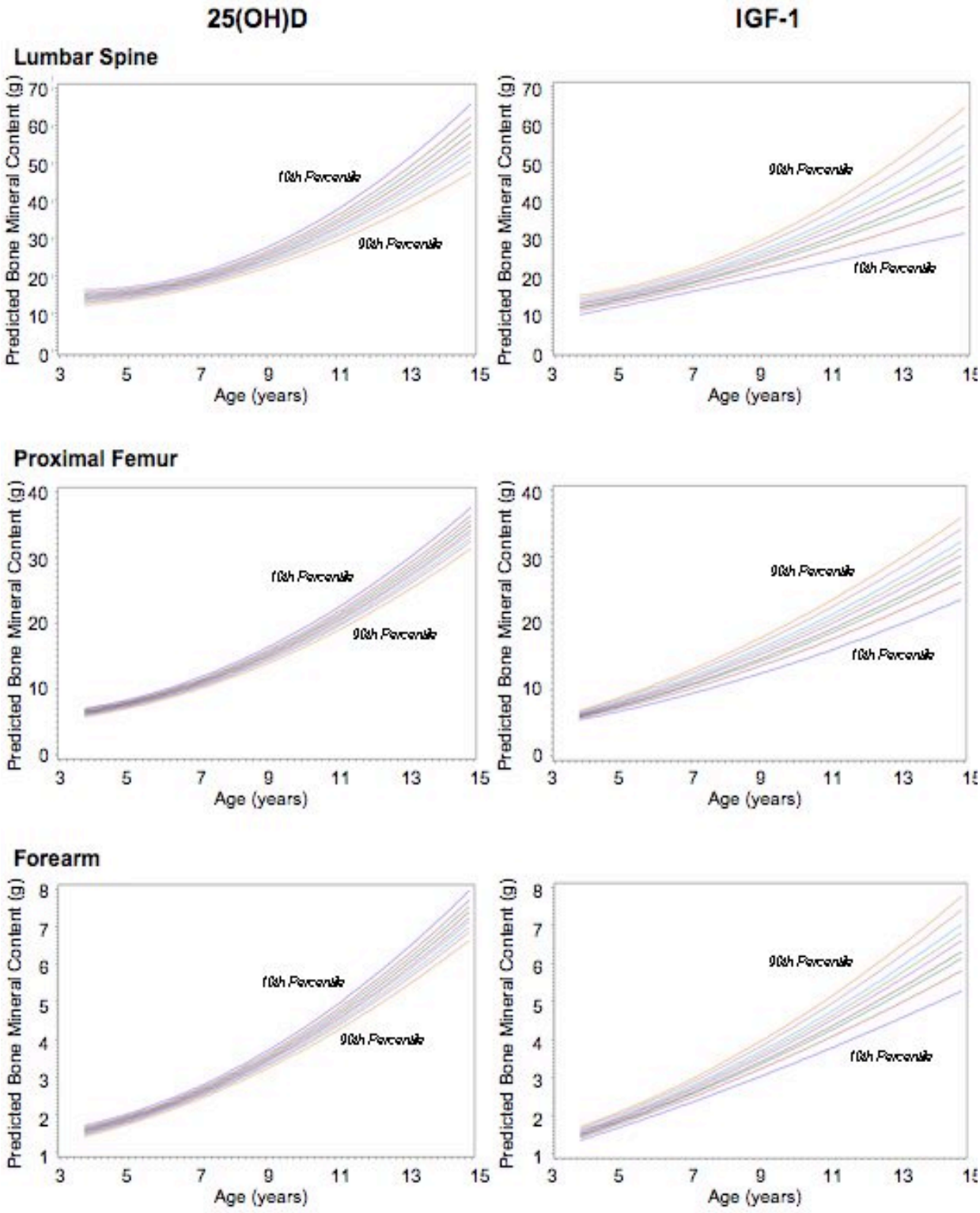


FIGURE 1. Plasma IGF-1 concentrations plotted by chronological age. All observations (N=326) were treated as cross-sectional data [i.e., each data point represented a participant's age and corresponding IGF-1 value at any time throughout the 9-year study].

FIGURE 2. Predicted curves for bone mineral content (BMC) accrual over 9 years. A linear mixed effects model shows predicted curves between BMC accrual and either baseline $\log_e$ 25(OH)D or $\log_e$ IGF-1 for a subject who entered the study at age 4 years. Each sequential curve represents a percentile (10^{th}, 20^{th}, 30^{th} … up to the 90^{th}) of either $\log_e$ 25(OH)D or $\log_e$ IGF-1 in the sample. The spread among these 9 curves is greater for the $\log_e$ IGF-1 vs. the $\log_e$ 25(OH)D plots, showing graphically that IGF-1 influences these growth curves more strongly than does 25(OH)D. The plots also demonstrate the greater positive effect of IGF-I on BMC accrual vs. the negative influence from 25(OH)D.


25(OH)D
IGF-1
Lumbar Spine
Proximal Femur
Forearm
Predicted Bone Mineral Content (g)
Age (years)
10th Percentile
90th Percentile

REFERENCES

1. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ 1992 Vitamin D_3 and calcium to prevent hip fractures in elderly women. New England Journal of Medicine **327:**1637-1642.
2. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B 2004 Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med **116**(9)**:**634-9.
3. Dawson-Hughes B, Bischoff-Ferrari HA 2007 Therapy of osteoporosis with calcium and vitamin D, pp V59-V63.
4. Matkovic V, Jelic T, Wardlaw GM, Ilich JZ, Goel PK, Wright JK, Andon MB, Smith KT, Heaney RP 1994 Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Invest **93**(2)**:**799-808.
5. Laing E, Voorhees C, Hall D, Hausman D, Lewis R 2006 A prospective analysis of plasma 25-hydroxyvitamin D and bone mass in white and black prepubertal females. Journal of Bone and Mineral Research **21**(Suppl 1)**:**S205.
6. Juul A, Holm K, Kastrup KW, Pedersen SA, Michaelsen KF, Scheike T, Rasmussen S, Muller J, Skakkebaek NE 1997 Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. Journal of Clinical Endocrinology and Metabolism **82**(8)**:**2497-2502.
7. Rosen CJ, Conover, C 1997 Growth hormone/insulin-like growth factor-I axis in aging: a summary of a National Institutes of Health aging-sponsored symposium. J Clin Endocrinol Metab **82:**3919-3922.
8. Hayden JM, Mohan S, Baylink DJ 1995 The insulin-like growth factor system and the coupling of formation to resorption. Bone **17:**93S-98S.
9. Canalis E 1993 Insulin like growth factors and the local regulation of bone formation. Bone **14:**273-276.
10. Sakata T, Halloran, B.P., Elalieh, H., Munson, S., Rudner, L., Venton, L., Ginsinger, D., Rosen, C., Bikle, D. 2003 Skeletal unloading induces resistance to insulin-like growth factor I on bone formation. Bone **32:**669-680.
11. Chlebna-Sokol D RA 2006 Insulin-like growth factor-I and mineral metabolism markers in children with idiopathic decrease in bone mass. Clinica Chimica Acta **366**(1/2)**:**257-263.
12. Kanbur NO DO, Kinik E 2005 The relationship between pubertal development, IGF-1 axis, and bone formation ion healthy adolescents. Journal of Bone MIneral Metabolism **23:**76-83.
13. Leger J 2007 The relationship between the GH/IGF-I axis and serum markers of bone turnover metabolism in healthy children. European Journal of Endocrinology **157**(5)**:**685-692.
14. Libanati C, Baylink DJ, Lois-Wenzel E, Srinvasan N, Mohan S 1999 Studies on the potential mediators of skeletal changes occurring during puberty in girls. J Clin Endocrinol Metab **84**(8)**:**2807-14.

15. Soyka LA, Grinspoon S, Levitsky LL, Herzog DB, Klibanski A 1999 The effects of anorexia nervosa on bone metabolism in female adolescents. J Clin Endocrinol Metab **84**(12)**:**4489-96.
16. Laing EM, Wilson AR, Modlesky CM, O'Connor PJ, Hall DB, Lewis RD 2005 Initial years of recreational artistic gymnastics training improves lumbar spine bone mineral accrual in 4- to 8-year-old females. J Bone Miner Res **20**(3)**:**509-19.
17. National Institutes of Health OoER NIH Policy and Guidelines on The Inclusion of Women and Minorities as Subjects in Clinical Research, vol. 2009.
18. Lohman TG, Roche AF, Martorell R 1988 Anthropometric Standardization Reference manual. Human Kinetics, Champaign, IL.
19. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, Wei R, Curtin LR, Roche AF, Johnson CL 2002 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11 (246)**:**1-190.
20. Tanner J 1962 Growth and Adolescence, 2nd ed. ed. Blackwell Scientific Publications, Oxford.
21. Hollis BW 2008 Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr **88:**(suppl)507S-10S.
22. Ito Y, Nakachi K, Imai K, Hashimoto S, Watanabe Y, Inaba Y, Tamakoshi A, Yoshimura T, Grp JS 2005 Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor beta, soluble fas, and superoxide dismutase activity for the JACC study. Journal of Epidemiology **15:**S67-S73.
23. Willis CM, Laing EM, Hall DB, Hausman DB, Lewis RD 2007 A prospective analysis of plasma 25-hydroxyvitamin D concentrations in white and black prepubertal females in the southeastern United States. Am J Clin Nutr **85**(1)**:**124-30.
24. Weaver CM, Fleet JC 2005 Vitamin D requirements: current and future (vol 80, pg 1735S, 2004). American Journal of Clinical Nutrition **81**(3)**:**729-729.
25. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B 2005 Fracture Prevention With Vitamin D Supplementation: A Meta-analysis of Randomized Controlled Trials. JAMA: Journal of the American Medical Association **293**(18)**:**2257-2264.
26. Tylavsky FA, Ryder KA, Lyytikainen A, Cheng S 2005 Vitamin D, parathyroid hormone, and bone mass in adolescents. J Nutr **135**(11)**:**2735S-8S.
27. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R 2006 Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab **91**(2)**:**405-12.
28. Viljakainen HT, Natri AM, Karkkainen M, Huttunen MM, Palssa A, Jakobsen J, Cashman KD, Molgaard C, Lamberg-Allardt C 2006 A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. J Bone Miner Res **21**(6)**:**836-44.
29. Mohan S, Baylink DJ 2005 Impaired skeletal growth in mice with haploinsufficiency of IGF-I: genetic evidence that differences in IGF-I expression

could contribute to peak bone mineral density differences. Journal of Endocrinology **185**(3)**:**415-420.

30. Mohan S, Richman C, Guo RQ, Amaar Y, Donahue LR, Wergedal J, Baylink DJ 2003 Insulin-like growth factor regulates peak bone mineral density in mice by both growth hormone-dependent and -independent mechanisms. Endocrinology **144**(3)**:**929-936.
31. Rosen CJ 1999 Serum Insulin-like growth factors and insulin-like growth factor-binding proteins: Clinical Implications. Clinical Chemistry **45**(8(B))**:**1384-1390.
32. Cadogan J, Blumsohn A, Barker ME, Eastell R 1998 A longitudinal study of bone gain in pubertal girls: Anthropometric and biochemical correlates. Journal of Bone and Mineral Research **13(10):**1602-1612.
33. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, Muller J, Hall K, Skakkebaek NE 1994 Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab **78**(3)**:**744-52.
34. Lofqvist C, Andersson E, Gelander L, Rosberg S, Blum WF, Albertsson Wikland K 2001 Reference values for IGF-I throughout childhood and adolescence: a model that accounts simultaneously for the effect of gender, age, and puberty. J Clin Endocrinol Metab **86**(12)**:**5870-6.
35. Gomez JM 2006 The role of insulin-like growth factor I components in the regulation of vitamin D. Current Pharmaceutical Biotechnology **7**(2)**:**125-132.
36. Rucker D, Ezzat S, Diamandi A, Khosravi J, Hanley DA 2004 IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. Clinical Endocrinology **60**(4)**:**491-499.
37. Bianda T, Hussain MA, Glatz Y, Bouillon R, Froesch ER, Schmid C 1997 Effects of short-term insulin-like growth factor-I or growth hormone treatment on bone turnover, renal phosphate reabsorption and 1,25 dihydroxyvitamin D-3 production in healthy man. Journal of Internal Medicine **241**(2)**:**143-150.
38. Abrams SA, Griffin IJ, Hawthorne KM, Gunn SK, Gundberg CM, Carpenter TO 2005 Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. J Clin Endocrinol Metab **90**(10)**:**5576-5581.
39. Bay K AA, Skakkebaek NE 2004 Estradiol levels in prepubertal boys and girls-analytical challenges. International Journal of andrology **27:**266-273.
40. Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston CC, Jr. 1991 Role of physical activity in the development of skeletal mass in children. J Bone Miner Res **6**(11)**:**1227-33.

CHAPTER 4

SUMMARY AND CONCLUSIONS

While PBM achieved in adolescence is determined primarily by genetic predisposition, hormonal and environmental influences can impact skeletal mineralization, bone expansion, and linear growth.[1] The extent to which hormones influence bone metabolism during growth is unknown. Vitamin D supplementation reduces bone loss and prevents fracture in the elderly, therefore the propensity is to assume likewise beneficial effects are occurring on bone during growth and development. However, an individual's hormonal status during maturational growth is uniquely different from all other life phases. IGF-I levels are at their highest concentration during this time period, and may significantly impact bone growth and PBM.

The present study was conducted to prospectively evaluate the influences of both IGF-I and 25(OH)D on bone mineral accrual in pre- and early-pubertal females. The primary finding from the study was that IGF-I is more strongly associated with BMC accrual at the total body, total proximal femur, radius, and lumbar spine regions in comparison to 25(OH)D. When IGF-I and 25(OH)D were included in the same regression equation, 25(OH)D did not have a significant predictive effect on BMC accrual at any site above and beyond that of IGF-I. At each skeletal site, the rate of BMC accrual was negatively associated with changes in 25(OH)D, but was positively associated with changes in IGF-I.

IGF is involved in both the bone formation and the bone resorption aspects of bone remodeling. *In vitro* and *in vivo* studies provide substantive evidence to indicate IGF-I involvement in osteoblast and osteoclast cell proliferation, but the process is immensely complex, and the precise role of the hormone has yet to be defined.[2,3,4] Animal model research in mice report that throughout puberty, IGF-I knockout mice (KO) display an 88-90% reduction in femoral BMC compared with corresponding control mice. Additionally, BMD increases ~40% during puberty in IGF-I mice, whereas it does not significantly increase in the IGF-I KO.[5] Most cross-sectional studies in children and adolescents support a positive correlation between IGF-I and BMD. Libanati et al[6] showed a pubertal stage dependent positive correlation between serum IGF-I and lumbar BMC, measured by DXA. The present study was the first investigation to demonstrate a significant positive correlation between changes in IGF-I and BMC. To our knowledge, only one other study has prospectively analyzed IGF-I and the change in BMC.[7] This study did not show a significant relationship between IGF-I and change in BMC and BMD over a two-year period in healthy adolescent females with a baseline age of 12 years. While this study did capture the significant changes in serum IGF-I that accompany puberty, significant changes in the girls' BMC and BMD were only observed during the final 6 months of the study.

The negative correlation between IGF-I and 25(OH)D observed in the present study lends support to the interrelationships observed between the two hormones. Cell culture studies indicate that IGF-I and $1,25(OH)_2D$ up regulate each other. $1,25(OH)_2D$ promotes the action of IGF-I by increasing IGF-I receptors. In turn, IGF-I stimulates the hydroxylation of the circulating 25(OH)D to the active $1,25(OH)_2D$ form in the kidneys

through the stimulation of the 1α-hydroxylase enzyme.[8,9] The high levels of IGF-I may explain the negative correlation of between 25(OH)D and BMC. It may be that in the presence of higher circulating levels of IGF-I leads to lower levels of 25(OH)D because IGF-I is stimulating hydroxylation to the active $1,25(OH)_2D$ form which is positively influencing bone. A limitation of this study was that the active vitamin D metabolite, $1,25(OH)_2D$, was not assessed. By focusing only on the 25(OH)D form, information on the functional role of vitamin D during childhood bone growth may not have been captured. These data were collected from an already completed trial and provide important information on vitamin D and IGF-I in association with bone mineral accrual.

In conclusion, these longitudinal data in early adolescent females indicate that both 25(OH)D and IGF-I have a significant impact on bone mineral accrual; the positive relationship between IGF-I on BMC is greater than the negative association of 25(OH)D with BMC. Furthermore, IGF-I and Vitamin D are significantly and negatively correlated in children Additional research studies are needed to determine if the influences of vitamin D on bone are mediated by IGF-I, and to delineate the relationship of IGF-I and $1,25(OH)_2D$ and their effects on bone growth in children.

References

1. Recker RR, Heaney RP 1993 Peak bone mineral density in young women. Jama **270**(24)**:**2926-7.

2. Hayden JM, Mohan S, Baylink DJ 1995 The insulin-like growth factor system and the coupling of formation to resorption. Bone **17:**93S-98S.

3. Canalis E 1993 Insulin like growth factors and the local regulation of bone formation. Bone **14:**273-276.

4. Sakata T, Halloran, B.P., Elalieh, H., Munson, S., Rudner, L., Venton, L., Ginsinger, D., Rosen, C., Bikle, D. 2003 Skeletal unloading induces resistance to insulin-like growth factor I on bone formation. Bone **32:**669-680.

5. Kasukawa Y, Baylink DJ, Wergedal JE, Amaar Y, Srivastava AK, Guo RQ, Mohan S 2003 Lack of insulin-like growth factor I exaggerates the effect of calcium deficiency on bone accretion in mice. Endocrinology **144**(11)**:**4682-4689.

6. Libanati C, Baylink DJ, Lois-Wenzel E, Srinvasan N, Mohan S 1999 Studies on the potential mediators of skeletal changes occurring during puberty in girls. J Clin Endocrinol Metab **84**(8)**:**2807-14.

7. Cadogan J, Blumsohn A, Barker ME, Eastell R 1998 A longitudinal study of bone gain in pubertal girls: Anthropometric and biochemical correlates. Journal of Bone and Mineral Research **13(10):**1602-1612.

8. Gomez JM 2006 The role of insulin-like growth factor I components in the regulation of vitamin D. Current Pharmaceutical Biotechnology **7**(2)**:**125-132.

9. Rucker D, Ezzat S, Diamandi A, Khosravi J, Hanley DA 2004 IGF-I and testosterone levels as predictors of bone mineral density in healthy, community-dwelling men. Clinical Endocrinology **60**(4)**:**491-499.

APPENDICES

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.

Additional questions or problems regarding your rights as a research participant should be addressed to The Chairperson, Institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 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.

a. The procedures are as follows:

a) Testing will be conducted at 10 different time points (the first five, each 6 months apart: months 0, 6, 12, 18 and 24) and (the last five, each 12 months apart: 36, 48, 60, 72 and 84). At 0, 12, 24, 36, 48, 60, 72 and 84 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).

Time point (months)	Sessions	Payment
0	1,2,3,4	Up to $65/quarter
6	3	Up to $65/quarter
12	1,2,3,4	Up to $65/quarter
18	3	Up to $65/quarter
24	1,2,3,4	$520 minus payments at 0, 6, 12, and 18 months
36	1,2,3,4	$100
48	1,2,3,4	$100
60	1,2,3,4	$100
72	1,2,3,4	$100
84	1,2,3,4	$100
		Total = $1,020

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 radiologic technologist (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

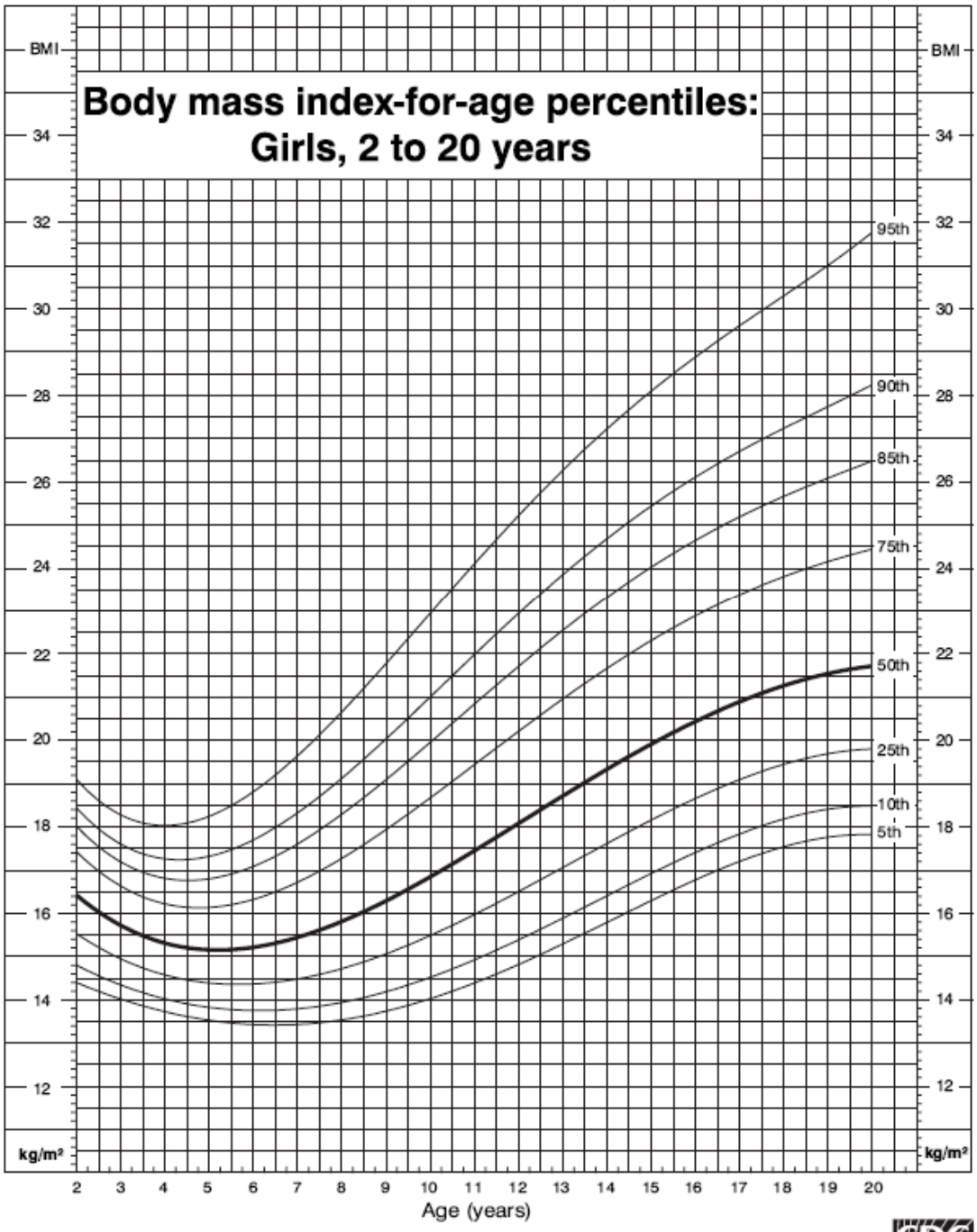
Please sign both copies, keep one and return one to the researcher.

Additional questions or problems regarding your child's rights as a research participant should be addressed to *The Chairperson, Institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu*

APPENDIX B

BMI chart

CDC Growth Charts: United States



Published May 30, 2000.
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

SAFER · HEALTHIER · PEOPLE™

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.

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

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

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

c. Each food that you ate.

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

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

3. 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?

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

_____

7. About how many glasses of water do you drink each day? _____

AY 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.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

DAY 2 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.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

DAY 3 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.

Time Eaten	Foods Eaten	Preparation Methods	Amount (cup, 1/2 cup, piece, etc)

APPENDIX D

Demographic Questionnaire

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

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. What was your birth weight? _____ Pounds _____ Ounces
4. Gender: (Circle One) Female Male
5. What is your grade in school?_____
6. How do you describe yourself? (Circle One or More: Mixed racial heritage should be indicated by checking more than one category)

Ethnicity: Hispanic or Latino
Non-Hispanic or Latino

Race: American Indian or Alaska Native
Asian
Black or African American
Native Hawaiian or other Pacific Islander
White
any combination of the above

7. Do you live with your parents? (Circle One) YES NO

   7a. If no, with whom do you live? _____

8. Do you have any brothers or sisters? (Circle One) YES NO

   8a. If yes, list ages of: _____Years (Brother) _____Years (Sister)
   _____Years (Brother) _____Years (Sister)
   _____Years (Brother) _____Years (Sister)

   8b. If yes, do they participate in sports? (Circle One) YES NO

   8c. If yes, list the sport and gender of sibling. Sport_____(Brother or Sister)
   Sport_____(Brother or Sister)

Sport_____(Brother or Sister)

Subject ID#: _____

Interviewer: _____

Date of Interview: ___

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

10. What is your mother's height and weight?_____Feet___Inches_____Pounds

11. What is your father's height and weight?_____Feet___Inches_____Pounds

12. 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
Over $100,000

13. What is your mother's occupation?_____

14. What is your father's occupation?_____

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

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. Have you gained or lost any weight (≥ 10 pounds) in the last 3 months? (Circle One)

   YES NO

   1a. If yes, how much? +_____ pounds OR -_____pounds

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

   2a. If yes, how much? _____ feet _____ inches

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

   Poor Fair Good Excellent

4. Have you started your menstrual cycles? (Circle One) YES NO

   If so, what date?

   4a. Are your menstrual cycles regular? YES or NO; *circle one*

   4b. If not, how long have they been irregular? _____

5. Have you ever used birth control pills? YES or NO; *circle one*

   5a. How old were you when you began using birth control pills? _____

   5b. How long have you been using them? _____

   5a. What periods of time did you stop using birth control pills? _____

   (Please give dates, if applicable)

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

6a. If yes, what diseases? _____

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

7a. If yes, what medications? _____ Amount per day _____

_____ Amount per day _____

_____ Amount per day _____

8. Do you currently smoke cigarettes? ___ YES or NO; *circle one*

8a. If yes, on the average, about how many cigarettes a day do you smoke?

____1-5, ____6-14, ____15-24, ____25-35, ____35 or more

9. If you used to smoke but do not smoke now, how long did you smoke?

_____months or years.

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.

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

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 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

   _____ years _____ months

6. Have you ever eaten a large amount of food and then vomited to get rid of the food? (Circle One) YES NO

   6a. If yes, how old were you? _____ years _____ months

   _____ years _____ months

7. Were you breastfed as an infant? (Circle One) YES NO

   7a. If yes, for how many months (exclusive)? _____

   7b. If yes, for how many months (supplemented with formula)?_____

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

Physical Activity

The next questions that I will ask you are about your physical activity such as P.E. and exercise. 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
   Below average
   Average
   Above average
   Very high

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 or do physical activity regularly (not including P.E. class)?
   (Circle One) YES NO
   3a. If yes, how often? _____ hours per day/week/month (Circle One)

4. Do you participate in P.E. at school? (Circle One) YES NO
   4a. If yes, how often? _____ hours per day/week/month (Circle One)

5. How many hours, on average, do you spend watching TV, or on the computer? ___

Subject ID#: _____

Interviewer: _____

Date of Interview: _____

GENERAL INFORMATION QUESTIONNAIRE

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? _____

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? _____

3. Have you ever had a bone fracture or broken bone? (Circle One) YES NO

   3a. If yes, which bone(s)? _____

   3b. If yes, how old were you? _____years _____months

   3c. If yes, in what type of circumstance did the fracture take place? _____

   3d. If yes, how was the fracture treated (casting, medication, rest, etc.)?____

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 E

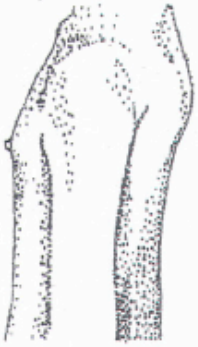
Tanner stages of sexual maturation

Lewis, Richard D.

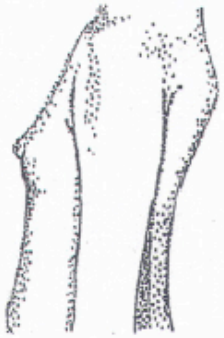
SEXUAL MATURATION QUESTIONNAIRE (GIRLS)

Subject ID#: _____
Date: _____

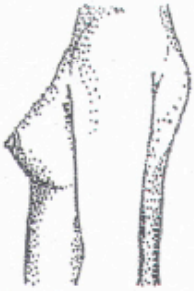
We need to find out what stage of sexual development you are in. Please look at the pictures and circle the one that looks most like you now.



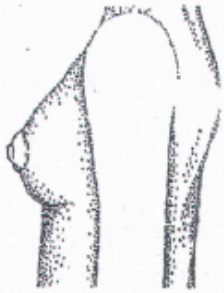
Stage 1: Elevation of papilla only.



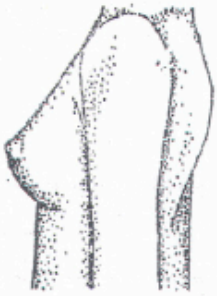
Stage 2: Elevation of breast and papilla as small mound, areola diameter enlarged.



Stage 3: Further enlargement without separation of breast and areola.



Stage 4: Secondary mound of areola and papilla above the breast.



Stage 5: Recession of areola to contour of breast.

Thank you for answering this question. Please send this questionnaire back to the researcher in the stamped envelope provided.

J. Tanner (1962) Growth and Adolescence, 2nd ed. Blackwell Scientific Publications, Oxford.

APPENXIX F

Anthropometric data recording sheet

Bone, Growth and Dietary Intakes in 4-8 Year Old Girls
(UGA BONE STUDY)
Participant Information Sheet

Anthropometrics/DXA

Subject ID: _____ timepoint:_____ Visit Date:_____

Race/Ethnicity: _____ Sex: _____

DOB: Month _____ Day _____ Year _____

Weight (kg): _____ _____ _____
Measure 1 Measure 2 Average of 1 and 2

Height (cm): _____ _____ _____
Measure 1 Measure 2 Average of 1 and 2

Sitting Ht (cm): _____ _____ _____
Measure 1 Measure 2 Average of 1 and 2

BMI (g/cm^2): _____

Length of Radius (cm): _____ **R** or **L** handed (circle one)

DXA operator use

- ❑ Total Body
- ❑ Lumbar Spine
- ❑ Proximal Femur
- ❑ Radius

Scan date: _____
Completed by: _____
initials of operator

Comments:_____
_____
_____
_____
_____.....