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Magnesium and bone mineral acquisition in ethnically diverse adolescents

Gretchen Kay Vannice
San Jose State University

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MAGNESIUM AND BONE MINERAL ACQUISITION IN ETHNICALLY DIVERSE ADOLESCENTS

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Presented to

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In Partial Fulfillment

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by

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APPROVED FOR THE DEPARTMENT OF
NUTRITION AND FOOD SCIENCE

Dr. May-Choo Wang

Dr. Clarie Hollenbeck

Dr. Lucy McProud

Dr. Laura Bachrach

APPROVED FOR THE UNIVERSITY

ABSTRACT

Magnesium and Bone Mineral Acquisition in Ethnically Diverse Adolescents.

by Gretchen K. Vannice

Magnesium (Mg) is known to play a role in skeletal health. Associations between Mg and bone mass were investigated in 306 ethnically diverse adolescents, who were a sub-set of a 4-year longitudinal study. Bone mass was measured by dual X-ray absorptiometry (DXA). Annualized gains in bone mass were calculated. Dietary data were gathered using a Food Frequency Questionnaire.

Dietary Mg was observed to be inversely associated with bone mineral density (BMD) and bone mineral apparent density (BMAD) at the hip in mature females. The regression coefficients (S.E.) were approximately .05 (.02) g/cm^2 per 100 mg of Mg for BMD and .015 (.006) g/cm^3 per 100 mg Mg for BMAD both years of data collection ($P < 0.05$). Associations were observed mostly in females.

Results suggest Mg may be negatively related to bone mass. Additional studies are needed to determine to what extent, if any, Mg influences skeletal health.

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PREFACE

The following is a publication style thesis. Chapter I and III are written according to guidelines outlined in the *Publication Manual of the American Psychological Association, 4th edition, 1994.* Chapter II is written in journal format and will be submitted to *The American Journal of Clinical Nutrition.*

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CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

Introduction

Osteoporosis is a substantial health problem in the United States (Looker et al., 1997; Melton, Chrischilles, Cooper, Lane, & Riggs, 1992). The prevalence of osteoporosis in the United States ranks among the highest in the world (World Health Organization, 1994). Among 50 year old Caucasians, the lifetime risk of skeletal fracture at the hip, spine or forearm is 39.7% in women and 13.1% in men (Melton et al., 1992). In 1995, an estimated 13.8 billion dollars were spent to treat skeletal fractures related to osteoporosis in adults over the age of 45 in the United States alone (Ray, Chan, Thamer, & Melton, 1997). While the majority of these costs were spent for treatment of Caucasian women, non-Caucasian women and men suffered from osteoporosis related skeletal fractures as well (Ray et al., 1997). Compounding the enormous medical costs associated with this condition are the devastating consequences to quality of life, morbidity and mortality (World Health Organization, 1994).

The fundamental pathogenesis of osteoporosis still remains unknown (Lonzer et al., 1996) although there is consensus that the etiology of osteoporosis is multifactorial (Marcus, 1996; Ott, 1990). Lifestyle and genetics factors, as well as gender factors appear to play powerful roles. The mechanisms by which these factors contribute to the occurrence of osteoporosis, and consequent skeletal fractures, appear to be based primarily on their impact on bone mass.

Increasingly, it is acknowledged that osteoporosis has its origins in childhood (Kreipe, 1995). Osteoporosis has been described as a pediatric condition, which manifests itself in old age (Lysen & Walker, 1997). Because skeletal mass in adulthood

is the result of both the amount of bone gained during growth and subsequent loss, factors affecting bone mass acquisition during growth and adolescence are important determinants of future resistance to osteoporotic fractures (Gilsanz et al., 1988). Identifying factors which contribute to optimizing bone mass, especially during periods of accelerated bone mineral acquisition, may contribute to a reduction in the incidence of osteoporosis and related fractures (Ott, 1990; Recker et al., 1992; Sowers & Galuska, 1993).

Data are insufficient regarding the role of diet and nutrition in optimizing bone mass (Bailey, 1997; New, Bolton-Smith, Grubb, & Reid, 1997). Dietary factors are of special interest to those investigating the etiology of osteoporosis for two reasons (Heaney, 1996). First, dietary factors are believed to play an integral role in the acquisition of bone mass and second, they are modifiable. To date, calcium is by far the most studied nutrient in skeletal health research (Angus, Sambrook, Pocock, & Eisman, 1988; Cumming, 1990; Heaney, 1992; Sowers & Galuska, 1993). Other nutrients include magnesium, phosphorus, sodium, zinc, copper, and vitamins A, C, D and K and dietary protein (Cooper et al., 1996; Heaney, 1996).

In the present study, associations were investigated between dietary magnesium and bone mineral acquisition in a sample of ethnically diverse adolescents and young adults. In particular, data gathered from Asian, Caucasian and African Americans during the first and second years of a 4-year longitudinal study of bone mineral acquisition were analyzed (Bhudhikanok et al., 1996; Wang et al., 1997). Since magnesium is involved in skeletal metabolism, it may be an important factor in qualitative changes of bone matrix

(Sojka & Weaver, 1995). It was hypothesized that dietary magnesium would be positively related to bone mineral acquisition, after taking into account the influences of gender, pubertal stage, race, body mass index, dietary calcium, protein, energy intake and weight-bearing activity.

Review of the Literature

Osteoporosis

Definition. Osteoporosis is defined as a systemic skeletal "disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" (World Health Organization, 1994, p.3).

Prevalence. Osteoporosis is worldwide, and prevalence varies by country, race and gender (Peck, 1993); it is increasing and projected to continue to increase (Kanis, Melton, Christiansen, Johnston, & Khaltaev, 1994). The incidence of osteoporosis in the United Sates ranks among the highest, along with New Zealand and Scandinavia; Asian countries rank at an intermediate level and the South African Bantu report the lowest incidence (Peck, 1993). Firm estimates of the prevalence of osteoporosis are challenging because of differing criteria used to define osteoporosis (Looker et al., 1997; Melton, 1997).

Across all races, osteoporosis-related fractures have been reported to occur much more frequently in women than men (Villa & Nelson, 1996). In the United States, Caucasians experience the highest incidence of osteoporosis-related skeletal fractures. Fracture incidence among Asian Americans is intermediate and lowest among African

Americans when compared to Caucasians (Gilsanz, Roe, Mora, Costin, & Goodman, 1991; Villa & Nelson, 1996)

Medical costs and outcomes. Osteoporotic fractures are a significant health problem in the United States and the costs associated with their treatment are enormous (Looker et al., 1997). The clinical significance of osteoporosis is manifested in osteoporosis-related skeletal fractures, which are then intensified by high medical costs and devastating effects on the quality of life (World Health Organization, 1994). In 1995, an estimated 13.8 billion dollars were spent to treat osteoporosis-related fractures in adults over the age of 45 years in the United States alone (Ray et al., 1997). While a disproportionate majority of these costs were spent for treatment of Caucasian women (75.1%, or $10.3 billion), about 5 % ($ 700 million) was spent to treat non-Caucasian women. Nearly 20% of the treatment expense was incurred by Caucasian and non-Caucasian men (18.4% and 1.3%, respectively) (Ray et al., 1997).

Skeletal fractures in old age often have devastating effects on quality of life and increase mortality. Unfortunately, at the usual age of diagnosis, only limited therapies are available (Lonzer et al., 1996). Five to twenty percent of individuals with a hip fracture die within one year of the fracture event, and more than 50% become permanently incapacitated (Peck, 1993). While many risk factors that predispose individuals to osteoporosis have been identified, the fundamental pathogenesis of osteoporosis still remains unknown (Lonzer et al., 1996). Because there is no cure for osteoporosis, the predominant focus must be on prevention through maximizing acquisition of bone mass (Kreipe, 1995)

Skeletal Development

Pubertal growth. Adolescence is a time of rapid growth and development, characterized by a unique and highly anabolic period. Height and weight increase, internal organs enlarge, and body composition changes as both lean mass and body fat increase in quantity and distribution. Body mass nearly doubles during adolescence (Shils, Olson, & Shike, 1994). Many areas of development and growth manifest during adolescence, including physical, psychological, intellectual and cognitive. The timing, intensity and extent of pubertal development and growth velocity, along with gender differences and changes in body composition, as well as individual variation all need to be considered when evaluating nutritional needs during adolescence (Shils et al., 1994).

Anthropometric gender differences appear during adolescence (Shils et al., 1994). Males acquire a larger skeleton, greater lean muscle mass, more weight and a lower percentage of body fat relative to females. Females gain both lean mass and adipose tissue, but females gain a greater percentage of body fat compared to males. Hormone activity increases during adolescence and leads to further physiological differences between males and females. Gender differences in body composition and hormones affect nutrient needs in the adolescent (Shils et al., 1994).

Males enter puberty about 2 years later than females, so their pubertal growth and peak height growth continue longer than females. The most rapid linear growth spurt occurs between the ages of 12 – 15 years in the average American male and ages 10 - 13 years in the average American female, with females experiencing the greatest growth the year prior to menarche. Males have longer legs than women because epiphyseal fusion

occurs sooner in females (Seeman, 1998). African Americans have longer legs and shorter trunks than Caucasians whereas Asians have longer trunks and shorter legs than Caucasians (Seeman, 1998). Peak nutritional requirements appear to occur simultaneously with peak growth velocity (Shils et al., 1994).

The tempo of maturation, in terms of intensity and extent of puberty and timing of growth velocity, varies from individual to individual (Ganong, 1997). Because there is so much individual variation, chronological age seems to be a poor indicator of skeletal status; hence, clinicians and researchers have found maturational age to be more useful. Standards have been developed for classifying maturational age. Tanner's Sexual Maturity Ratings, based on breast development and pubic hair stage in girls and genitalia and pubic hair stage in boys, are widely used to classify individuals by stage of sexual development (Tanner, 1962). Furthermore, differences in the tempo of pubertal development among racial groups exist and must be considered when classifying subjects of different race (Eveleth & Tanner, 1990; Villa, 1994)

Bone acquisition. It is becoming recognized that pre-puberty and adolescence are critical periods of time for bone mineral acquisition (Ott, 1991). Acquisition of bone mass occurs gradually throughout childhood, and accelerates during adolescence until sexual maturity is reached (Katzman, Bachrach, Carter, & Marcus, 1991). Bone mineral density, (BMD), has been shown to increase considerably during puberty and puberty clearly influences BMD (Gilsanz et al., 1988). Nearly half of the adult skeletal mass is laid down in the second decade of life (Kreipe, 1995) and an estimated 90% of adult skeletal mass is attained by the end of adolescence (Lonzer et al., 1996). Investigators

report that as much bone mineral may be deposited during the short period of maximum skeletal growth in adolescence as may be lost over the adult lifetime (Bailey, 1997).

There are essentially three phases of bone development. These include growth, consolidation, and senescence. The growth phase, which predominantly occurs during the first two decades of life, results in an increase in length and width of bones as well as acquisition of bone mineral. During this time, bone formation exceeds bone resorption. Bone modeling creates the adult shape and skeletal dimensions. Ninety percent of adult skeletal mass is acquired during the growth phase. The growth spurt at puberty represents the final period of extensive growth of healthy bones (Kreipe, 1995). Bone size appears to increase faster than bone mineral; during puberty, bone size may more closely proximate its peak adult value than does the mineral acquired within it (Bailey, 1997). Bone epiphyses close near the time peak height velocity is reached.

Researchers have observed bone mineral content (BMC) and BMD values to be similar among 9-10 year old boys and girls (Bonjour, Theintz, Buchs, Slosman, & Rizzoli, 1991), with gender differences becoming expressed during adolescence (Gilsanz et al., 1994). Females experience their peak height velocity and widening of the bony pelvis around 12 years of age, and investigators have reported substantial increases in bone mass during the first 2 years after menarche in girls (Bonjour et al., 1991; Theintz et al., 1992). Males experience peak height velocity and widening of the shoulders around 14 years of age. After this time, bones continue to increase in density and thickness and this marks the beginning of the consolidation phase (Kreipe, 1995).

The consolidation phase usually occurs during the second and third decades of

life. This is when bone remodeling begins. Repeated cycles of bone resorption (by osteoclast activity) and bone formation (by osteoblastic activity) on localized surface areas of bone characterize bone remodeling. The process of bone remodeling is regulated by incompletely understood cellular, hormonal and other mechanisms (Kreipe, 1995). This distinguishes the time when bone mineral is being deposited, resulting in increases in bone mineral density. Bone is primarily comprised of both cortical (compact) and trabecular (porous) components, and the trabecular component is believed to be more metabolically active (Rodin et al., 1990).

Senescence begins around the age of 40 in women and 50 in men. This is when natural losses of bone occur. Age-related bone losses occur in adults although the natural progression is still unclear (Rodin et al., 1990). Women lose a greater percentage of bone over a lifetime compared to men, 25% and 12% respectively (Pollitzer & Anderson, 1989). It is believed that greater quantities of bone mineral acquired during growth and consolidation will allow for more mineral loss before the net loss of bone mass puts one at risk for osteoporosis-related skeletal fractures (Kreipe, 1995).

Peak bone mass. Peak bone mass (PBM) is described as the highest quantity of bone one achieves in a lifetime (L.K. Bachrach, personal communication, 1998; Kreipe, 1995). Researchers hypothesize that individuals who attain a greater PBM will have a reduced risk of osteoporosis-related skeletal fractures (Hui, Slemenda, & Johnston, 1988). Epidemiological studies of osteoporosis risk fractures have begun to emphasize the importance of achieving adequate bone mass during growth since maximizing bone mineral deposits during adolescence and young adulthood seems to be one way to

minimize risk for osteoporosis (Kreipe, 1995). It is not clear if, or to what extent, gains in BMD occur after adolescence (Recker et al., 1992). Elucidation of precise mechanisms contributing to achievement of PBM is essential in order to effectively develop and implement preventive measures (Matkovic et al., 1994). The primary focus of osteoporosis prevention involves maximizing peak bone mass during the growth and consolidation phases, with a secondary focus on bone mass preservation during senescence (Lysen & Walker, 1997).

Bone Measurement

Interpreting bone mineral data in adolescents and young adults is challenging because changes in bone mineral are occurring simultaneously with changes in overall bone size (Katzman et al., 1991). This challenge is further complicated by racial differences in bone mass which are partially due to variations in bone size (Seeman, 1998).

Dual energy X-ray absorptiometry (DXA). Bone mineral at sites throughout the skeleton is often assessed using dual energy X-ray absorptiometry (DXA) which reports bone mineral density based on a projected two-dimensional image. DXA is a non-invasive method which makes use of ionizing radiation (the amount of radiation attenuation is directly related to the amount of bone present). The most common sites measured are spine and hip. Once the data are acquired, a region of interest (ROI) is defined and calculations are completed, providing values of bone mineral content (BMC) and bone mineral density (BMD) (Carter, Bouxsein, & Marcus, 1992).

DXA is a preferred method for bone mineral assessment because exposure to x-ray radiation is relatively low (about 3mrem) and resolution is high (1.5mm) (Ho, Kim,

Schaffler, & Sartoris, 1990). High precision (1-2%) is well-documented using phantoms and the accuracy of DXA has been validated using cadavers (Ho et al., 1990). The major limitation of DXA is that it projects a two-dimensional image, providing a measurement of projected areal density, not true volumetric density (Ho et al., 1990).

Expression of bone mass. Bone mass is usually expressed as BMC (g) or BMD (g/cm^2). Dividing the bone region's apparent bone mineral content (BMC), in grams, by its projected area in a specified region of interest (ROI) derives the latter (BMD). Using DXA, the depth of the ROI is not "seen," so bone volume is not taken into account. Areal BMD does not reflect differences in bone size among measured subjects (Seeman, 1998), nor bone thickness (Katzman et al., 1991). In summary, the bigger the bone, the higher the areal BMD. (Seeman, 1998). This is of clinical importance because bones of larger width and length tend to be thicker and bone thickness cannot be factored into BMD. As a result, simply using BMD as a measurement for bone density overestimates BMD in taller individuals and underestimates BMD in shorter individuals (Katzman et al., 1991). For pubertal subjects whose bones are growing, and individuals of different races, it is important that bone size be considered. (Seeman, 1998).

Several algorithms for estimating volumetric ("true") bone mineral density have been proposed. One such algorithm assumes that the bone is cylindrical in shape; estimates of bone volume are mathematically derived and used to estimate volumetric bone mineral density. This estimate is referred to as "bone mineral apparent density," or BMAD (g/cm^3) (Carter et al., 1992; Katzman et al., 1991). BMAD is especially appropriate in longitudinal studies when individuals with changing skeletal dimensions,

such as adolescents and young adults, are being followed. BMAD can also be used for cross-sectional analyses comparing differently sized subjects, such as Asians and Caucasians (Carter et al., 1992).

Factors Associated with Bone Mass

Identifying factors that influence bone mass during skeletal growth is of great interest because the amount of bone mass gained during childhood and adolescence may be an important determinant of whether an individual develops osteoporosis-related skeletal fractures later in life (Gilsanz et al., 1994). The fact that such a large accumulation of bone mineral occurs during adolescence suggests that both genetic and environmental factors operating at this stage in life may play critical roles in determining bone mass, and impact risk for osteoporosis (Young et al., 1995). The exact timing at which adolescents reach peak bone mass is unclear (Bonjour et al., 1991; Matkovic et al., 1994; Recker et al., 1992; Rodin et al., 1990). Non-modifiable factors, including genetics, puberty, gender and race all appear to uniquely contribute to skeletal development (Gilsanz et al., 1994; Gilsanz et al., 1988; Kelly, Twomey, Sambrook, & Eisman, 1990; Lonzer et al., 1996; Pollitzer & Anderson, 1989; Young et al., 1995). Diet and physical activity are modifiable environmental factors which appear to play important roles as well (Eisman, Sambrook, Kelly, & Pocock, 1991; Kreipe, 1995; Lysen & Walker, 1997; Ott, 1990; Slemenda, Miller, Hui, Reister, & Johnston, 1991; Sowers & Galuska, 1993). While the relative contributions of genetic and environmental factors to bone mass is uncertain (Angus et al., 1988), it has been suggested that genetic potential may be reached only if environmental factors are favorable (Bachrach, 1996).

Timing of peak bone mass (PBM). The age at which PBM occurs in the lifespan is unclear and continues to be researched. Investigators have observed PBM to occur as early as adolescence and as late as the fourth decade of life (Bonjour et al., 1991; Matkovic et al., 1994; Recker et al., 1992; Rodin et al., 1990). One investigation of bone mineral acquisition in Caucasian adolescents aged 9-18 years using DXA suggested that PBM occurs during adolescence in boys and girls, although at different ages (Bonjour et al., 1991). In this cross-sectional study, PBM was observed in 14-15 year old girls, and in 17-18 year old boys. Other researchers have observed adolescent gender differences in PBM as well (Gordon, Halton, Atkinson, & Webber, 1991).

PBM has been observed to occur in the third and fourth decades of life (Recker et al., 1992; Rodin et al., 1990). A five-year longitudinal study of 156 healthy, non-pregnant college-aged women indicated BMD, measured by dual-photon absorptiometry, increased into the third decade of life, ceasing at 28.3-29.5 years of age. (Recker et al., 1992). Lastly, a cross-sectional study which investigated 225 healthy, premenopausal Caucasian women, ages of 18 –52 years, suggested PBM to occur at the spine in the middle of the fourth decade of life (Rodin et al., 1990). In contrast, bone losses were observed at the femoral neck (FN) commencing in the late twenties.

Non-modifiable Factors

Genetics. Bone mass is strongly influenced by both genetic and familial factors (Kelly et al., 1990; Lonzer et al., 1996; Young et al., 1995). It has been suggested that genetics may have the greatest influence on bone mineral acquisition and loss (Kreipe, 1995). Genetic influences on BMD may be skeletal site specific (Pocock et al., 1987).

Differences in BMD between mothers and daughters have been observed. Seeman and colleagues have reported that premenopausal daughters of women with osteoporosis may have a relatively low bone mass, when compared to non-osteoporotic, healthy controls (Seeman et al., 1989)

Puberty. In a cross sectional investigation of the effects of puberty on BMD during skeletal growth in 101 children aged 2-18 years, puberty has been shown to have a greater effect on BMD than that of gender alone (Gilsanz et al., 1988). In another cross sectional investigation in children and adolescents, weight in boys and pubertal stage (Tanner Staging), in girls, were observed to be the strongest determinants of BMD at the lumbar spine, even when adjusted for bone size, using bone mineral apparent density (BMAD) (Boot, de Ridder, Pols, Krenning, & de Muinck Keizer-Schrama, 1997). No associations with diet and bone mass were observed among girls; calcium was positively associated with total body bone mass in boys. Racial differences in dietary intake were observed. Asian subjects had a significantly lower mean intake of calcium when compared to the Caucasian subjects (759 mg/day vs. 1180 mg/day, respectively). Puberty influences BMD in both boys and girls, and gender may confound these effects as well.

Gender. Gender appears to influence BMD (Gilsanz et al., 1994; Kelly et al., 1990). At all ages, women have less BMD than men (Pollitzer & Anderson, 1989). Gender differences in bone mass may be a reflection of differences in bone size, rather than bone density (Gilsanz et al., 1994). When gender differences in BMD and bone size were investigated in children and adolescents (aged 4 – 20 years) using quantitative computed tomography (QCT), data indicated no difference in bone mass between boys

and girls, but substantial differences in vertebral dimensions. Among pre-pubertal subjects, both the cross-sectional area and volume of the vertebra were found to be 17% larger in the boys than girls, even after controlling for confounding variables. The authors concluded that gender differences in bone size begin before puberty and are further expressed during puberty. This study illuminates the importance of considering volumetric bone volume when investigating skeletal growth in adolescents.

Race. Racial differences in bone mass exist (Pollitzer & Anderson, 1989). African Americans have greater bone mass at all ages when compared to Caucasians (Pollitzer & Anderson, 1989). This is true for both men and women, even after controlling for anthropometric differences. Racial differences have been shown to exist in children under the age of 6 (Li, Specker, Ho, & Tsang, 1989). When 75 African American and 53 Caucasian children ages 1-6 were investigated, BMC was found to be significantly higher in the African American children (both male and female), even after controlling for height and weight, and these findings were highly significant. Additionally, bone width was greater among African Americans than in Caucasians. Whether the tempo of prepubertal and pubertal growth differs between African American and Caucasian subjects is not clear (Seeman, 1998).

Among adults, African American men have been shown to have 7% greater BMD than Caucasian men; African American women presented 13% greater BMD when compared to Caucasian women (Pollitzer & Anderson, 1989). Liel, Edwards, Shary, Spicer, Gordon & Bell, also reported that African American women have greater BMD at all sites when compared to Caucasian women, even when controlling for height and

weight (Liel et al., 1988).

Data comparing bone mass between Caucasians and Asians are scarce (Bhudhikanok et al., 1996; M-C. Wang, personal communication, 1998). Boot and colleagues (Boot et al., 1997), reported total body BMD of Asian females to be lower than Caucasian females and hypothesized this difference may be due to differences in dietary intake, particularly calcium. Others have reported similar bone densities among Asians and Caucasians when corrected for height and weight (Villa, 1994).

Modifiable Factors

Physical activity and diet appear to contribute significantly to acquisition of bone mass among adolescents (Kreipe, 1995; Lysen & Walker, 1997; Ott, 1990; Sowers & Galuska, 1993).

Physical activity. Physical activity is positively associated with bone mass (Eisman et al., 1991; Slemenda et al., 1991). The impact of physical activity on bone mass in adults has been modestly investigated with positive outcomes (Houtkooper et al., 1995; Slemenda et al., 1991), but the impact of physical activity and bone mass in growing children has not been well-researched (Slemenda et al., 1991). It is generally accepted that children are much more active than adults, but this has not been adequately quantified (Slemenda et al., 1991). Lack of physical activity in children is considered to be a risk factor for osteoporosis (Lysen & Walker, 1997).

In a prospective investigation of children between the ages of 5.3 – 14 years, it was discovered that total hours of weight-bearing activity significantly impacted BMD at the radius and hip, independent of age and gender (Slemenda et al., 1991). There is

evidence that excessive amounts of physical activity which lead to estrogen deficiency among women of child-bearing age (menstruating-age), can have deleterious and non-reversible effects on bone mass (Bachrach, 1996; Eisman et al., 1991).

Diet. Nutrition plays a potentially dominant role in bone mass acquisition (New et al., 1997; Ott, 1990). It has been suggested that the impact of osteoporosis could potentially be reduced by as much as 50% or more through adequate nutrition (Lysen & Walker, 1997). The timing and precision of nutrient needs during skeletal growth and development are uncertain (Miller, Groziak, & DiRienzo, 1996), but appear to vary (Shils et al., 1994). Bone growth, development, and mineralization directly influence nutritional requirements (Miller et al., 1996).

Skeletal health is affected by nutrition in primarily two distinct ways (Heaney, 1996). First, bone cells responsible for deposition, maintenance and repair of bone tissue are nutrient dependent for proper functioning. Second, bone acts as a mineral reserve, specifically for calcium, phosphorous, and magnesium, and the amount of mineral available in reserve is in part dependent on the absorbed intake and excretion of these minerals.

Dietary choices as well as the efficiency of absorption and retention of nutrients, and sun exposure influence skeletal development (Miller et al., 1996). Additionally, nutrients interact with each other and affect requirements for one another (Heaney, 1996). Nutrient-nutrient interactions ought to be considered in regard to skeletal health because interactions may impact nutrient requirements (Heaney, 1996).

Malnutrition during childhood and adolescence appears to negatively impact bone

mass. An investigation of Spanish women revealed that individuals raised during a period of "normal" nutrition have significantly greater bone mass, measured at the hand and spine, than those individuals raised during a period of widespread malnutrition (Revilla et al., 1997).

Poor nutritional status has been observed in women with osteoporosis. Reduced plasma levels of common biochemical markers of nutrition, including prealbumin, transferrin, and retinol-binding protein, and fibronectin, have been strongly associated with total body bone mineral content (g) in osteoporotic women, when compared to controls (Rico et al., 1993).

Nutrients and Skeletal Health.

Several nutrients are thought to be involved with bone mineral acquisition, including calcium, magnesium, phosphorus, sodium, zinc, copper, vitamins A, C, D and K and dietary protein (Cooper et al., 1996; Heaney, 1996). Calcium is by far the most highly researched nutrient in the area of skeletal health (Heaney, 1992; Sowers & Galuska, 1993); definitive associations between calcium intake and bone mass remain controversial (Cumming, 1990). Other nutrients, needed to support optimal skeletal health, must be considered (Angus et al., 1988; Freudenheim, Johnson, & Smith, 1986; New et al., 1997). Further, it has been suggested that dietary recommendations for bone health be placed in context of the total diet, rather than a singular emphasis on calcium (Miller et al., 1996).

Magnesium

Magnesium (Mg) is a macromineral, and is the fourth most abundant in the

human body. There are 20-28 grams of magnesium in the adult male (Groff, Gropper, & Hunt, 1995). About 55-60% of total body magnesium is found in bone, one percent exists in extracellular fluid and the rest (39-40%) is found intracellularly in soft tissue, mainly the liver and striated muscle (intracellular). Levels of serum magnesium are similar in men and women and remain constant with advancing age (Prasad, 1976).

Food sources. Magnesium is found in both plant and animal foods (Groff et al., 1995). Good dietary sources of magnesium include nuts, unpolished cereal grains and rice, soybeans and other legumes, seafood, chocolate, tea, molasses, peas, carrots, and green leafy vegetables. Refinement and processing of foods decrease magnesium content significantly; foods comprised of refined sugars and fats are essentially void of Mg. Chlorophyll, present in green leafy vegetables, contains magnesium.

Digestion and absorption. Magnesium absorption occurs throughout the small intestine, predominantly in the ileum (Groff et al., 1995). Adults generally absorb 30 - 60% of the magnesium found in foods. Absorption appears to be poorly controlled and largely determined by dietary intake. Intestinal absorption is accomplished by a saturable, carrier mediated facilitated transport mechanism at levels of normal dietary intake, and by simple diffusion at elevated, or pharmacological levels of intake. The kidney seems to be the organ responsible for maintaining total body levels. As dietary intake increases, urinary excretion increases and vice versa.

It has been reported that magnesium deficiency does not occur in individuals with healthy kidneys (Prasad, 1976). There are many causes of magnesium deficiency. Most, however, occur as a result of conditions involving inadequate intake, malabsorption,

excessive loss, or prolonged parenteral feeding. These conditions are not present in generally healthy children, adolescents, and young adults. Magnesium deficiency was first recognized among starvation victims during World War II. In addition, there are endocrine causes of magnesium deficiency such as hyperthyroidism, primary and secondary aldosteronism and hypoparathyroidism. Magnesium deficiency is difficult to detect and establishing a diagnosis of deficiency has eluded scientists (Prasad, 1976).

Function. Magnesium is involved in over 300 enzyme systems, including energy production and transfer, protein synthesis, hepatic and muscle glucose utilization, the formation, degradation and transcription of genetic nucleic acids, and some membrane transfer systems (Prasad, 1976; Wester, 1987). Specifically, one of magnesium's most significant roles is its involvement in the complex adenosine triphosphate (ATP). ATP is considered to be the metabolic fuel of life (Groff et al., 1995) and is produced in the mitochondrial membrane in animal cells during the process of oxidative phosphorylation, or energy production. The pathways by which magnesium enters the mitochondria have been well described (Jung & Brierley, 1994). Magnesium binds to phosphate in ATP and forms a complex; all enzyme reactions which utilize ATP show an absolute requirement for magnesium (Prasad, 1976).

Magnesium and calcium. Calcium (Ca) intake influences magnesium absorption, probably because they share common gastrointestinal absorption pathways (Seelig, 1990). Both high calcium intake (1900 mg/day) and a diet with a high Ca:Mg ratio have been shown to decrease gastrointestinal absorption and retention of magnesium (Norman et al., 1981; Seelig, 1990). Furthermore, it is thought that consumption of recommended

higher levels of calcium (>1000 mg/day), accompanied by proportionately lower levels of magnesium (adult RDA: women, 310 mg/day and men, 400 mg/day), may lead to reduced plasma levels of magnesium (Dreosti, 1995).

Independently, magnesium, in its ATPase role, is essential for calcium absorption (Ganong, 1997). It has been demonstrated in young men with adequate calcium intake that increasing magnesium intake increases calcium absorption (Seelig, 1990). Further, magnesium supplementation can correct hypocalcemia and hypomagnesemia (Seelig, 1990).

The metabolic pathways of magnesium and calcium are tightly linked because of their apparent metabolic interdependency (Wallach, 1990). Magnesium has been referred to as both a mimic and antagonist of calcium (Levine & Coburn, 1984). Magnesium may bind competitively to the same binding sites as calcium, and produce appropriate physiological responses, or exert no effect; it may alter intracellular calcium distribution by changing the flux of calcium across cell membranes (Levine & Coburn, 1984).

Magnesium and Skeletal Development

There is evidence to suggest dietary magnesium may play a role in the development of bone mass (New et al., 1997; Prasad, 1976; Wallach, 1990; Wester, 1987). Magnesium is an important minor cation in bone (Tsuboi et al., 1994) and nearly sixty-percent of magnesium in the human body is found in bone. Mineral composition of bone is predominantly calcium (approximately 32.2%) and phosphorous (Martin, Bailey, McKay, & Whiting, 1997); bone concentration of magnesium is less than 1%. Magnesium is found in two distinct areas within bone and is believed to influence both

matrix and mineral metabolism (Sojka & Weaver, 1995). The bone surface is believed to represent one body pool of magnesium, and this is thought to be the site where magnesium exchange takes place between plasma and bone. Additionally, magnesium appears to be incorporated into hydroxyapatite crystal during bone formation (Groff et al., 1995).

Magnesium plays an integral role in the formation of hydroxyapatite, the crystalline structure within bone (Blumenthal, Betts, & Posner, 1977; Posner, Betts, & Blumenthal, 1977). Magnesium may influence vitamin D activity (Dreosti, 1995) and endogenous hormones (Wester, 1987) associated with skeletal metabolism. Magnesium may also play a role in osteocyte proliferation, tissue organization, and bone resorption (Kenney, McCoy, & Williams, 1994).

Magnesium concentration within bone. The concentration of magnesium in human bone was analyzed in Japan in 1994 (Tsuboi et al., 1994). The transverse section of the rib of 60 humans, in the age range of 10 – 80 years, was obtained from patients undergoing surgery for the removal of neoplastic tissue. Results showed that the magnesium content of bone did not change, in general, but was slightly higher in adolescent bone when compared to the oldest bones. Conversely, reduced concentrations of magnesium (by approximately 12%) in bone (primarily the trabecular constituent) have been consistently observed in postmenopausal women with osteoporosis, even though overall bone mineral concentrations of calcium were not affected (Cohen, 1988; Dreosti, 1995). Further, along with the lower than normal magnesium concentrations, the hydroxyapatite crystals were found to be greater in size and perfection; a direct functional

link between magnesium concentration and bone fragility has been suggested (Sojka & Weaver, 1995).

Magnesium status on biochemical bone function was investigated in rats (Kenney et al., 1994). The timing of the investigation was planned during periods of active bone growth and calcification in the life cycle of a rat. In this controlled study, the rats fed magnesium deficient diets exhibited slower overall growth, reduced bone strength and significantly less magnesium concentrations in femur ash (all significant at $P<0.05$). Compared to the controls, the rats fed magnesium deficient diets developed shorter femurs, but there was no difference in the diameter and midfemoral cross-sectional areas between the two groups. A similar study observed reduced magnesium concentration in the bones of rats fed magnesium deficient diets, but bone concentrations of calcium were not affected (Howarth, Waring, & Singh, 1993). Furthermore, it was observed that the magnesium concentration in the heart did not change among any of the study groups, suggesting magnesium conservation in the heart muscle, despite reduced dietary intake and bone concentrations.

Magnesium and hydroxyapatite. The formation of hydroxyapatite begins with calcium phosphate [$3Ca_3(PO_4)_2$] in an amorphous phase, as detected by x-ray and electron diffraction, and is called amorphous calcium phosphate (ACP). ACP transforms to poorly crystallized hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] to create hydroxyapatite (Posner, 1978). Hydroxyapatite (HA) in bone is considered "poorly crystallized" due to the presence of significant amounts of foreign ions, (Weiner & Traub, 1992), one of which is magnesium (Tsuboi et al., 1994).

Magnesium has been shown to play an essential role in both the formation of ACP and the transformation of ACP to HA (Blumenthal et al., 1977; Posner, 1978; Posner et al., 1977). ACP is found within the mitochondria of certain cells, especially cells involved with tissue calcification; ACP can accumulate within the mitochondria in a variety of animal species. Synthetic ACP has been shown to be similar to cytoplasmic ACP found in the mitochondria of the hepatopancreas of the blue crab. Significant quantities of both magnesium and ATP have been identified in the mitochondria of the blue crab (Blumenthal et al., 1977). Magnesium is required for the formation of ACP; the presence of magnesium and ATP are essential for the initial formation of intramitochondrial ACP (Blumenthal et al., 1977; Posner, 1978).

Magnesium, functioning with ATP, has been demonstrated to stabilize ACP, in vitro (Posner et al., 1977; Tomazic, Tomson, & Nancollas, 1975). The presence of magnesium substantially decreases the hydrolysis rate of ATP in solution (Blumenthal et al., 1977) and it is not until ATP concentrations begin to approach zero (are nearly undetectable) that ACP begins conversion to crystalline HA; HA formation does not appear to occur when ATP is present. The presence of ATP appears to be the primary factor responsible for delaying the conversion of ACP to HA (Blumenthal et al., 1977). Because magnesium decreases the rate of ATP hydrolysis, magnesium can both prolong the stabilization effect on ACP and slow the conversion rate of ACP to HA. Furthermore, this conversion appears to be under allosteric regulation, as evidenced by the sigmoid curve demonstrating the conversion of ACP to HA; this suggests involvement of additional mechanisms. In vivo, magnesium may inhibit transformation of ACP to

calcium-rich HA in young rat bones (Nielsen, 1973).

In bone, HA crystals are intimately associated with the collagen network and form a highly complex yet orderly mineral-organic composite material. Investigating the crystalline structure of bone is very challenging because the crystals are extremely small, roughly a few hundred angstroms long and wide, and just a few tens of angstroms thick. They are considered to be probably the smallest biologically formed crystal (Weiner & Traub, 1992).

Magnesium is a potent inhibitor of HA crystallization in vitro (Blumenthal et al., 1977). The effects of dietary magnesium on bone composition were investigated in rats (Burnell, Liu, Miller, & Teubner, 1986). Thirty-six rats were randomized into two groups and placed on control diets, containing necessary vitamins and minerals, for 7 days. After 7 days, one group was placed on a high magnesium/normal calcium diet and the other group remained on the control diet. The animals were sacrificed at 10 weeks. Results confirmed what has been learned in vitro; magnesium was a HA crystal growth inhibitor in vivo. It was concluded that the final composition and crystal structure of bone was strongly influenced by magnesium.

Magnesium affects the stability of the HA crystal (Tsuboi et al., 1994). In vitro, magnesium has been shown to inhibit HA crystallization and destabilize HA structure and stability. The role and effect of magnesium on biological HA was investigated on deproteinated turkey leg (Bigi et al., 1992). Magnesium content was measured at varying degrees of calcification in the leg, from tendon to bone. Magnesium content was found to be higher in the tendon than the calcified tissue. Furthermore, as the HA crystal size

became larger with increasing calcification, the magnesium content simultaneously fell and then remained constant. The smaller HA crystals were richer in magnesium than the larger HA crystals. A higher concentration of magnesium in smaller HA crystals when compared to larger crystals has been demonstrated in rat bone as well (Burnell et al., 1986).

Magnesium and endocrine control. Active vitamin D, (1,25 $[OH]_2$ vitamin D_3), known as calcitriol, and parathyroid hormone (PTH) and calcitonin (CT) are endogenous hormones involved in calcium metabolism and skeletal mineralization and maintenance (Ganong, 1997). Quantitative and functional interactions between magnesium and endogenous hormones related to skeletal health are suspected to impact bone mineral accretion (Dreosti, 1995) but remain unclear.

Calcitriol may increase gastrointestinal absorption and extra- to intracellular transport of magnesium (Wester, 1987). Animal studies on pigs fed normal levels of magnesium demonstrated that vitamin D supplementation increases intestinal absorption of magnesium (Pointillart, Denis, & Colin, 1995). Magnesium also appears to be an important factor in vitamin D metabolism in man (Fatemi, Ryzen, Flores, Endres, & Rude, 1991; Zofková & Kancheva, 1995). The hydroxylase enzyme required for hepatic and renal conversion of vitamin D to its active form is a magnesium dependent enzyme (Wester, 1987). Inadequate magnesium consumption may affect activation of vitamin D_3, and thereby impair intestinal absorption of calcium and magnesium, as well as calcium regulation (Dreosti, 1995). Because increased levels of calcium intake have been shown to reduce magnesium absorption and retention (Norman et al., 1981), increased calcium

consumption may result in less plasma magnesium available for bone metabolism and turnover (Dreosti, 1995).

PTH reportedly stimulates intestinal absorption and renal reabsorption of magnesium, as well as release of magnesium from bone (Zofková & Kancheva, 1995). On the other hand, magnesium is essential for PTH secretion from the parathyroid gland (using adenylate cyclase to convert ATP to cyclic AMP), and for PTH directed action on bone, kidney and gut (Wester, 1987). Conditions of magnesium deficiency and severe hypomagnesemia appear to impair PTH sensitivity and function (Wester, 1987). Magnesium deficiency is known to affect bone turnover, and may impair bone mineralization (Fatemi et al., 1991). Experimental intravenous injections of magnesium into hypomagnesemic individuals resulted in increases in serum PTH levels, whereas similar injections into normomagnesemic individuals resulted in decreases in serum PTH (Fatemi et al., 1991). Excess magnesium appears to inhibit PTH secretion (Zofková & Kancheva, 1995).

Magnesium Intake and Bone Mass

Scant epidemiological investigation of associations between dietary magnesium and bone mass have been reported (Angus et al., 1988; Freudenheim et al., 1986; New et al., 1997). Among premenopausal women, positive associations between dietary magnesium and forearm BMC were noted in one longitudinal study (Freudenheim et al., 1986); another cross sectional study reported similar findings (Angus et al., 1988). More recently, researchers observed significant associations between magnesium and BMD at the lumbar spine (New et al., 1997). Furthermore, BMD at both the spine and hip

increased with increased quartile of magnesium intake. One investigation of children reported no associations of magnesium, or any other nutrient, with bone mass (Gilsanz et al., 1998). Additionally, two magnesium supplementation trials completed in small groups of postmenopausal women have reported positive results (Abraham & Grewal, 1990; Stendig-Lindberg, Tepper, & Leichter, 1993).

Epidemiological investigation. Associations between dietary intake of fourteen nutrients and bone mass at the arm (radius and humerus), measured by bone mineral content (BMC) and bone mineral content/bone width (BMC/BW), were investigated in seventeen premenopausal Caucasian women (age 42.3 ±3.4 years) in a four-year longitudinal study (Freudenheim et al., 1986). Calcium supplements were given in a double-blind placebo controlled style. Bone mass was measured using single-photon absorptiometry. Dietary intake was assessed using 24-hour dietary recalls, completed over two cycles of 29 randomized days each year, over 3 years. Up to 72 records were collected for each subject. Mean magnesium intake in both the non-supplemented (NS) group (n=9) and supplemented (S) group (n=8) were similar, 243 ±44 mg/day and 210 ± 39 mg/day, respectively. In the longitudinal analysis, magnesium correlated with the slope of loss in BMC at both the radius ($r=0.657$, $P=0.05$) and the humerus ($r=0.718$, $P<0.03$) in the NS group; there were no significant correlations with calcium (mean calcium intake 793±189 mg/day). Conversely, in the S group, in which subjects consumed an average of 1930±228 mg/day of calcium, no associations were found with magnesium or any other nutrient. The authors suggested that several nutrients may help to minimize bone loss, and the correlations observed in the NS group, but not the S

group, may indicate a beneficial effect on bone from overall diet quality.

In another study, associations between nutrient intake and bone mass at the proximal femur, lumbar spine (L2-4) and distal radius (forearm) were investigated in 88 premenopausal Caucasian women (mean age 37.8±0.8 years) (Angus et al., 1988). Dual photon absorptiometry was used to measure BMD (g/cm^2) at the proximal femur and L2-4; single photon absorptiometry was used to measure BMC at the forearm. Nutrient intake was assessed using 4-day diet records, recorded over four consecutive days, including a weekend day. Mean magnesium intake was 243±9 mg/day; mean calcium intake was 738±32mg/day. Magnesium correlated with forearm BMC ($r=0.23$, $P<0.05$); no associations were observed with calcium at any skeletal site. The authors suggested that bone mass in appendicular sites, those most "fracture prone," may be influenced by a variety of dietary factors.

New and associates (New et al., 1997) conducted a cross-sectional population based study investigating associations between dietary intake and BMD at clinically important sites in 994 healthy premenopausal women (mean age 47.1 ± 1.43 years). BMD at both lumbar spine (L2-4) and femoral neck (FN) were measured using DXA. Usual dietary intake was assessed with a validated Food Frequency Questionnaire; mean magnesium intake was 311 ± 85 mg/day. After adjustment for age, weight, height, physical activity, smoking and social status, magnesium intake correlated with L2-4 ($r=0.06$, $P<0.05$). Analysis by stepwise multiple regression (including age, weight, height, physical activity, smoking, social status, and several nutrients, specifically protein, fiber, magnesium, calcium, potassium, phosphorous, sodium, zinc, vitamin C and

D as independent factors), revealed that magnesium was the only independent predictor of BMD at L2-4 (β=0.018 g/cm^2 per 100 mg magnesium; $P < 0.05$). No dietary associations were observed at FN. When magnesium intake was analyzed by quartile, BMD at both L2-4 and FN increased significantly with increased magnesium intake.

Gilsanz and associates (Gilsanz et al., 1998) recently reported results from a cross-sectional investigation of racial differences in skeletal density and size of African American (n=40) and Caucasian (n=40) children, ages 8-18 years. Computed tomography (CT) was used to measure density and cross-sectional area of the lumbar spine and midshaft of the femur. Nutrient intake data were collected using three-day food records. There were no significant differences in nutrient intake between the African American and Caucasian children. Mean magnesium intake (mg/day) in African American boys was 240±85 and girls 170±53; in Caucasian boys 238±57 and girls 191±42. Multivariate linear regression analysis showed no associations between any nutrient variable and bone density at the spine or femur, when analyzed separately by gender or race, or as a total group.

Magnesium supplementation trials. Nineteen postmenopausal women receiving hormonal replacement therapy (either estrogen alone or cyclic progestogen/estrogen) ingested a multivitamin, multimineral supplement containing 500 mg of calcium (citrate) and 200 mg magnesium (oxide), as well as an additional supplement of 400 mg magnesium (oxide) for 6-12 months (Abraham & Grewal, 1990). A control group consisted of seven women, also on hormone replacement therapy. There were no significant differences in age, height, weight, years since menopause, duration of

hormone therapy, baseline BMD, or duration of follow-up between the two groups. BMD at the calcaneous bone was measured with single photon absorptiometry and nutritional advice regarding dietary recommendations for postmenopausal women was provided. An 11% increase in mean BMD was observed in the supplement group ($P<0.01$). Limitations included lack of randomization, a relatively short study period, confounding hormone replacement therapy, and lack of nutrient assessment from diet.

Increases in trabecular bone density of the distal radius were observed in a controlled supplementation trial using magnesium (250 mg magnesium hydroxide) in 31 postmenopausal osteoporotic women (Stendig-Lindberg et al., 1993). Limitations included a small number of subjects, high attrition, a non-randomized sample, and exclusion of reproductive histories and time since the onset of menopause. However, in subjects who completed the two-year trial, no side-effects from supplementation were reported, and the observed increases in mean trabecular bone density were significant. There are no reported studies of magnesium supplementation in adolescents and young adults, and osteoporotic, postmenopausal subjects are substantially different physiologically than healthy youth.

Magnesium Intake Among Adolescents

Very little is known about micronutrient intake among adolescents even though adequate nutrients are essential for proper growth and development (Zive, Nicklas, Busch, Myers, & Berenson, 1996). Dietary consumption of magnesium among adolescents and young adults has been modestly investigated (Groff et al., 1995). Results of investigations completed indicate inadequate magnesium consumption among

adolescents and young adults (Pennington, 1996; Zive et al., 1996), when based on previous Recommended Dietary Allowances (RDA) (National Research Council, 1989) and current Dietary Reference Intakes (Institute of Medicine, 1997). This is consistent with the 1977-1978 United States Department of Agriculture (USDA) National Food Consumption Survey which reported the lowest intake of magnesium, among all surveyed (newborn to elderly), in teenagers and adults (Marier, 1986). Similarly, USDA dietary surveys recorded since 1965 have indicated that the magnesium content of self-selected diets among a significant percentage of adults is marginal or suboptimal, based on the RDA levels (Lakshmanan, Rao, Kim, & Kelsay, 1984).

Food sources. Results from the School Nutrition Dietary Assessment Study (Gordon & McKinney, 1995) indicate that milk and milk products, followed by grain products, are the foods which contribute the greatest amount of magnesium to diets of school age children in grades 1-12 (by percent of RDA); magnesium contribution from vegetables was negligible (0.2-0.3 % RDA). Among the Bogalusa Heart study cohort (mean age 23 years), breads and grains, vegetables and soups, followed by milk, were the foods which contributed the greatest amounts of dietary magnesium (Zive et al., 1996).

Dietary intake. Magnesium intake among young adult men and women from self-selected diets was assessed over a one-year period (Lakshmanan et al., 1984). Dietary records were maintained for 365 days. Mean magnesium intake among women ages 20-34 years was 238 mg/day; intake was 333 mg/day among the same age men. The higher consumption among men reflected greater caloric consumption for their greater body weights; magnesium content per kilocalorie (kcal) was similar among men and women at

0.14/kcal.

Between 1988-1991, dietary intake of nutrients among young adults, ages 19-28 years (mean age 23 years), was assessed in a cross-sectional sample (n=504) using 24 – hour dietary recall methods (Zive et al., 1996). The authors noted that dietary intakes based on one 24-hour recall do not reflect habitual dietary intake, but are useful in gathering assessment data. In the sample, which was a sub-set of the Bogalusa Heart Study cohort, 58% were female, 70% were Caucasian and 30% were African American. The Bogalusa Heart Study was an epidemiological investigation of cardiovascular risk factors among a biracial pediatric population in Bogalusa, Indiana (Zive et al., 1996). The RDA were used to assess nutrient intake, and consumption of less than 60% of the RDA was considered to be inadequate; nutrient intake of magnesium was inadequate in 40% of the sample. Mean magnesium intake was 285 ± 153 mg per day in males and 189 ±102 mg per day in females in a combined analyses of all subjects. When analyzed by race, Caucasians consumed more magnesium, 236±135 mg per day, compared to African Americans, 214± 132 mg per day. The RDA was 350 mg/day for males and 280 mg/day for females in this age group (National Research Council, 1989). Overall, fewer than 20% of the subjects consumed less than one-third of the RDA for magnesium, and most of the subjects consumed between one-third and two-thirds of the RDA for magnesium.

The Total Diet Study is an annual survey administered by the Food and Drug Administration (FDA) with the purpose of identifying changes and trends in the mineral content of foods present in the United States food supply (Pennington, 1996). A group of 260 foods is selected to represent the "core" foods of the US population (based on

consumption data from national food surveys); these foods are purchased quarterly and analyzed for selected mineral content. The results of the mineral composition analysis are merged with national food consumption data to provide an estimate of nutrient intake in eight age-sex categories. Results of the Total Diet Study indicated that nutrient intake among adolescent and young adult females were below the RDA (National Research Council, 1989) in five of eight minerals, including magnesium (Pennington, 1996). Females in both groups, 14-16 years of age, and 25-30 years of age, reportedly consumed approximately 195 mg/day of magnesium, which represents 65% and 70% of the RDA (National Research Council, 1989), respectively. Intake among adolescent and young adult males was below the RDA for magnesium as well. Both the younger males (14-16 years of age) and older males (aged 25-30 years) consumed approximately 300 mg/day of magnesium, representing 75% and 86% of RDA (National Research Council, 1989), respectively.

Dietary reference intake. The Food and Nutrition Board of the National Research Council recently published newly created standards for Dietary Reference Intakes (DRI) (Institute of Medicine, 1997). Magnesium was included in this flagship publication. The estimated average requirement (EAR) is a value estimated to meet the nutrient needs of 50% of the population in a specific life-stage and gender group. The RDA is a recommended level of dietary intake sufficient to meet the nutrient requirements of 97-98% of individuals. In order to establish a RDA for a nutrient, a functional endpoint must be defined. For magnesium the functional endpoint was metabolic balance. While the board agreed that positive magnesium retention during periods of rapid growth is

desirable, the extent of "positive retention" is unknown and difficult to quantify, so metabolic balance was agreed upon as the functional endpoint. The EAR for females, aged 14-18 years is 300 mg/day and for same age males, 340 mg/day; the RDA is 360 and 410 mg/day, respectively. For females aged 19-30 years of age, the EAR is 255 mg/day and same age males, 330 mg/day; the RDA is 310 and 400 mg/day, respectively. The 1996 Total Diet Study indicates that mean magnesium intake among adolescents and young adults is below both EAR and RDA values for magnesium (Pennington, 1996).

Proposed food fortification. Increases in calcium intake have been recommended to support adequate acquisition of bone mass in adolescents (Institute of Medicine, 1997), without concomitant increases in magnesium. One proposed avenue for increasing calcium intake is national food fortification. Children and adolescents are among the most sensitive sub-groups to food fortification programs. As previously reported, magnesium and calcium are known to share common pathways of absorption and regulation (Seelig, 1990). Further, nutrients interact with each other and alter requirements (Heaney, 1996). Little investigation into the effect of increases in calcium intake on magnesium metabolism has been completed among adolescents (Andon, Ilich, Tzagournis, & Matkovic, 1996). The impact of these proposed recommendations, particularly to skeletal metabolism, is unclear.

In the following pages, one of the first epidemiological investigations of associations between dietary magnesium and bone mass, as well as gains in bone mass, among ethnically diverse adolescents and young adults, is presented.

CHAPTER II

JOURNAL ARTICLE

Title: Magnesium and bone mineral acquisition in ethnically diverse adolescents and young adults

Gretchen K. Vannice[1], May-Choo Wang[1], Clarie B. Hollenbeck[1], Robert Marcus[3,4], Lucy M. McProud[1], Laura K. Bachrach[2]

from the Department of Nutrition and Food Science, San Jose State University, San Jose, California[1] and Departments of Pediatrics, [2] and Medicine,[3] Stanford University School of Medicine, Stanford California, and the Musculoskeletal Research Laboratory, Geriatric Research, Education and Clinical Center, [4] Veteran's Affairs Medical Center, Palo Alto, California

Address all correspondence and reprint requests to:

Gretchen K. Vannice, M.Sc.
c/o May-Choo Wang, Dr. P.H., R.D.
Department of Nutrition and Food Science
San Jose State University
One Washington Square
San Jose, CA 95192-0058
Tel: (408) 924-3100
FAX: (408) 924-3116
E-mail: gvannice@jps.net

ABSTRACT

Magnesium and Bone Mineral Acquisition in Ethnically Diverse Adolescents and Young Adults

G. K. Vannice, M-C. Wang, C.B. Hollenbeck, R. Marcus, L.M. McProud, and L.K. Bachrach. San Jose State University, San Jose, CA, Stanford University, Stanford, CA, and VA Medical Center, Palo Alto, CA.

Magnesium (Mg) is known to play an essential role in skeletal health, yet there are few reports on the relationships between dietary Mg and bone mineral acquisition. We investigated associations of dietary Mg with bone mass as well as annualized gains in bone mass at the femoral neck (FN) and lumbar spine (L2-4) in 306 ethnically diverse youths who constituted a sub-set of participants of a four-year longitudinal study on bone mineral acquisition. Subjects were categorized by pubertal stage based on self-ratings using Tanner stages. Bone mass was measured by dual X-ray absorptiometry (DXA) and expressed as areal (BMD, g/cm^2) and volumetric (bone mineral apparent density or BMAD, g/cm^3) bone mineral density. Bone mineral acquisition was calculated over a one-year period from measurements made during the first and second annual examinations. Dietary data were gathered using the Health Habits and History Questionnaire of the National Cancer Institute. Data were stratified by gender and pubertal stage, and analyzed using multiple linear regression techniques with dietary Mg, calcium, protein, energy, weight-bearing activity, race and BMI as independent variables.

Dietary Mg was observed to be negatively associated with BMD and BMAD at the hip of mature females both years of data collection. The regression coefficients (S.E.) were approximately .05 (.02) g/cm^2 per 100 mg of Mg for BMD and .015 (.006) g/cm^3 per 100 mg Mg for BMAD ($P < 0.05$). Dietary calcium was positively associated with FN BMD in mature females.

Our findings suggest that dietary Mg may be negatively related to bone mass. Because Mg has been demonstrated to play a role in hydroxyapatite formation, additional studies are needed to determine if and how dietary Mg influences skeletal health either through effects on calcium metabolism, or independently.

KEY WORDS: Magnesium, diet, calcium, BMAD, bone mass, bone density, bone mineral acquisition, race, ethnicity, African Americans, Asians, Caucasians, adolescents, young adults

Introduction

Osteoporosis is a significant public health problem in the United States (1, 2). In 1995, it cost nearly 14 billion dollars for treatment alone in adults over the age of 45 years (3). Osteoporosis-related skeletal fractures lead to devastating effects on quality of life, and increases in mortality (4). It is increasingly acknowledged that osteoporosis may have its origins in childhood (5). Indeed, it has been described as a pediatric condition which manifests itself in old age (6). Because there is no cure for osteoporosis (5), and current therapies provide only limited improvement (7), prevention of this disorder is an important focus (5).

Bone mass has been identified as a major predictor of osteoporosis risk (1). Identifying factors affecting attainment of peak bone mass, especially during growth and adolescence is essential (8-10). Modifiable factors, such as diet and physical activity, and non-modifiable factors, including genetics and gender appear to play important roles in the etiology of osteoporosis (9, 10).

There is insufficient information regarding the role of diet and nutrition in optimizing bone mass, especially among adolescents and young adults (11-13). Although calcium has been the most studied nutrient in skeletal health research (14), other nutrients may also contribute to bone mass (9, 15, 16); among these nutrients is magnesium (17).

Magnesium is an essential nutrient for humans (18) because it is required for hundreds of vital metabolic functions (19, 20). Magnesium is a constituent of bone (21, 22) and appears to be involved in the acquisition of bone mass (17, 19). Preliminary evidence from animal model and epidemiological studies suggests that magnesium may

play a role in skeletal health (13, 17, 23-26). Surveys of magnesium intake indicate that adolescents and young adults consistently consume less than recommended levels (27-31).

In the present study, associations between dietary magnesium and bone mass, as well as gains in bone mass, in a group of ethnically diverse adolescents and young adults were investigated, using data gathered during a 4-year longitudinal study on bone mineral acquisition (32, 33). We hypothesized that positive associations between dietary magnesium and bone mass would be observed.

Subjects and Methods

Subjects

Our convenience sample included 100 Asian Americans (51 females, 49 males), 102 non-Hispanic Caucasians (53 females, 49 males) and 104 African American (62 females, 42 males) between the ages 9-25 years. Mexican Americans were also recruited. However, because data gathered for Mexican Americans were insufficient in the second year of the study, the present analysis excluded Mexican American participants. Individuals who were clinically symptomatic of anorexia nervosa or bulimia were excluded. A detailed description of recruitment methods is reported elsewhere (32, 34). The Stanford University Panel on Human Subjects in Medical Research approved the study protocol. Written informed consent was obtained from all subjects over age 18; written parental consent was obtained for all minor participants.

Data Collection

Clinical data and bone mass measurements were gathered at 4 examinations, at

11-18 month intervals.

1. Bone Mass

Bone mass was measured at the lumbar spine (L_{2-4}) and femoral neck (FN) using dual energy X-ray absorptiometry (DXA; QDR-1000W, software version 6.10, Hologic Corp., Waltham, MA), in the pencil beam mode. In our laboratory, the in vivo coefficient of variation for replicate measurements is approximately 0.6% for the femoral neck and lumbar spine in adolescents and young adults. Bone mass was expressed in conventional terms of bone mineral content (BMC, g) and bone mineral density (BMD, g/cm^2). Both BMC and BMD are influenced by bone size and underestimate true volumetric bone mineral density in smaller individuals and overestimate it in larger individuals (35). To compensate for differences in bone size, we also used an estimate of volumetric bone density, bone mineral apparent density (BMAD, g/cm^3), for the spine and femoral neck (36). We used bone measurement data collected in the first and second annual examinations. Bone gains were analyzed on individuals who completed the second year examination; subject retention rate was 72%.

2. Height, Weight and Pubertal Stage

All subjects had weight and stadiometer-derived height measurements from which BMI was calculated. Pubertal stage was determined by a self-assessment questionnaire with drawings and written descriptions of Tanner's breast/genital and pubic hair classifications (37). This method has been shown to be comparable to pubertal staging by physician examination in Caucasians (38). However, because of possible ethnic differences in the quantity and timing of pubic hair development (39), and because

growth of pubic hair reflects adrenal as opposed to gonadal activity, we used the breast/genital Tanner ratings alone to group subjects into one of three stages of pubertal develoment. These were pre-/early puberty (Stages 1-2), mid-puberty (Stages 3-4), and maturity (Stage 5). Menstrual history was obtained by questionnaire. Information regarding reproductive history and use of oral contraceptives was not collected. The impact of oral contraceptive use on bone mass remains controversial (8, 40).

3. Diet

Diet was assessed using the self-administered 97-item National Cancer Institute Health Habits and History Questionnaire (41). This food frequency questionnaire has been validated for selected nutrients in Caucasian women (42), African American women (43) and Asian Americans (Wang, M-C., personal communication, 1998). Only nutrient data collected in the first year of data collection were included in the analyses. Nutrient analysis was performed using the Dietary Analysis System (Dietsys) version 3.0, 1994 (National Cancer Insitute, Bethesda, MD) (44). Questionnaires reporting energy intakes of less than 500 kcal or more than 6000 kcal were excluded from all analyses involving nutrient intake. Since few subjects reported use of dietary supplements, the data on supplements were excluded from the nutrient analyses.

4. Physical Activity

Subjects were interviewed about their recreational activities during the previous year. The number of hours spent each week in weight-bearing and in non-weight-bearing exercise were estimated; weight-bearing exercise was considered to be any activity that loads the body with at least body weight (45). Walking was excluded from this

assessment because of subjects' difficulty in estimating time spent in this activity, and because some subjects reported only recreational while others included utilitarian walking.

Statistical Analysis

Statistical analysis was performed on SAS/Graph 6.12 (SAS Institute, Cary, NC, U.S.A.). Subjects were grouped by pubertal stage rather than age because puberty has been shown to be more highly correlated to bone mass (35, 36, 46, 47) and the tempo of puberty may vary across races (39, 48). Gender and pubertal stage differences in nutrient intake and bone mass were determined with ANOVA; comparisons between groups were assessed using Bonferroni's multiple comparisons procedure at a significance level of 0.05 (49). Racial comparisons of nutrient intake and bone mass in subjects were assessed using the nonparametric Wilcoxon test (50), since the distribution of several of these variables deviated from normality.

After subjects were stratified by pubertal stage and gender, multiple linear regression analysis was applied to simultaneously consider the effects of magnesium, calcium, protein, and energy intake, weight-bearing activity, body mass index and race on each bone mass measurement. Race was treated as a dummy variable (Asian and African American compared with Caucasian) (51). The outcome (y) variable was BMD and BMAD at the hip and spine, measured in year 1 and year 2, as well as annualized gains in BMD and BMAD between year 1 and year 2. Bone gain was calculated as follows:

$$\frac{BMADyr_2 - BMADyr_1}{months\,between\,visits / 12} X \frac{100}{BMADyr_1}$$

Additional details of the statistical analyses can be found elsewhere (33).

Results

Table 1 summarizes clinical characteristics of the cohort by gender, pubertal stage and race. Mean BMI was within normal range for subjects, except in mature African American females whose BMI was 27 ± 6.3 kg/m^2. Midpubertal subjects spent the greatest amount of time in weight bearing physical activity. Additional information regarding clinical characteristics can be found elsewhere (values may vary slightly from previous reports since this study included data from the second year examinations) (32, 33).

Diet

Mean magnesium intake exceeded current recommended levels (30) only in pre-/early pubertal females and males, and midpubertal males (Table 1). Among females, magnesium intake did not differ by pubertal stage or by race. In contrast, among males, mean magnesium and mean calcium intake were significantly higher among midpubertal African Americans than Asians ($P < 0.05$). Mean calcium intake in mature Caucasian males was significantly higher than in Asians ($P < 0.05$). Mean intake of magnesium, calcium and energy was lowest among Asian females in all pubertal groups, when compared to Caucasians and African Americans; this was not true among Asian males, although they demonstrated a similar trend.

In females, in the pre-/early pubertal groups, mean calcium:magnesium (Ca:Mg) ratio among Caucasians was significantly higher than African Americans. In the mid-pubertal groups, mean Ca:Mg ratio among Caucasians was higher than both African Americans, and Asians ($P < 0.05$). In males, mean Ca:Mg ratio among Caucasians was

significantly higher than in Asians, but only in the mature group ($P < 0.05$). In males and females, calcium:protein, calcium:phosphorus, as well as calcium density (mg calcium/1000kcal) all differed significantly by race among all pubertal stages ($P < 0.05$) (data not shown).

Bone Mass

Bone mass measurements by gender, pubertal stage and race are presented in Tables 2 and 3 (a & b). FN will be referred to as the "hip", and L_{2-4} as the "spine". Among females, BMC was significantly higher among African Americans than Asians at the hip, in both the pre-/early puberty and mature groups ($P < 0.05$), both years of data collection. In the mature group, BMD at the hip, and BMAD at the spine were significantly higher among African Americans than both Caucasians and Asians, both years.

Among males, mature African Americans were observed to have significantly higher BMD and BMAD at the hip, than Asians ($P < 0.05$), for both years. In contrast, BMD in the spine was significantly lower in mid-pubertal African Americans than in Asians, both years of data collection.

Bone Gains

When analyzed by pubertal stage (Table 4) the greatest mean % gains in BMAD at both the hip and spine were observed mid-puberty. Among midpubertal females, Caucasians showed the greatest mean % gain in BMAD at the hip, and African Americans demonstrated the greatest mean % gain in BMAD at the spine. Racial differences in mean % bone gains among females, within pubertal stage, did not reach

statistical significance. In contrast, among mid-pubertal males, Caucasians demonstrated the greatest mean % gain in BMAD at the spine, and it was significantly higher than in African Americans ($P < 0.05$). Asians demonstrated the greatest mean % gain in BMAD at the hip, and the difference between Asians and African Americans was also significant.

Multiple Linear Regression Analysis

Data are reported by gender and pubertal stage. Regression coefficients adjusted for BMI, race, and weight-bearing activity for the nutrient variables are shown in Table 5. No associations were observed with nutrient or energy intake and bone mass among mid-pubertal groups. The nutrients studied were not associated with gains in BMAD.

Femoral neck

In mature females, magnesium was observed to be negatively associated with BMD and BMAD at the hip ($P < 0.05$), at the first and second examinations. In the same group, calcium was observed to be positively associated with BMD at the hip, both years. Calcium was positively associated with BMAD at the hip in pre-/early pubertal females, both years. In contrast, calcium was negatively associated with BMD at the hip in pre-/early pubertal males, in the first year ($P < 0.05$), and in the second year ($P < 0.10$).

Lumbar spine

No associations were observed with magnesium and bone mass. Among mature females, calcium intake was positively related to BMD (g/cm^2 per 100 mg calcium) at the spine in the first year of data collection ($\beta = 0.0152$, SEE = 0.0067, $P < 0.05$), and marginally associated in the second year ($\beta = 0.0129$, SEE = 0.0065, $P < 0.06$). No significant associations at the spine were observed among males.

Discussion

Major gains in bone mineral occur during childhood and adolescence when the amount of bone mass accrued is equivalent to that lost in adulthood (12). While genetic factors play a major role in the attainment of peak bone mass (5, 7, 52, 53), diet, physical activity and other modifiable factors are also important determinants (16, 54, 55).

This study represents one of the first epidemiological investigations of dietary magnesium and bone mass acquisition in growing adolescents and young adults. We hypothesized that dietary magnesium would be positively associated with bone mass. In fact, few associations between magnesium and bone mass were observed. Associations between magnesium, as well as calcium, and bone density were mostly observed among females. In mature females, magnesium was observed to be negatively related to FN BMD and FN BMAD, while calcium was noted to be positively associated with FN BMD. In pre-/early puberty, calcium was also positively related to FN BMAD in females, but negatively associated with FN BMD in males.

That we found dietary magnesium to be negatively associated with bone mass was somewhat surprising. Magnesium is the fourth most abundant mineral in the human body, and although the magnesium content of bone is less than 1%, sixty percent of magnesium within the body exists in bone (18, 19). Magnesium is believed to influence both bone matrix and mineralization (56), perhaps through the formation of hydroxyapatite crystal (57, 58). Magnesium also shares pathways of absorption and regulation with calcium (59), and influences endocrine control of skeletal metabolism (19, 60).

Other groups have also attempted to investigate the role of magnesium in skeletal health, but only in animals and adults (13, 17, 23, 24, 61). Animal studies suggest that magnesium may have a positive effect on bone formation and calcification (17). However, a few studies in chicks, rabbits and rats have indicated decreases in bone density and osteoblastic activity with magnesium supplementation (17).

Among humans, magnesium supplementation trials in postmenopausal women have shown significant increases in BMD of the calcaneous and distal radius bones (25, 26). A recent cross-sectional epidemiological investigation by New and associates (13) reported dietary magnesium, and not calcium, to be an independent positive predictor of bone mass at the lumbar spine in premenopausal women. Another cross-sectional investigation in premenopausal women found positive correlations between magnesium and forearm BMC, but no association between calcium and bone mass (23).

It is possible that magnesium may affect bone density differently, depending on calcium intake levels. A 4-year longitudinal investigation by Freudenheim and associates (24) showed positive associations between the rate of change in radius and humerus BMC and magnesium, but not calcium, in non-calcium-supplemented premenopausal women (mean calcium intake = 793 ± 189 mg/d). In the calcium supplemented group (mean calcium intake = 1930 ± 228 mg/d), change in bone mass was not associated with magnesium or calcium. Thus it would appear that when calcium intakes are low, magnesium may positively influence bone density. In our study, we did not investigate interactions between magnesium and calcium. Our observations may also differ from those in adults because children and adolescents are still actively depositing bone.

Mean magnesium intake among mature females was below recommended levels (30); this is consistent with other reports in this population (29, 62). That magnesium intake met current recommended levels in the younger, but not the older sub-groups, suggests changes in diet. Dietary changes may occur along with increased independence during adolescence. Mean calcium intake tended to be the lowest among mature females. Calcium consumption among adolescents reportedly decreases with increasing age and this appears to be related to decreases in milk consumption (63).

There is concern regarding the recommendation for increases in calcium without concomitant increases in magnesium (59, 64). It is known in nutritional science that coingested nutrients interact with each other (16), and magnesium and calcium share metabolic pathways (65). It has been shown in adults, that calcium:magnesium (Ca:Mg) ratio of 2:1 or greater, may interfere with magnesium absorption (59). All of the subjects were observed to consume Ca:Mg ratio greater than 2:1. The extent to which nutrient interactions affect healthy children is unknown (64).

We acknowledge several limitations of this study. First, because BMD is dependent on dietary influences over the lifetime, it is likely that assessments over a one year period are inadequate for capturing dietary influence on bone mass. Second, stratification by gender and pubertal stage reduced statistical power to detect nutrient relationships with bone mass. Third, while we attempted to control for weight bearing physical activity as stated earlier, walking was excluded. Finally, our dietary assessment method may not have adequately captured usual magnesium intake in our subjects. The Food Frequency Health and Habits History Questionnaire has been validated for selected

nutrients, including calcium, among different racial groups (42, 43, 66), but only in adults, and it has not been validated for magnesium.

This research is exploratory; by observing associations we cannot draw specific conclusions. Nutrients other than calcium deserve greater investigation. That several nutrients, in addition to calcium, are related to bone mass is not a new idea (23, 24). Here, we suggest skeletal development may be influenced by magnesium within certain thresholds of calcium intake. More specifically, magnesium's influence on bone density may depend on calcium intake levels.

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TABLE 1

Characteristics of subjects by race and pubertal stage[1,4]

	Females									Total		
	Asian			*Caucasian*			*African American*					
	Pre-/Early Puberty	Mid-Puberty	Mature	Pre-/Early Puberty	Mid-Puberty	Mature	Pre-/Early Puberty	Mid-Puberty	Mature	Pre-/Early Puberty	Mid-Puberty	Mature
N	6	19	26	14	16	23	7	29	26	27	64	75
Age (y)	9.9±0.6	14.0±1.5	20.9±2.5	10.5±1.2	13.6±1.7	20.4±3.7	9.9±0.9	13.0±1.9	19.2±3.8	10.2±1.0	13.4±1.8	20.2±3.4
BMI (kg/m^2)	16±0.5	21±2.4	21±1.9[a]	18±2.8	21±2.5	21±2.5[b]	21±7.2	22±4.6	27±6.3[a,b]	18±4.3	21±3.6	23±4.9
Magnesium (mg/d)	199±48	248±107	225±73	216±83	265±113	234±93	271±192	249±130	236±140	226±115	253±118	232±105
Calcium (mg/d)	743±262	824±524	708±383	1094±438	1239±687	800±342	851±673	813±574	673±444	953±487	923±610	724±392
Ca:Mg (mg:mg)	3.79±0.86	3.23±1.22[d]	3.03±0.94	5.07±1.26[c]	4.51±1.24[d,e]	3.65±1.32	2.93±0.90[c]	3.11±0.82[e]	2.87±1.05	4.21±1.10	3.50±1.06	3.17±1.11
Protein (g/d)	63±19	82±42	73±32	62±21	75±26	57±20	89±59	86±43	77±38	69±35	82±39	69±32
Energy (kcal/d)	1549±641	1948±893	1709±701	1457±490	1732±586	1422±543	2424±1925	2251±1134	1982±1053	1728±1110	2032±964	1716±825
Activity (hrs/wk)[2,3]	1.5±1.5	4.2±3.4	3.8±3.6	5.5±3.5	7.9±7.6	9.9±9.6	6.2±4.8	10.1±10.6	4.8±6.1	4.9±3.9	7.9±8.7	6.0±7.2

[1] mean ±SD

[2] weight-bearing activity only

[3] sample sizes for activity may vary slightly due to missing values

[4] similar letter superscripts indicate significant racial differences within pubertal group at $P < 0.05$

TABLE 1, continued from previous page

Characteristics of subjects by race and pubertal stage[1,4]

	Males									Total		
	Asian			*Caucasian*			*African American*					
	Pre-/Early Puberty	Mid-Puberty	Mature	Pre-/Early Puberty	Mid-Puberty	Mature	Pre-/Early Puberty	Mid-Puberty	Mature	Pre-/Early Puberty	Mid-Puberty	Mature
N	13	15	21	20	16	13	5	21	16	38	52	50
Age (y)	10.7±1.6	15.7±1.7	20.7±2.1	11.0±1.3	14.6±1.5	20.7±3.1	10.7±1.9	12.7±2.7	19.2±4.1	10.9±1.5	14.1±2.4	20.2±3.1
BMI (kg/m^2)	18±2.7	20±1.4	22±2.8	19±2.9	20±2.5	25±3.3	17±2.1	19±2.5	23±3.9	18±2.7	20±2.2	23±3.5
Magnesium (mg/d)	297±89	277±107[a]	261±113	327±85	328±114	376±146	357±168	416±193[a]	338±172	321±99	349±158	315±148
Calcium (mg/d)	1086±555	843±265[b]	659±320[c]	1440±494	1293±621	1260±591[c]	1403±1011	1407±798[b]	916±436	1314±603	1209±663	898±495
Ca:Mg (mg:mg)	3.52±1.03	3.11±0.57	2.54±0.69[d]	4.37±0.89	3.80±1.07	3.49±1.32[d]	3.80±1.19	3.31±1.02	2.80±0.74	4.00±0.98	3.40±0.93	2.87±0.91
Protein (g/d)	96±25	96±38	87±29	94±21	96±34	109±42	114±51	128±54	100±54	97±28	109±46	97±42
Energy (kcal/d)	2260±555[e]	2179±669[f]	2090±783	2381±634	2472±768[g]	2782±991	3306±1622[e]	3501±1376[f,g]	2567±1282	2461±840	2803±1178	2423±1040
Activity (hrs/wk)[2,3]	6.9±5.9	10.3±7.2	5.6±5.5	11.7±9.2	13.0±7.8	6.8±5.2	3.9±2.4	14.1±12.3	9.8±6.9	9.1±8.1	12.7±9.6	7.2±6.1

[1] mean ±SD

[2] weight-bearing activity only

[3] sample sizes for activity may vary slightly due to missing values

[4] similar letter superscripts indicate significant racial differences within pubertal group at $P < 0.05$

TABLE 2

First and second year bone measurement values, reported by pubertal stage[1]

Year 1						
	Females			*Males*		
Pubertal Stage	*n*	*LS BMC (g)*	*FN BMC (g)*	*N*	*LS BMC (g)*	*FN BMC (g)*
Pre-/Early Puberty	27	19.63±3.56	2.89±0.53	38	21.21±4.08	3.28±0.47
Mid-Puberty	64	37.98±9.06	4.15±0.73	52	37.70±13.48	4.64±1.09
Mature	75	46.40±7.39	4.42±0.80	50	52.02±9.59	5.51±0.92
	n	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)	*N*	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)
Pre-/Early Puberty	27	0.70±0.07	0.68±0.09	38	0.69±0.07	0.74±0.08
Mid-Puberty	64	0.97±0.14	0.85±0.12	52	0.88±0.17	0.88±0.13
Mature	75	1.07±0.11	0.89±0.14	50	1.05±0.14	0.97±0.15
	N	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)	*N*	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)
Pre-/Early Puberty	27	0.13±0.01	0.16±0.02	38	0.12±0.01	0.17±0.02
Mid-Puberty	64	0.16±0.02	0.18±0.03	52	0.13±0.02	0.17±0.02
Mature	75	0.16±0.02	0.18±0.03	50	0.15±0.02	0.17±0.03

Year 2						
	Females			*Males*		
Pubertal Stage	*n*	*LS BMC (g)*	*FN BMC (g)*	*N*	*LS BMC (g)*	*FN BMC (g)*
Pre-/Early Puberty	24	21.38±8.93	3.22±0.51	31	19.17±10.27	3.53±0.48
Mid-Puberty	51	33.66±18.62	4.48±0.69	37	30.20±22.30	4.90±1.10
Mature	45	27.31±23.24	4.46±0.80	32	34.53±27.28	5.64±0.97
	n	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)	*N*	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)
Pre-/Early Puberty	24	0.76±0.08	0.71±0.08	31	0.71±0.08	0.76±0.07
Mid-Puberty	51	1.03±0.13	0.90±0.14	37	0.92±0.16	0.90±0.14
Mature	45	1.06±0.11	0.89±0.16	32	1.07±0.14	0.98±0.15
	n	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)	*N*	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)
Pre-/Early Puberty	24	0.13±0.01	0.16±0.02	31	0.12±0.01	0.16±0.02
Mid-Puberty	51	0.16±0.02	0.18±0.03	37	0.14±0.01	0.17±0.02
Mature	45	0.16±0.02	0.18±0.04	32	0.15±0.02	0.17±0.03

[1]Mean (±SD) values of bone mineral content (BMC), bone mineral density (BMD), and bone mineral apparent density (BMAD). LS; lumbar spine (L 2-4). FN; femoral neck

TABLE 3a
First year bone measurement values reported by race and pubertal stage[1,2]

	Females			*Males*		
Pubertal Stage	*n*	*LS BMC (g)*	*FN BMC (g)*	*N*	*LS BMC (g)*	*FN BMC (g)*
Asians						
Pre-/Early Puberty	6	17.48±3.33	2.36±0.32[a,b]	13	20.67±5.44	3.09±0.50
Mid-Puberty	19	38.42±8.42	3.85±0.51	15	45.17±10.59[a]	4.78±0.84
Mature	26	43.94±5.58	3.98±0.45[c]	21	51.04±9.07	5.21±0.90
Caucasians						
Pre-/Early Puberty	14	20.25±3.40	2.98±0.32[a]	20	21.56±3.57	3.36±0.48
Mid-Puberty	16	37.40±7.34	4.23±0.59	16	38.23±10.93	4.74±0.88
Mature	23	46.83±7.66	4.34±0.61[d]	13	56.87±10.62	5.79±1.03
African Americans						
Pre-/Early Puberty	7	20.24±3.85	3.15±0.74[b]	5	21.22±1.85	3.42±0.20
Mid-Puberty	29	38.00±10.48	4.29±0.87	21	31.97±14.78[a]	4.45±1.39
Mature	26	48.48±8.24	4.93±0.93[c,d]	16	49.37±8.45	5.67±0.81

	Females			*Males*		
Pubertal Stage	*n*	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)	*N*	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)
Asians						
Pre-/Early Puberty	6	0.67±0.05	0.61±0.07[a]	13	0.69±0.09	0.72±0.07[a]
Mid-Puberty	19	0.98±0.13	0.82±0.11	15	0.96±0.14[a]	0.87±0.12
Mature	26	1.01±0.09[a]	0.82±0.09[b]	21	1.02±0.14	0.90±0.13[b]
Caucasians						
Pre-/Early Puberty	14	0.69±0.07	0.68±0.05	20	0.68±0.06	0.74±0.07
Mid-Puberty	16	0.96±0.12	0.83±0.09	16	0.88±0.13	0.90±0.11
Mature	23	1.05±0.10[b]	0.86±0.12[c]	13	1.10±0.16	1.00±0.16
African Americans						
Pre-/Early Puberty	7	0.73±0.07	0.74±0.13[a]	5	0.74±0.05	0.82±0.06[a]
Mid-Puberty	29	0.97±0.15	0.89±0.14	20	0.83±0.18[a]	0.88±0.15
Mature	26	1.13±0.12[a,b]	0.98±0.16[b,c]	16	1.04±0.13	1.03±0.13[b]

	Females			*Males*		
Pubertal Stage	*n*	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)	*N*	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)
Asians						
Pre-/Early Puberty	6	0.13±0.01	0.16±0.03	13	0.12±0.01	0.17±0.02[a]
Mid-Puberty	19	0.16±0.02	0.17±0.02	15	0.14±0.02	0.16±0.02
Mature	26	0.15±0.01[a]	0.17±0.03[a]	21	0.14±0.02	0.16±0.02[c]
Caucasians						
Pre-/Early Puberty	14	0.13±0.01	0.16±0.02	20	0.12±0.01[a]	0.16±0.02[b]
Mid-Puberty	16	0.15±0.01	0.16±0.02[c]	16	0.13±0.01	0.17±0.02
Mature	23	0.16±0.01[b]	0.17±0.03[b]	13	0.15±0.02	0.17±0.03
African Americans						
Pre-/Early Puberty	7	0.14±0.01	0.18±0.03	5	0.14±0.01[a]	0.20±0.03[a,b]
Mid-Puberty	29	0.16±0.02	0.18±0.03[c]	20	0.13±0.02	0.18±0.02
Mature	26	0.17±0.02[a,b]	0.20±0.03[a,b]	16	0.15±0.02	0.19±0.03[c]

[1] Mean (±SD) values for bone mineral content (BMC), bone mineral density (BMD), and bone mineral apparent density (BMAD) measured at LS (lumbar spine: L2–4) and FN (femoral neck)
[2] similar letter superscripts indicate significant racial differences within pubertal stage at $P < 0.05$

TABLE 3b
Second year bone measurement values reported by race and pubertal stage[1,2]

	Females			*Males*		
Pubertal Stage	*n*	*LS BMC (g)*	*FN BMC (g)*	*N*	*LS BMC (g)*	*FN BMC (g)*
Asians						
Pre-/Early Puberty	5	18.99±10.29	2.71±0.25[a]	8	14.80±13.49	3.61±0.57
Mid-Puberty	13	28.64±20.86	4.13±0.52	13	42.03±18.39	5.03±0.66
Mature	16	26.02±21.64	4.00±0.49[b]	14	34.61±26.22	5.29±0.94
Caucasians						
Pre-/Early Puberty	14	24.03± 5.13	3.30±0.44	20	23.15±4.12	3.48±0.48
Mid-Puberty	15	39.06±12.19	4.52±0.66	13	36.74±20.58	5.32±1.01
Mature	18	37.17±21.07	4.49±0.71	9	39.66±28.96	5.88±1.05
African Americans						
Pre-/Early Puberty	5	18.13±12.89	3.49±0.63[a]	3	14.62±13.47	3.68±0.19
Mid-Puberty	23	33.98±19.67	4.64±0.75	11	16.10±19.21	4.26±1.38
Mature	11	19.89±24.32	5.08±0.91[b]	9	30.26±28.31	5.93±0.87

	Females			*Males*		
Pubertal Stage	*n*	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)	*N*	*LS BMD* (g/cm^2)	*FN BMD* (g/cm^2)
Asians						
Pre-/Early Puberty	5	0.74±0.07	0.65±0.07	8	0.72±0.11	0.76±0.06
Mid-Puberty	13	1.03±0.13	0.86±0.13	13	0.97±0.08[a]	0.91±0.10
Mature	16	1.00±0.09[a]	0.82±0.08[a]	14	1.03±0.14	0.91±0.14[a]
Caucasians						
Pre-/Early Puberty	14	0.75±0.09	0.72±0.07	20	0.70±0.07	0.75±0.07
Mid-Puberty	15	1.01±0.11	0.88±0.11	13	0.97±0.13[b]	0.96±0.12
Mature	18	1.06±0.10	0.88±0.13[b]	9	1.11±0.15	1.01±0.15
African Americans						
Pre-/Early Puberty	5	0.80±0.09	0.78±0.09	3	0.75±0.07	0.83±0.09
Mid-Puberty	23	1.06±0.14	0.94±0.15	11	0.81±0.19[a,b]	0.87±0.15
Mature	11	1.14±0.11[a]	1.02±0.23[a,b]	9	1.09±0.12	1.06±0.12[a]

	Females			*Males*		
Pubertal Stage	*n*	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)	*N*	*LS BMAD* (g/cm^3)	*FN BMAD* (g/cm^3)
Asians						
Pre-/Early Puberty	5	0.13±0.01	0.16±0.03	8	0.13±0.01	0.16±0.02
Mid-Puberty	13	0.16±0.02	0.18±0.04	13	0.14±0.01	0.16±0.02
Mature	16	0.15±0.02[a]	0.17±0.02	14	0.14±0.02	0.16±0.02[a]
Caucasians						
Pre-/Early Puberty	14	0.13±0.01	0.16±0.02	20	0.12±0.01	0.16±0.02
Mid-Puberty	15	0.16±0.01	0.17±0.02	13	0.14±0.01	0.17±0.02
Mature	18	0.16±0.01[b]	0.17±0.03	9	0.16±0.02	0.17±0.03
African Americans						
Pre-/Early Puberty	5	0.14±0.01	0.17±0.01	3	0.13±0.01	0.19±0.04
Mid-Puberty	23	0.17±0.02	0.19±0.03	11	0.13±0.02	0.17±0.02
Mature	11	0.18±0.02[a,b]	0.21±0.06	9	0.15±0.02	0.19±0.02[a]

[1] Mean (±SD) values of bone mineral content (BMC), bone mineral density (BMD), and bone mineral apparent density (BMAD). LS; lumbar spine (L2-4). FN; femoral neck.
[2] similar letter superscripts indicate significant racial differences within pubertal stage at $P < 0.05$

TABLE 4

Percent gains in volumetric bone mineral apparent density (BMAD), between year one and year two[1,2,3] among subjects by race and pubertal stage.

	Females			*Males*		
Pubertal Stage	*n*	*LS BMAD % Gain*	*FN BMAD % Gain*	*n*	*LS BMAD % Gain*	*FN BMAD % Gain*
Asians						
Pre-/Early Puberty	5	2.10 ± 1.82	-0.46 ± 2.54	8	1.44 ± 3.28	-2.91 ± 4.62
Mid-Puberty	13	2.75 ± 2.76	2.90 ± 7.47	13	1.67 ± 2.96	3.10 ± 3.80[a]
Mature	16	2.64 ± 6.67	1.41 ± 3.41	14	-0.24 ± 2.07	-0.63 ± 3.26
Caucasians						
Pre-/Early Puberty	14	2.98 ± 5.07	-0.89 ± 7.04	20	0.34 ± 2.69	-0.81 ± 5.65
Mid-Puberty	15	2.90 ± 1.90	3.81 ± 4.93	13	3.96 ± 3.13[a]	0.69 ± 5.04
Mature	18	-0.02 ± 1.37	-0.65 ± 3.78	9	0.02 ± 2.97	-3.57 ± 4.05[b]
African Americans						
Pre-/Early Puberty	5	0.33 ± 3.08	-2.78 ± 5.08	3	-1.58 ± 0.78	-3.00 ± 3.83
Mid-Puberty	23	4.23 ± 3.04	3.04 ± 4.85	11	0.39 ± 3.50[a]	-1.93 ± 5.51[a]
Mature	11	0.39 ± 2.13	0.89 ± 9.11	9	2.33 ± 2.54	1.15 ± 2.31[b]
Total						
Pre-/Early Puberty	24	2.24 ± 4.23	-1.11 ± 5.88	31	0.41 ± 2.81	-1.57 ± 5.22
Mid-Puberty	51	3.46 ± 2.72	3.23 ± 5.54	37	2.09 ± 3.44	0.76 ± 5.09
Mature	45	1.03 ± 4.29	0.46 ± 5.40	32	0.56 ± 2.65	-0.96 ± 3.66

[1] mean ±SD. LS; lumbar spine (L2-4). FN; femoral neck.

[2] % gains in BMAD: $[(BMAD_{yr\,2} - BMAD_{yr1})/ BMAD_{yr1}] \times 100 \div$ time interval (in years).

[3] similar letter superscripts indicate significant racial differences within pubertal groups at $P < 0.05$

TABLE 5

Associations of nutrient intake with bone measurements in pre-/early pubertal and mature subjects at the femoral neck (FN)[1,2]

Bone measurement (Dependent variable)	Nutrient Intake (Independent variable)			
	Magnesium (100 mg/d)	Calcium (100 mg/d)	Protein (g/d)	Energy (kcal/d)
FN BMD year 1				
Females				
Pre-/Early Puberty	NS	NS	NS	NS
Mature	-0.0575±0.023	0.0156±0.0074	NS	NS
Males				
Pre-/Early Puberty	NS	-0.0099±0.0039	NS	NS
Mature	NS	NS	NS	NS
FN BMD year 2				
Females				
Pre-/Early Puberty	NS	NS	NS	NS
Mature	-0.0508±0.022	0.0159±0.0072	NS	NS
Males				
Pre-/Early Puberty	NS	NS	NS	NS
Mature	NS	NS	NS	NS
FN BMAD year 1				
Females				
Pre-/Early Puberty	NS	0.0062±0.0028	NS	NS
Mature	-0.0169±0.0059	NS	NS	NS
Males				
Pre-/Early Puberty	NS	NS	NS	NS
Mature	NS	NS	NS	
FN BMAD year 2				
Females				
Pre-/Early Puberty	NS	NS	NS	NS
Mature	-0.0158±0.0059	0.0044±0.0019	NS	NS
Males				
Pre-/Early Puberty	NS	NS	NS	NS
Mature	NS	NS	NS	NS

[1] Values represent regression coefficients (± SEE) derived from multiple regression analyses which included the following independent variables: weight-bearing activity, BMI, race (African Americans and Asians compared to Caucasians), and the nutrients of interest. All values reported are significant at $P < 0.05$. BMD; bone mineral density (g/cm^2). BMAD; bone mineral apparent density (g/cm^3). NS; not significant.

[2] No significant associations were observed between nutrients and gains in BMAD.

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CHAPTER III

SUMMARY AND RECOMMENDATIONS

REFERENCES

Summary and Recommendations

Magnesium plays an integral role in skeletal metabolism. It was hypothesized that a positive association between dietary magnesium and bone mass, as well as gains in bone mass, would be observed. However, negative associations were observed between magnesium and bone mass at the femoral neck among mature females only. Interestingly, no associations were observed between magnesium and bone mass in the pre-/early and mid-pubertal subjects, even though bone mineral is rapidly accruing in these younger sub-groups. No associations between nutrient intake and gains in bone mass were observed.

Future studies could be improved by addressing the study's limitations. Because bone mass appears to be influenced by dietary intake over the lifetime, a one year assessment of nutrient influences on bone mass may be inadequate for capturing dietary influences on bone mass. Further analysis of the diet and bone mass data gathered for the longitudinal study, of which this study was a sub-set, could be informative. Because gender and pubertal stage have been shown to impact bone mass, it is important to stratify subjects by gender and pubertal group. Stratification reduced statistical power of the data. It would behoove future researchers to attempt to make observations among larger groups of individuals. At the present time it is difficult for researchers to accurately assess time spent walking; attempts to control for this ought to be made in future research. Finally, although progress has been made, accurately assessing dietary intake among children and adolescents remains challenging for nutrition scientists. Validated assessment tools for these purposes are still needed.

The inadequate intake of magnesium consistently documented in this population is disconcerting. Adolescents and young adults routinely underconsume foods which are good sources of magnesium. Increased consumption of refined and processed foods has further decreased magnesium intake. The nutritional impact of consistently low levels of magnesium intake, when compared to recommended levels, deserves investigation.

Due to recent concerns regarding inadequate calcium intake among youths, food fortification with calcium is increasingly prevalent. While this may be a prudent measure, there are serious concerns regarding substantial increases in one nutrient without concomitant increases in another. Nutrient-nutrient interactions do exist and there is inadequate information regarding these effects in humans. Although bone mineral is predominantly calcium, magnesium and phosphorus are essential skeletal nutrients. All three of these nutrients are intimately involved with absorption, metabolism and regulation of each other. Vitamins are involved as well, and the extent to which other minerals, including fluoride and manganese, may be involved in skeletal health remains largely unknown.

This study was unable to document clear associations with magnesium and bone mineral density, perhaps, in part, because of the limitations previously described. Future studies are warranted. Independent metabolic effects of magnesium and calcium need further investigation. Clearer knowledge of the effects of magnesium on skeletal development is needed. Well-controlled human magnesium supplementation trials could be informative. Interactions between magnesium and other nutrients should be investigated. Finally, the impact of foods and food groups on bone mineralization

deserve investigation.

Diet is one of the many factors contributing to bone mass. Nonetheless, bone is more than 30% mineral, diet is modifiable, and osteoporosis may have its origins in childhood.

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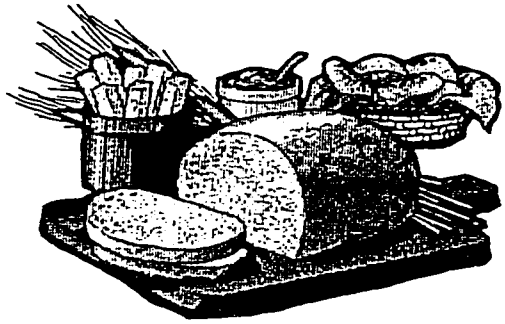
Appendix A

FOOD QUESTIONNAIRE



This form asks about your usual food intake.
It takes about 30 minutes to complete. Please follow these instructions:

- Answer each question as best you can – estimate if you aren't sure.
- Use only a No. 2 pencil.
- Be certain to completely blacken in each of your answers, and erase completely if you make any changes.

CORRECT MARK: ●
INCORRECT MARKS: ⊘ ⊗ ◒ ⊙

PLEASE PRINT YOUR NAME IN THIS BOX.
PLEASE DO NOT WRITE OUTSIDE THE BOXED AREA.

1. IDENTIFICATION NUMBER

2. SEX
○ Male
○ Female

3. AGE
○ Less than 20
○ 20-29
○ 30-39
○ 40-49
○ 50-59
○ 60-69
○ 70+

4. TODAY'S DATE

	DAY	YEAR	
○ Jan			
○ Feb			
○ Mar	01	91	○
○ Apr	02	92	○
○ May	03	93	○
○ Jun	04	94	○
○ Jul	05	95	○
○ Aug	06	96	○
○ Sep	07	97	○
○ Oct	08	98	○
○ Nov	09	99	○
○ Dec	10	00	○

5. WEIGHT pounds

6. HEIGHT ft. in.

HHHQ. FULL.JAN 92

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7. Do you smoke cigarettes now?
   - ○ No
   - ○ Yes **IF YES, on the average, about how many cigarettes a day do you smoke now?**
     ○ 1 - 5 ○ 6 - 14 ○ 15 - 24 ○ 25 - 34 ○ 35 or more

8. About how many times have you gone on a diet to lose weight?
   ○ Never ○ 1 - 2 ○ 3 - 5 ○ 6 - 8 ○ 9 - 11 ○ 12 or more

9. During the past year have you taken any vitamins or minerals?
   ○ No ○ Yes, fairly regularly ○ Yes, but not regularly
   IF YES, what do you take fairly regularly?

VITAMIN TYPE	NUMBER OF TABLETS								FOR HOW MANY YEARS?				
	NONE	1-3 PER WEEK	4-6 PER WEEK	1 PER DAY	2 PER DAY	3 PER DAY	4 PER DAY	5+ PER DAY	LESS THAN 1 YR	1-2 YEARS	3-5 YEARS	6-9 YEARS	10+ YEARS
Multiple Vitamins													
Stress-tabs type	○	○	○	○	○	○	○	○	○	○	○	○	○
Therapeutic, Theragran type	○	○	○	○	○	○	○	○	○	○	○	○	○
One-a-day type	○	○	○	○	○	○	○	○	○	○	○	○	○
Other Vitamins													
Vitamin A	○	○	○	○	○	○	○	○	○	○	○	○	○
Vitamin E	○	○	○	○	○	○	○	○	○	○	○	○	○
Calcium or Tums	○	○	○	○	○	○	○	○	○	○	○	○	○
Vitamin C	○	○	○	○	○	○	○	○	○	○	○	○	○

10. If you take Vitamin E or Vitamin C:
    How many units per Vitamin E tablet? ○ 100 ○ 200 ○ 400 ○ 1000 ○ Don't know
    How many milligrams per Vitamin C tablet? ○ 100 ○ 250 ○ 500 ○ 1000 ○ Don't know

11. Do you regularly take pills containing any of these nutrients?
    ○ No or don't know ○ Iron ○ Beta-carotene
    ○ Zinc ○ Selenium ○ _____

12. What kinds of fat do you *usually* use in cooking (to fry, stir-fry, or saute)? Mark only one or two.
    ○ Don't know or don't cook ○ Lard, fatback, baconfat ○ Pam or no oil ○ Crisco
    ○ Stick margarine ○ Butter ○ Soft tub margarine ○ Oil
    ○ 1/2 butter, 1/2 margarine ○ Low calorie margarine

13. What kinds of fat do you *usually* add to vegetables, potatoes, etc.? Mark only one or two.
    ○ Don't add fat ○ Lard, fatback, baconfat ○ Low calorie margarine
    ○ Stick margarine ○ Soft tub margarine ○ 1/2 butter, 1/2 margarine
    ○ Butter ○ Whipped butter ○ Crisco

14. When you eat the following foods, how often do you eat a low-fat or non-fat version of that food?
    CHEESE ○ Always low-fat ○ Sometimes ○ Rarely low-fat
    ICE CREAM/YOGURT ○ Always low-fat ○ Sometimes ○ Rarely low-fat
    SALAD DRESSING ○ Always low-fat ○ Sometimes ○ Rarely low-fat

15.

	SELDOM/NEVER	SOMETIMES	OFTEN/ALWAYS
a. How often do you add salt to your food?	○	○	○
b. How often do you add pepper to your food?	○	○	○
c. How often do you eat the skin on chicken?	○	○	○
d. How often do you eat the fat on meat?	○	○	○

16. **About how often do you eat the following foods from restaurants or carry-outs? Remember to think about all meals (breakfast, lunch, dinner or snacks).**

RESTAURANT FOOD	NUMBER OF VISITS LAST YEAR						
	NEVER IN PAST YEAR	1-4 TIMES PAST YEAR	5-11 TIMES PAST YEAR	1-3 TIMES A MONTH	ONCE A WEEK	2-4 TIMES A WEEK	ALMOST EVERY DAY
Fried chicken	○	○	○	○	○	○	○
Burgers	○	○	○	○	○	○	○
Pizza	○	○	○	○	○	○	○
Chinese food	○	○	○	○	○	○	○
Mexican food	○	○	○	○	○	○	○
Fried fish	○	○	○	○	○	○	○

17. **This section is about your *usual* eating habits over the past year.**

FIRST: Mark the column to show how often, on the average, you ate the food during the past year. Please BE CAREFUL which column you put your answer in.

SECOND: Mark whether your usual serving size is small, medium or large. Please DO NOT OMIT serving size.

ADDITIONAL COMMENTS:

- Please DO NOT SKIP any foods. If you never eat a food, mark "Never or less than once a month."
- A small serving is about one-half the medium serving size shown, or less.
- A large serving is about one-and-a-half times the medium serving size shown, or more.

SAMPLE: This person ate a medium serving of rice about twice per month and never ate squash.

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	NEVER OR LESS THAN ONCE PER MONTH	1 PER MON	2-3 PER MON	1 PER WEEK	2 PER WEEK	3-4 PER WEEK	5-6 PER WEEK	1 PER DAY	2+ PER DAY	MEDIUM SERVING	YOUR SERVING SIZE		
											S	M	L
Rice	○	○	●	○	○	○	○	○	○	3/4 cup	○	●	○
Winter squash, baked squash	●	○	○	○	○	○	○	○	○	1/2 cup	○	○	○

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TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	NEVER OR LESS THAN ONCE PER MONTH	1 PER MON	2-3 PER MON	1 PER WEEK	2 PER WEEK	3-4 PER WEEK	5-6 PER WEEK	1 PER DAY	2+ PER DAY	MEDIUM SERVING	YOUR SERVING SIZE S	M	L
FRUITS AND JUICES													
EXAMPLE: Apples, etc.	○	○	○	●	○	○	○	○	○	1 medium or 1/2 cup	○	●	○
Apples, applesauce, pears	○	○	○	○	○	○	○	○	○	1 medium or 1/2 cup	○	○	○
Bananas	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Peaches, apricots (fresh or canned	○	○	○	○	○	○	○	○	○	1 medium or 1/2 cup	○	○	○
Cantaloupe (in season)	○	○	○	○	○	○	○	○	○	1/4 medium	○	○	○
Cantaloupe (rest of year)	○	○	○	○	○	○	○	○	○	1/4 medium	○	○	○
Watermelon (in season)	○	○	○	○	○	○	○	○	○	1 slice	○	○	○
Strawberries (in season)	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Oranges	○	○	○	○	○	○	○	○	○	1 medium	○	○	○
Grapefruit	○	○	○	○	○	○	○	○	○	1/2 medium	○	○	○
Orange juice or grapefruit juice	○	○	○	○	○	○	○	○	○	6 ounce glass	○	○	○
Fruit drinks with added vitamin C, such as Hi-C	○	○	○	○	○	○	○	○	○	6 ounce glass	○	○	○
Any other fruit, including berries, fruit cocktail, grapes	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
BREAKFAST FOODS													
High fiber, bran or granola cereals, shredded wheat	○	○	○	○	○	○	○	○	○	1 medium bowl	○	○	○
Highly fortified cereals, such as Total, Just Right or Product 19	○	○	○	○	○	○	○	○	○	1 medium bowl	○	○	○
Other cold cereals, such as corn flakes, Rice Krispies	○	○	○	○	○	○	○	○	○	1 medium bowl	○	○	○
Cooked cereal, or grits	○	○	○	○	○	○	○	○	○	1 medium bowl	○	○	○
Milk on cereal	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Sugar added to cereal	○	○	○	○	○	○	○	○	○	2 teasp	○	○	○
Eggs	○	○	○	○	○	○	○	○	○	1 egg=sm 2 eggs=med	○	○	○
Bacon	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
Sausage	○	○	○	○	○	○	○	○	○	2 patties or links	○	○	○

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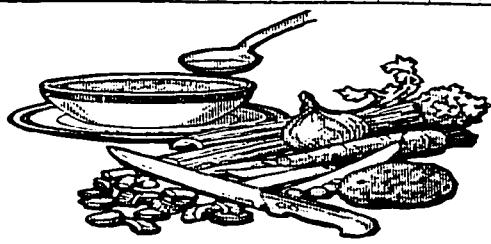
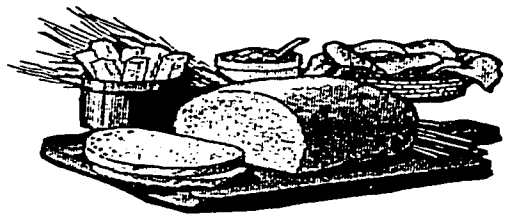
TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	NEVER OR LESS THAN ONCE PER MONTH	1 PER MON	2-3 PER MON	1 PER WEEK	2 PER WEEK	3-4 PER WEEK	5-6 PER WEEK	1 PER DAY	2+ PER DAY	MEDIUM SERVING	YOUR SERVING SIZE		
											S	M	L
VEGETABLES													
String beans, green beans	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Peas	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Chili with beans	O	O	O	O	O	O	O	O	O	3/4 cup	O	O	O
Other beans such as baked beans, pintos, kidney, limas, and lentils	O	O	O	O	O	O	O	O	O	3/4 cup	O	O	O
Corn	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Winter squash/baked squash	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Tomatoes, tomato juice	O	O	O	O	O	O	O	O	O	1 medium or 6 oz. glass	O	O	O
Red chili sauce, taco sauce, salsa picante	O	O	O	O	O	O	O	O	O	2 tablesp	O	O	O
Broccoli	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Cauliflower or brussels sprouts	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Spinach (raw)	O	O	O	O	O	O	O	O	O	3/4 cup	O	O	O
Spinach (cooked)	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Mustard greens, turnip greens, collards	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Cole slaw, cabbage, sauerkraut	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Carrots, or mixed vegetables containing carrots	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Green salad	O	O	O	O	O	O	O	O	O	1 medium bowl	O	O	O
Regular salad dressing & mayonnaise, including on sandwiches or on potato salad, etc.	O	O	O	O	O	O	O	O	O	2 tablesp	O	O	O
French fries and fried potatoes	O	O	O	O	O	O	O	O	O	3/4 cup	O	O	O
Sweet potatoes, yams	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Other potatoes, including boiled, baked, mashed & potato salad	O	O	O	O	O	O	O	O	O	1 medium or 1/2 cup	O	O	O
Rice	O	O	O	O	O	O	O	O	O	3/4 cup	O	O	O
Any other vegetable, including cooked onions, summer squash	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Butter, margarine or other fat added to veg., potatoes, etc.	O	O	O	O	O	O	O	O	O	2 pats	O	O	O

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	NEVER OR LESS THAN ONCE PER MONTH	1 PER MON	2-3 PER MON	1 PER WEEK	2 PER WEEK	3-4 PER WEEK	5-6 PER WEEK	1 PER DAY	2+ PER DAY	MEDIUM SERVING	YOUR SERVING SIZE		
											S	M	L
MEAT, FISH, POULTRY, LUNCH ITEMS													
Hamburgers, cheeseburgers, meatloaf, beef burritos, tacos	○	○	○	○	○	○	○	○	○	1 medium or 4 oz.	○	○	○
Beef, (steaks, roasts, etc., including sandwiches)	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Beef stew or pot pie with carrots or other vegetables	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Liver, including chicken livers	○	○	○	○	○	○	○	○	○	4 ounces	○	○	○
Pork, including chops, roasts	○	○	○	○	○	○	○	○	○	2 chops or 4 ounces	○	○	○
Fried chicken	○	○	○	○	○	○	○	○	○	2 small or 1 large pce	○	○	○
Chicken or turkey (roasted, stewed or broiled, including on sandwiches)	○	○	○	○	○	○	○	○	○	2 small or 1 large pce	○	○	○
Fried fish or fish sandwich	○	○	○	○	○	○	○	○	○	4 ounces or 1 sandwich	○	○	○
Tuna, tuna salad, tuna casserole	○	○	○	○	○	○	○	○	○	1/2 cup	○	○	○
Oysters	○	○	○	○	○	○	○	○	○	5 pieces, 1/4 cup or 3 oz.	○	○	○
Shell fish, (shrimp, crab, lobster, etc.)	○	○	○	○	○	○	○	○	○	5 pieces, 1/4 cup or 3 oz.	○	○	○
Other fish (broiled or baked)	○	○	○	○	○	○	○	○	○	2 pieces or 4 ounces	○	○	○
Spaghetti, lasagna, other pasta with tomato sauce	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Pizza	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
Mixed dishes with cheese (such as macaroni and cheese)	○	○	○	○	○	○	○	○	○	1 cup	○	○	○
Liverwurst	○	○	○	○	○	○	○	○	○	2 slices	○	○	○
Hot dogs	○	○	○	○	○	○	○	○	○	2 hot dogs	○	○	○
Ham, bologna, salami and other lunch meats	○	○	○	○	○	○	○	○	○	2 slices or 2 ounces	○	○	○
Vegetable and tomato soups, including vegetable beef, minestrone	○	○	○	○	○	○	○	○	○	1 medium bowl	○	○	○
Other soups	○	○	○	○	○	○	○	○	○	1 medium bowl	○	○	○



TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	NEVER OR LESS THAN ONCE PER MONTH	1 PER MON	2-3 PER MON	1 PER WEEK	2 PER WEEK	3-4 PER WEEK	5-6 PER WEEK	1 PER DAY	2+ PER DAY	MEDIUM SERVING	YOUR SERVING SIZE		
											S	M	L
BREADS, SNACKS, SPREADS													
Biscuits, muffins, (including fast foods)	O	O	O	O	O	O	O	O	O	1 medium piece	O	O	O
White bread (including sandwiches, bagels, burger rolls, French or Italian bread	O	O	O	O	O	O	O	O	O	2 slices	O	O	O
Dark bread, such as wheat, rye, pumpernickel, (including sandwiches)	O	O	O	O	O	O	O	O	O	2 slices	O	O	O
Corn bread, corn muffins, corn tortillas	O	O	O	O	O	O	O	O	O	1 medium piece	O	O	O
Salty snacks, such as potato chips, corn chips, popcorn	O	O	O	O	O	O	O	O	O	2 handfuls or 1 cup	O	O	O
Peanuts, peanut butter	O	O	O	O	O	O	O	O	O	2 tablesp	O	O	O
Margarine on bread or rolls	O	O	O	O	O	O	O	O	O	2 pats	O	O	O
Butter on bread or rolls	O	O	O	O	O	O	O	O	O	2 pats	O	O	O
Gravies made with meat drippings, or white sauce	O	O	O	O	O	O	O	O	O	2 tablesp	O	O	O
DAIRY PRODUCTS													
Cottage cheese	O	O	O	O	O	O	O	O	O	1/2 cup	O	O	O
Other cheeses and cheese spreads	O	O	O	O	O	O	O	O	O	2 slices or 2 ounces	O	O	O
Flavored yogurt, frozen yogurt	O	O	O	O	O	O	O	O	O	1 cup	O	O	O
SWEETS													
Ice cream	O	O	O	O	O	O	O	O	O	1 scoop or 1/2 cup	O	O	O
Doughnuts, cookies, cake, pastry	O	O	O	O	O	O	O	O	O	1 piece or 3 cookies	O	O	O
Pumpkin pie, sweet potato pie	O	O	O	O	O	O	O	O	O	1 medium slice	O	O	O
Other pies	O	O	O	O	O	O	O	O	O	1 medium slice	O	O	O
Chocolate candy	O	O	O	O	O	O	O	O	O	1 small bar or 1 oz	O	O	O
Other candy, jelly, honey, brown sugar	O	O	O	O	O	O	O	O	O	3 pieces or 1 tblsp.	O	O	O



TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	NEVER OR LESS THAN ONCE PER MONTH	1-3 PER MON	1 PER WEEK	2-4 PER WEEK	5-6 PER WEEK	1 PER DAY	2-3 PER DAY	4-5 PER DAY	6+ PER DAY	MEDIUM SERVING	YOUR SERVING SIZE		
											S	M	L
BEVERAGES (Please note that the categories for these columns are different.)													
Whole milk and beverages with whole milk (not incl. on cereal)	○	○	○	○	○	○	○	○	○	8 oz. glass	○	○	○
2% milk and beverages with 2% milk (not including on cereal)	○	○	○	○	○	○	○	○	○	8 oz. glass	○	○	○
Skim milk, 1% milk or buttermilk (not including on cereal)	○	○	○	○	○	○	○	○	○	8 oz. glass	○	○	○
Regular soft drinks (not diet soda)	○	○	○	○	○	○	○	○	○	12 oz can or bottle	○	○	○
Beer	○	○	○	○	○	○	○	○	○	12 oz can or bottle	○	○	○
Wine or wine coolers	○	○	○	○	○	○	○	○	○	1 medium glass	○	○	○
Liquor	○	○	○	○	○	○	○	○	○	1 shot	○	○	○
Coffee, regular or decaf	○	○	○	○	○	○	○	○	○	1 medium cup	○	○	○
Tea (hot or iced)	○	○	○	○	○	○	○	○	○	1 medium cup	○	○	○
Lemon in tea	○	○	○	○	○	○	○	○	○	1 teaspo	○	○	○
Non-dairy creamer in coffee or tea	○	○	○	○	○	○	○	○	○	1 tablesp	○	○	○
Cream (real) or Half-and-Half in coffee or tea	○	○	○	○	○	○	○	○	○	1 tablesp	○	○	○
Milk in coffee or tea	○	○	○	○	○	○	○	○	○	1 tablesp	○	○	○
Sugar in coffee or tea	○	○	○	○	○	○	○	○	○	2 teaspoons	○	○	○
Glasses of water	○	○	○	○	○	○	○	○	○	8 oz. glass	○	○	○

18.

SUMMARY QUESTIONS	AVERAGE USE LAST YEAR								
	LESS THAN ONCE PER WEEK	1-2 PER WEEK	3-4 PER WEEK	5-6 PER WEEK	1 PER DAY	1 1/2 PER DAY	2 PER DAY	3 PER DAY	4+ PER DAY
a. How often do you use fat or oil in cooking?	○	○	○	○	○	○	○	○	○
b. About how many servings of vegetables do you eat, not counting salad or potatoes?	○	○	○	○	○	○	○	○	○
c. About how many servings of fruit do you eat, not counting juices?	○	○	○	○	○	○	○	○	○
d. About how many servings of cold cereal do you eat?	○	○	○	○	○	○	○	○	○

THANK YOU VERY MUCH FOR TAKING THE TIME TO FILL OUT THIS QUESTIONNAIRE

Please take a moment to fill in any questions you may have skipped.

62173

Appendix B

STANFORD UNIVERSITY
Stanford, California 94305-5532

DAVID GABA, M.D.
CHAIR, PANEL ON MEDICAL HUMAN SUBJECTS
c/o Administrative Panels Office

(415)-725-5873
(415)-725-8013 (fax)

CERTIFICATION OF HUMAN SUBJECTS APPROVAL

DATE: July 8, 1997

TO: L.K. Bachrach, M.D.
and R. Marcus, M.D.
Department of Pediatrics

FROM: Chairman, Administrative Panel
on Human Subjects in Medical Research

PROTOCOL TITLE:
Bone Mineral Accretion in Healthy Adolescents

The Panel approved human subjects involvement in your research project on July 8, 1997.

The expiration date of this approval is July 7, 1998. If this project is to continue beyond that date, please submit an updated proposal in advance for the Panel's re-approval. If this proposal is used in conjunction with any other human experimentation or if it is modified in any way, it must be re-approved for these special circumstances. In addition, the Panel requests prompt notification of any complications which may occur during any experimental procedure.

All continuing projects and activities must be reviewed and re-approved at least annually by the Panel. Panel approval of any project is for a maximum period of one year. It is the responsibility of the investigator to resubmit the project to the Panel for annual review.

David M. Gaba, M.D. Chairman

cc: R. Marcus
D. Feldman

Funding Agency: (Under DK45226,LSPCH AR-91-03,Marcus,#9831&Feldman DK50802)(R)
Period of Time: 07/08/97 through 07/07/98
Investigational New Drugs: N ,
Investigational New Device: N ,
Cooperating Institution: N
Full Board Review
Assurance Number: M1272-03
IRB #01

Appendix C

San José State
UNIVERSITY

Office of the Academic Vice President
Associate Vice President Graduate Studies and Research
One Washington Square
San Jose, CA 95192-0025
Voice: 408-924-2480
Fax: 408-924-2477
E-mail: gstudies-awards@sisu.edu
http://www.sisu.edu

TO: Gretchen Vannice
P.O. Box 1900
Soquel, CA 95073

FROM: Nabil Ibrahim,
Acting AVP, Graduate Studies & Research

DATE: April 27, 1999

The Human Subjects-Institutional Review Board has approved your request to use human subjects in the study entitled:

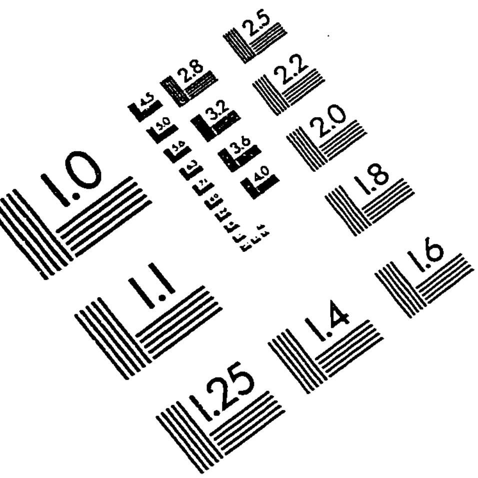
"Magnesium and Bone Mineral Acquisition in Ethnically Diverse Adolescents"

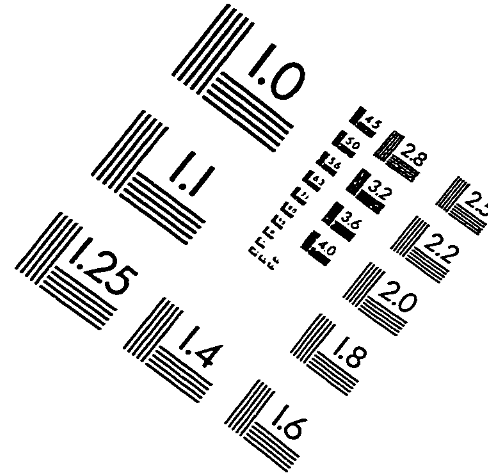
This approval is contingent upon the subjects participating in your research project being appropriately protected from risk. This includes the protection of the anonymity of the subjects' identity when they participate in your research project, and with regard to any and all data that may be collected from the subjects. The Board's approval includes continued monitoring of your research by the Board to assure that the subjects are being adequately and properly protected from such risks. If at any time a subject becomes injured or complains of injury, you must notify Nabil Ibrahim, Ph.D., immediately. Injury includes but is not limited to bodily harm, psychological trauma and release of potentially damaging personal information.

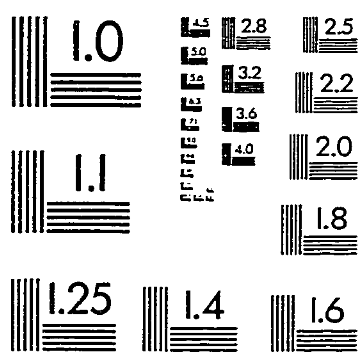
Please also be advised that all subjects need to be fully informed and aware that their participation in your research project is voluntary, and that he or she may withdraw from the project at any time. Further, a subject's participation, refusal to participate, or withdrawal will not affect any services the subject is receiving or will receive at the institution in which the research is being conducted.

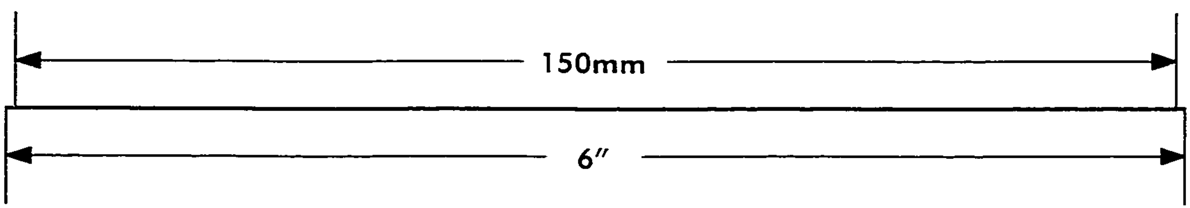
If you have any questions, please contact me at (408) 924-2480.

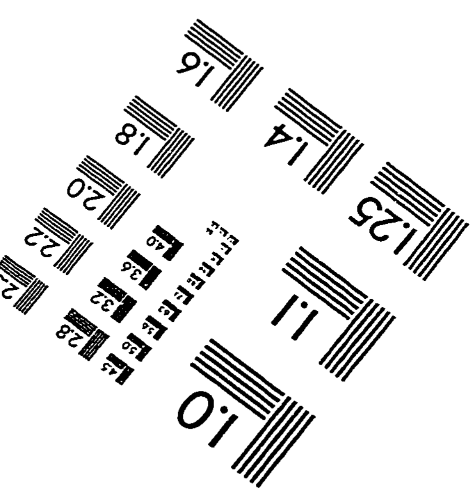
IMAGE EVALUATION TEST TARGET (QA-3)

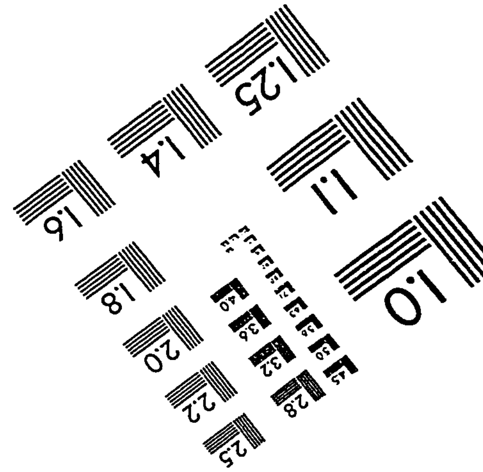












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