ZINC SUPPLEMENTATION AND BONE TURNOVER IN YOUNG ADOLESCENT GIRLS

by

VALERIE CHERTIN

(Under the Direction of Richard D. Lewis)

ABSTRACT

Pubertal growth is characterized by high bone formation rates. Zinc (Zn), a trace mineral required for skeletal development, has been shown to augment bone formation during growth. Our laboratory found that supplementation over 4 weeks with 9 mg Zn/day increased the bone formation marker, N-terminal propeptide of type I procollagen (P1NP). The purpose of this study was to examine if a higher Zn dose, 24 mg/day, over 4 weeks would further increase P1NP, as well as insulin-like growth factor-I (IGF-I) concentrations in healthy, non-obese, white females in sexual maturation breast stage 2/3 (N=39). Plasma Zn and serum P1NP, crosslinked telopeptide of type I collagen (bone resorption marker), and IGF-I were assessed. Over 4 weeks, Zn supplementation significantly increased plasma Zn, but not P1NP or IGF-I. This research shows that a high Zn dose of 24mg did not augment bone formation during pubertal growth.

INDEX WORDS: Zinc (Zn), N-terminal propeptide of type I procollagen (P1NP), crosslinked telopeptide of type I collagen (ICTP), insulin-like growth factor-I (IGF-I), bone biomarkers
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DEDICATION

I would like to dedicate this work to my great-grandmother, Lyubov Elkina. I am so lucky to have known you and I admire your strength in all that you have experienced. I believe that your spirit lives on with me forever, and I hope to be half the woman and matriarch that you were.
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CHAPTER 1
INTRODUCTION

Osteoporosis is a debilitating condition characterized by low bone mass and increased risk of fracture. Bone mineral content (BMC) in childhood is an important determinant for adult osteoporosis risk (1, 2). Peak BMC accrual in females occurs at approximately 12 years of age (1) and a substantial amount of adult BMC is accrued throughout adolescent growth spurt (2). Specifically, 39% of the total body and 43%, 46% and 33% of the lumbar spine, total hip and femoral neck BMC (males and females), respectively, is accrued during the young-adult years (2). Therefore, it is hypothesized that childhood is a critical time to maximize bone mineral accrual and reduce risks for fractures later in life.

Several strategies have aimed to improve bone mineral accrual during childhood, most notably physical activity and calcium supplementation. There is considerable evidence linking physical activity to increased bone mineral accrual during childhood. Physical activity, particularly high-impact, weight-bearing exercises (gymnastics and volleyball), are highly osteogenic and thus are associated with greater bone size and strength (3). It is known that calcium is essential for maintaining bone mass. There have been several short-term trials suggesting that calcium might be an important determinant of peak bone mass in young adults by influencing bone accretion during growth (4, 5). A longer-term trial was able to show improvements in areal bone mineral density after calcium supplementation for adolescent females, particularly during the pubertal growth spurt (6). Another strategy to maximize bone mineral accrual has been through vitamin D supplementation. Meta-analyses of vitamin D intervention trials in children and adolescents have concluded that vitamin D supplementation in children and adolescents with serum 25(OH)D concentrations defined as deficient could improve
total peak body BMC (7). To date, vitamin D and calcium have been the most widely studied nutrients, particularly benefiting deficient populations. There has been less focus on the effects of trace minerals on bone.

Zinc (Zn) is a trace mineral found in many tissues of the body, with 20% stored in the skeleton (8) and is essential for bone development and maintenance (9). In cell culture, Zn has been shown to have a stimulatory effect on bone formation and mineralization and an inhibitory effect on bone resorption (9-11), although the mechanisms for these effects are unclear. Studies using various animal models have shown improvements in bone mineralization and strength following Zn supplementation (12-14). For example, Rhesus monkeys fed low levels of dietary Zn (2 ug/g diet) had slowed skeletal growth and development that was most apparent toward the end of the skeletal growth spurt (13). Ovesen et al (14) demonstrated that supplementation with Zn to growing male rats improved bone strength via biomechanical testing over 4 weeks.

Human studies show relationships between dietary Zn intake and bone, and supplementation trials show similar results as the above animal studies. For instance, Zn intake has shown to be positively correlated to arial BMD (aBMD) as well as other measures of bone geometry, size and strength in both adults and children (15-18). Supplementation trials in adults and children investigating the effects of Zn on bone showed that daily Zn supplementation (50 mg/day) in healthy men over 12 weeks significantly increased markers of bone formation, particularly bone specific alkaline phosphatase (BSAP) activity (an index of total osteoblast activity) (19). In children and adolescents, most Zn supplementation trials have been conducted in children with short stature. Imamoglu et al (20) observed that daily Zn supplementation of 50 mg elemental Zn over a period of 6 weeks in healthy pre-pubertal children,
ages 7.7 ± 2.6 years old, with idiopathic short stature increased bone formation markers [osteocalcin (OC)] and serum Zn.

The influence of Zn on bone formation may be via its action on circulating insulin-like growth factor-I (IGF-I). IGF-I is synthesized by bone cells and is just one of a network of local growth factors and systemic hormones that mediate the process of bone remodeling (21, 22). Zn may stimulate the production of IGF-I, ultimately leading to improved bone mass. Zn supplementation has been shown to increase circulating IGF-I concentrations in both animal and human models, but only in children of short stature (20, 23).

Two Zn supplementation trials have been conducted in healthy, normal stature, non-deficient populations with bone outcomes. Clark et al (24) supplemented healthy adolescent non-Hispanic white females, ages 12.2 ± 0.3 years with 15 mg elemental Zn/day (as citrate), (~0.3 mg/kg body weight) over six weeks. Serum Zn increased vs. placebo (P<0.001), but no significant differences were found in bone formation markers, OC or deoxypyridinoline (DPD), following supplementation.

A randomized control trial (RCT) was conducted in our laboratory in early-pubertal females, 9 - 11 years of age, of both non-Hispanic white and black races (see Chapter 3). Subjects were administered 9 mg/day elemental Zn (~0.2 mg/kg body weight) (n=75) or a placebo (n=72) over 4-weeks to determine effects on plasma Zn, bone formation markers [OC, N-terminal propeptide of type I procollagen (P1NP)], bone resorption markers [crosslinked telopeptide of type I collagen (ICTP), pyridinoline (PYD), DPD], and IGF-I. Plasma Zn and P1NP increased significantly in the Zn vs. placebo group (p<0.05). However, no differences in resorption markers or IGF-I were found. These results provide preliminary evidence that Zn supplementation at a dose of 9 mg/day may be a feasible nutritional strategy for improving bone
formation during growth. Another pilot project conducted in our laboratory using slightly older adolescent females, showed that Zn supplementation with a higher dose (24 mg/day) significantly increased IGF-I. Because of the modest increase in plasma Zn and the fact that IGF-I was not significantly changed with the 9 mg/d supplement, in the RCT it may be that a higher Zn dose would elicit more positive changes in bone remodeling.

The purpose of the study presented in this thesis was to examine the effects of Zn supplementation on bone turnover markers and IGF-I concentrations in females in the early stages of puberty. The main hypothesis was that supplementation with 24 mg/day elemental Zn will alter markers of bone formation and circulating IGF-I in white females, 9 - 12 years of age, in the early stages of puberty.

The specific aims were to: 1) determine if Zn alters markers of bone turnover, in favor of formation and 2) determine if Zn supplementation increases circulating IGF-I. The proposed project fills knowledge gaps regarding a strategy that may improve bone formation in healthy adolescents, which may ultimately lead to enhanced bone strength and reduced risk for adult osteoporosis.
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Introduction

Peak bone mineral content (BMC) accrual in females occurs at approximately 12 years of age (1) and a substantial amount (33-46%) of adult BMC is accrued throughout adolescent growth (2). Therefore, adolescence may be an ideal timeframe to conduct nutrition interventions to maximize BMC accrual and reduce the risk of adult osteoporosis. While it is well known that calcium and vitamin D have positive effects on bone accrual in childhood and prevention of osteoporosis (3-6), novel approaches to optimizing bone health and growth are currently being researched.

Zinc (Zn) is required for skeletal development and maintenance and has a potential stimulatory effect on bone formation and an inhibitory effect on bone resorption (7). In cell culture, Zn has been shown to stimulate bone formation by stimulating the osteoblast. Cell culture has also shown that Zn may inhibit bone resorption by inhibiting resorbing factors or inhibiting the synthesis of osteoclasts (bone resorbing cells) (7). Studies using various animal models have shown improvements in bone mineralization and strength following Zn supplementation (8, 9). Rhesus monkeys fed low levels of dietary Zn (2 ug/g diet) had slowed skeletal growth and development that was most apparent after the completion of the skeletal growth spurt (9). Total body, lumbar spine and pelvis BMD were significantly higher in the control group vs the low Zn group by 39 months of age. The reason why Zn has this effect on bone is not understood, however certain mediating factors such as hormones may have a role. Insulin like growth factor-I (IGF-I) is synthesized by bone cells and is just one of a network of
local growth factors and systemic hormones that mediate the complex process of bone remodeling (10, 11). Zn may stimulate the production of IGF-I, ultimately leading to improved bone mass.

Most Zn intervention trials involving bone health have focused on deficiency populations and many intervention trials have focused primarily on calcium or vitamin D. Therefore, this project fills knowledge gaps regarding a novel strategy that may improve bone formation in healthy adolescents, which may ultimately lead to enhanced bone strength and reduced risk for adult osteoporosis. The goal of this study was to determine if Zn supplementation alters markers of bone turnover and IGF-I among females in the early stages of puberty. The hypothesis was that Zn supplementation would alter circulating markers of bone turnover, in favor of formation, and alter circulating IGF-I. The research question was, “does supplementation with 24 mg elemental Zn alter bone turnover markers and IGF-I concentrations in early adolescent females?”

This literature review provides background on childhood BMC accrual, bone turnover and growth factors in pediatric bone, Zn and bone, dietary intake of Zn, and the effects of Zn supplementation on circulating bone formation markers and IGF-I.

**Osteoporosis**

Osteoporosis, a severe musculoskeletal disease, can result in skeletal fractures due to low bone mass. It is among the most common conditions for which adults seek medical care. It is estimated that US health costs (of one in three US adults being treated with a musculoskeletal impairment or injury each year) approximate $250 billion and that by 2025 osteoporosis related costs are projected to grow to $25 billion yearly (12, 13). The encumbering effects on society and healthcare resources produce a significant burden globally and in the US. Risk factors for
osteoporosis include age, body composition, family history of osteoporosis, race, and taking certain medications. There is a strong rationale for studying females since deteriorating bone health and risk of osteoporotic fractures is much higher in females than males. According to the National Osteoporosis Foundation (NOF), approximately 50% of women over the age of 50 year with osteoporosis will experience a fracture; the risk of mortality increases following a hip fracture. Also, non-Hispanic blacks, compared to non-Hispanic whites, have higher bone mass which reduces their risk for experiencing fractures and mortality due to fractures. This may be a result of their higher peak bone mass in early adulthood and lower rates of loss with aging (14).

Areal bone mineral density (aBMD) testing, using dual energy X-ray absorptiometry (DXA), measures the amount of mineral content in the bones and is a good predictor of fracture risk. The International Society for Clinical Densitometry reported that skeletal sites recommended for assessment are the lumbar spine and total body (15). Pediatric DXA reports should report Z-scores, and should not include T-scores, since T-scores are scores based on young adult norms and the scans may not only be used to diagnose osteoporosis, but could also predict the risk of future fractures. The NOF suggests the best way for delaying onset in adulthood is getting enough calcium, vitamin D and regular exercise. However, as the origins of osteoporosis are linked to childhood skeletal growth, it is vital to determine modifiable factors, such as cost effective nutritional strategies, that will optimize bone mineral accretion and development in the earlier stages of life.

**Bone mineral accrual in children**

The development of bone mass during the growing years is an important determinant for risk of osteoporosis in later life (1). Childhood is a particularly important time to maximize bone
accrual because the skeleton undergoes many changes (2). The Saskatchewan Pediatric Bone Mineral Accrual Study was conducted in 1991 and included over 220 male and female children ages 8 to 14 years. The primary goal was to investigate bone accrual in children. Bone mineral was measured yearly until 1997. A subgroup of subjects have a complete collection of data spanning their age of peak height velocity (PHV). PHV is a measure of the maximum rate of growth in stature during a growth spurt. The age of maximum velocity of growth is called the age at PHV and it is the most commonly used indicator of somatic (body height growth) maturity in longitudinal studies. BMC is primarily determined by height and body weight. The Pediatric Bone Mineral Accrual Study subjects’ height and weight were compared to a reference standard for children to determine the stage of most bone accrual. Approximately 90% of adult bone mass is achieved by the end of adolescence. It was determined that peak bone accrual in females occurs at approximately 12 years of age (1). From the Pediatric Bone Mineral Accrual Study, Whiting et al (1) calculated that bone growth is maximal 6 months after PHV. In the second year of peak skeletal growth, adolescents accumulate over 25% of adult bone. Depending on the skeletal site, 33% to 46% of the adult BMC is accrued over the entire 5 years of adolescent growth (2). Therefore, this stage of adolescence may be an ideal stage to conduct a nutrition intervention as a preventive measure to reduce the risk of osteoporosis later in life.

Bone turnover and growth factors in pediatric bone

A bone’s cross-sectional size increases through the action of osteoblasts that add mineralized tissue on the outer (periosteal) bone surface, a process called periosteal apposition. The periosteum surrounds the bone like a stocking, which in children is thick and is only loosely attached to the diaphysis (16). Histomorphometric studies of rib and iliac bone have
demonstrated that periosteal bone formation is much more active in children than in adults (17). However, there may be a more fundamental difference between periosteal bone metabolism in children and adults. In children, bone formation is continuous, which is the hallmark of modeling (18). Bone modeling is achieved by appositional growth along the periosteal surface and by the calcification of cartilage adjacent to the growth plate (19). The periosteal apposition rates show changes with age that resemble percentile charts for height velocity (20). Bone modeling adapts structure to loading by changing bone size and shape and removes damage and thus maintains bone strength. During early life growth is rapid, but then continuously slows down until it reaches a low slope during early school age. This is then followed by a pubertal peak, after which periosteal growth almost comes to a standstill. In adults and children, bone undergoes cyclical coupling of resorption and formation, which is characteristic of remodeling (18). Resorption occurs by cells called osteoclasts that degrade the bone. This is followed by the reversal phase, when the osteoblasts are recruited. The formation phase follows, when osteoblasts begin to lay down the new bone. Bone remodeling, is responsible for damage prevention and repair and formation of new bone for during growth to maintain bone mass.

In healthy young children, particularly during puberty, bone growth and mineralization, as well as bone turnover increase dramatically (21). There has been interest in the clinical potential of markers of bone turnover both as tools to assess fracture risk and for monitoring treatment of osteoporosis (22). Increased bone turnover in children is reflected by an increase in markers of formation and markers for resorption. High levels of bone turnover markers (BTM) represent linear growth at end-plates, modeling at the periosteum, and remodeling. Type I collagen constitutes approximately 90% of the organic matrix of bone. N-terminal propeptide of type I procollagen, P1NP, a marker of bone formation, is released from type I procollagen before
the collagen molecules are incorporated into collagen fibers (19). It has been recognized by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine as the reference standard for bone formation. PINP reflects the synthesis of the most abundant protein of bone tissue (22). Thus, serum concentrations of P1NP are related to the amount of newly formed collagen laid down in the bone. Gracia-Marco et al (23) were able to demonstrate that PINP was strongly and negatively associated with BMC, aBMD and bone area in pubertal girls. Although we would expect a positive association, we see the opposite because high levels of estrogen inhibit bone turnover in late puberty, thus marked by an increase in remodeling over modeling leading to a decrease in BTM (21). However, in early adolescent females, increased hormone levels, such as estrogen and IGF-I, should not affect the clearance rate of P1NP, but increase the clearance rate of BTM carboxy-terminal propeptide of type I procollagen by scavenger receptors (24). Crosslinked telopeptide of type I collagen, ICTP, a marker of bone resorption, is released during the degradation of type I collagen (19) and is not further degraded in the bloodstream. Therefore, the production rate of ICTP reflects the rate of type I collagen breakdown during bone resorption (25).

Collagen type I cross-linked N-telopeptide is another marker of resorption, but it is more effective in monitoring changes in osteoclast activity, rather than ICTP, which is more associated with the degradation of bone (19). There are controllable and non-controllable factors that account for variability of bone turnover and BTMs. The controllable factors include circadian rhythm, menstrual and seasonal variability, fasting status as well as exercise. Circadian rhythm has a very strong impact on the variability of some of the BTM levels. For instance, bone resorption peaks in the second half of the night (between 3 and 7 a.m.) (26). Non-controllable factors include age, chronic diseases and specific medications. BTMs are not specific for either
bone modeling or remodeling. Thus, these BTMs might be valuable tools to assess fracture risk and evaluate the effects of growth hormone treatment or the efficacy for nutrition interventions. There are other factors which regulate the process of bone modeling and remodeling, the concentrations of BTM, and ultimately bone mass.

The process of bone remodeling is regulated by systemic hormones, growth factors, nutrition and physical activity and other factors. In puberty longitude growth is stimulated by the proliferation of chondrocytes in the epiphyseal growth plate (21). In early puberty chondrocytes are stimulated by low levels of estrogen and high levels of IGF-I and GH. At the end of puberty when estrogen levels are increased they inhibit chondrocyte proliferation and reduce bone turnover, despite the high levels of circulating IGF-I (21). For the purposes of this thesis, the focus will be on growth factors, such as IGF-I, and the nutrient, Zn. IGF-I is available to bone through exocrine delivery via the circulation, paracrine and autocrine synthesis, and liberation from stores in the bone matrix. Locally it is synthesized by osteoblasts, it diffuses into newly deposited osteoid and is stored in the bone matrix (7). IGF-I has an important role in maintaining bone mass by stimulating osteoblast precursors and promoting bone matrix formation (11). Studies comparing IGF-I knockout (KO) mice with controls show IGF-I is involved in bone mineral accrual. Mohan et al (27) reported no significant increase in BMD in IGF-I KO mice during the pubertal period, whereas the control mice exhibited a 40% increase in BMD. As mentioned previously, aBMD can be an important predictor of adult osteoporosis risk later in life. Additionally, clinical studies in humans exhibiting mutations in IGF-I or its receptor resulted in intrauterine and postnatal growth retardation (28). Typically, adolescence is marked by a rapid increase in IGF-I concentrations, which later declines with increasing age and mirrors the slope of bone accrual and age-related bone loss (11). Consistent with the timing of the
pubertal period, peak concentrations occur at the ages of 13–14 years in girls, with slightly higher values in girls than in boys (29). Thus, the pubertal stage is the most important predictor of serum IGF-I concentrations. A prospective study over a period of 9 years during childhood and adolescence by Breen et al (30) demonstrated that plasma IGF-I was positively and significantly associated with BMC accrual at all skeletal sites. The predicted BMC accrual was greatest in those children in the highest plasma IGF-I percentiles. It is during this rapid growth period that Zn needs are high and supplementation may have the potential to influence both plasma IGF-I and BMC gains. IGF-I may continue to affect bone turnover and BMD in adulthood through osteoblast activation. It may have an effect in cortical bone to maintain bone strength through optimal cortical geometry (12). The significant link between changes in IGF-I and BMC may be related to the roles of IGF-I on bone formation. The effect that IGF-I may have on BTMs is still uncertain. Although it is generally assumed that increases in levels of BTMs during puberty are consequent to changes in IGF-I secretion, recent studies have demonstrated relatively weak associations between IGF-I levels and markers of bone turnover in puberty (31). The studies that do show a strong and positive relationship between IGF-I and specifically P1NP have been in populations that are growth hormone deficient and have received hormone replacement therapy. Therefore more research needs to be conducted to see if IGF-I levels are correlated with BTMs in healthy adolescents.

**Zinc intake in the US**

Zn is an abundant mineral that is available throughout the body and in the diet. Good sources of Zn in the diet are red meat, certain seafood, fortified cereals, and some dairy products. The recommended dietary allowance (RDA) for early adolescent females, ages 9-13 years, is 8
mg/day. The current mean dietary Zn intakes for non-Hispanic white girls 6-11 years of age, is 9.9 mg/day and 9.73 mg/day for black girls of the same age (32).

Zn is considered a relatively nontoxic micronutrient in moderate supplementation levels, and the potential for Zn toxicity is very small at 24 mg/day (33). The Institute of Medicine (IOM) defines the upper limit (UL) as "the highest daily level of chronic nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population” (33). The basis for the 23 mg UL of Zn in children 9-13 years of age is the No Observed Adverse Effects (NOAEL) derived for infants and corrected for older children based on body weight. It is based on a value in infants in which no adverse affects were observed. The majority of Zn supplementation trials in adults report no adverse effects, including a meta-analysis study of several Zn supplementation trials in which studies utilizing Zn supplementation levels above 22 mg/d reported no adverse effects (34). Adverse effects associated with chronic intake of supplemental Zn may include suppression of immune response, decrease in high-density lipoprotein cholesterol, and reduced copper (Cu) status. There have been reports of Zn supplementation reducing Cu status based on erythrocyte superoxide dismutase (ESOD) activity. In 2 studies reporting reduced ESOD activity, the level of supplementation was 50 mg Zn gluconate (35, 36). The Lowest-Observed Adverse-Effect Level (LOAEL) of Zn in adults was determined to be 60 mg/day, based on the 50 mg supplement in the 2 studies combined with an estimated 10 mg of dietary Zn. The UL or the NOAEL of 40 mg/d for adults was determined after adjusting the LOAEL with an uncertainty factor of 1.5, to account for inter-individual variability (33). Impaired Cu status and other symptoms of Zn toxicity such as gastric distress, dizziness, and nausea are typically not observed with intakes under 150 mg Zn/day. In the current study Zn supplementation with 24 mg/day will provide 0.67 mg Zn/kg body weight for a
female, 10.5 years of age, with a body weight of 36 kg (50th percentile BMI/age). This level of Zn supplementation is at least twice the mean dietary Zn intakes for most females 6-19 years of age. The combination of 24 mg of supplemental Zn and the average dietary Zn intakes of 9-13 years old children is above the estimated UL of 23 mg/day for children 9-13 years of age. Therefore, this should be safe and provide a level of Zn that would be expected to elicit positive changes in bone turnover.

**Zinc and bone**

Zn is required for the growth, development and maintenance of the skeleton (7). In cell culture, Zn has been shown to have a stimulatory effect on bone formation and mineralization and an inhibitory effect on bone resorption (37). The mechanism by which Zn may stimulate bone formation is still being investigated. In cell culture, Yamaguchi et al (37) showed that Zn stimulates bone formation by stimulating the osteoblast, thereby having the potential to increase bone mass and improve bone strength. The proliferation of osteoblasts produced a remarkable increase in alkaline phosphatase activity. Alkaline phosphatase is an osteoblastic Zn metalloenzyme that is involved in bone calcification in the femoral–metaphyseal tissues (37). Hall et al (38) demonstrated that *in vitro* Zn stabilizes alkaline phosphatase activity, rather than increase it. Zn has been shown to have an inhibitory effect on bone resorption. In vitro, Zn has been shown to inhibit osteoclast-like cell formation in mouse bone marrow cells cultured in a bone-resorbing agent. Specifically, by suppressing the effects of osteoclastogenesis (formation of osteoclasts) that are generated by bone marrow cells (39). Zn is also a highly potent and selective inhibitor of rat bone osteoclast activity *in vitro* (40). However, the effect that Zn has on resorption *in vitro* is not applicable to all studies. In human studies Zn has been shown to not
have an effect on bone resorption markers following supplementation (41, 42). However, if Zn supplementation enhances bone formation and suppresses resorption, this could ultimately lead to an increase in bone mass.

**Dietary zinc**

Several studies in adult men and women have demonstrated positive relationships between dietary Zn and bone indices. A cross sectional study investigated the association between dietary intake and aBMD in 994 healthy premenopausal women ages 45-49 years. New et al (43) found that Zn intake was positively associated with aBMD confirming prior evidence of a positive association between dietary intake of Zn and bone mass in pre- and postmenopausal women. Another study examined 99 women, ages 35-65 over 4 years. The longitudinal analysis showed that Zn intake was positively associated with gains in radial BMC in the postmenopausal group (44). High levels of intake correlated with slower loss (p<0.05) A cross sectional study was conducted to correlate the effects of Zn intake to bone structure and strength. Laudermilk et al (45) found a positive association with objective measures of bone geometry, size, and strength in fourth-grade girls. Specifically, Zn intake was correlated with diaphysial volumetric BMD. The negligible relationships between intake of micronutrients and bone status observed in sixth-graders might underscore the increasing and pre-dominant influence of hormones (e.g., estrogen) on bone accrual as females begin to approach puberty. If supplemental Zn improves skeletal health, it is likely that the effect is heightened during pubertal growth, when Zn needs are highest.
Preliminary zinc supplementation studies

Zn supplementation in deficient populations has been previously studied. Zn deficiency has been associated with skeletal abnormalities in fetal and postnatal development. In Iranian schoolboys, ages 12-18, Zn supplementation (28 mg/day as sulfate) improved metacarpal cortical bone width compared to supplementation without Zn, ultimately, restoring both skeletal growth and maturation in supplemented participants (46). Other studies have examined the effects of Zn supplementation on bone turnover in children. Imamoglu et al (41) observed healthy pre-pubertal children, ages 7.7 ± 2.6 years, with idiopathic short stature for 6 weeks receiving Zn supplementation of 50 mg/day (>0.6 mg/kg bw). The researchers observed an increase in osteocalcin (OC) and BSAP (P<0.05) in the Imamoglu study (2005).

Few Zn supplementation trials have been studied in healthy, non-deficient populations. A 12-week study in 20 healthy men found that daily Zn supplementation (50 mg/day) significantly increased markers of bone formation, including bone specific alkaline phosphatase (BSAP) activity (47). Another study by Clark et al (42) examined adolescent non-Hispanic white females, ages 12.2 ± 0.3 yr, receiving 15 mg Zn/day, (~0.3 mg/kg bw). The researchers observed greater increases in serum Zn vs. placebo (P<0.001), but no differences in OC or deoxypyridinoline (DPD) after 6 weeks (42). The inconsistencies between these studies could be the result of the differences in supplementation dose, showing more significant results (p<0.05) when the Zn dose was at least 0.6 mg/kg bw vs. 0.3 mg/kg bw. OC has been used as a marker of bone formation in many bone intervention and supplementation trials. It is incorporated in the bone matrix and is released into the circulation from the matrix during bone resorption. Thus, it may be a better maker of bone turnover that than formation (48).
Therefore, P1NP is potentially more specific and sensitive in monitoring changes in bone formation in response to Zn supplementation than OC in this age group.

A pilot study conducted in our laboratory by (49) administered 30 mg elemental Zn supplement to females, 11-14 years of age, was approximately 0.6 mg/kg body weight and significantly increased plasma Zn and plasma IGF-I and IGFBP-3 over 3 weeks, without any reported side effects. Unfortunately, there was no control group in the pilot study. For the current study, a 24 mg Zn supplement was selected based on the data from pilot study, which will provide 0.67 mg Zn/kg body weight for a female, 10.5 years of age, with a body weight of 36 kg (50th percentile BMI/age). This amount was hypothesized to elicit positive changes in both serum BTMs and growth factors.

**Zinc supplementation and growth factors**

The elongation and structural adaptations that occur in the skeleton during childhood and pubertal growth are influenced by IGF-I. As stated previously, circulating levels of IGF-I increase significantly throughout puberty, peaking during the maturational period associated with peak height velocity (11, 29). IGF-I has been shown to stimulate proliferation of osteoblast precursors and early-stage osteoblasts and promote bone matrix formation by mature osteoblasts (11, 12). IGF-I also stimulates bone resorption through enhancing the recruitment, synthesis and activation of osteoclasts. The effects of Zn on bone mineralization may be mediated through changes in circulating IGF-I. Zn may stimulate the production of IGF-I, ultimately leading to increased bone mass. Zn is proposed to stimulate osteoblastic cell proliferation by enhancing the effect of IGF-I and acting on transcriptional sites regulated by IGF-I (37). Zn supplementation has been shown to increase circulating IGF-I concentrations in both animal and human models.
Clark et al (42) found that circulating IGF-I was unchanged after healthy adolescent white females received 15 mg Zn/day for 6 weeks. Imamoglu et al (41) observed increases in IGF-I (p<0.001) in children ages 7.7 ± 2.6 years, after 6 weeks of Zn supplementation (50 mg/day). A study conducted by Hamza et al (50) showed that after 3 months of Zn supplementation (50 mg/day) serum IGF-I levels increased in the Zn deficient and short-statured children. These studies report mixed findings with respect to Zn supplementation and IGF-I and the discrepancies may be related to the fact that short-stature children were used in one study, the other used a healthy population administering a relatively small Zn dose and the most recent study looked at Zn deficient children who were also short-statured. Also, the possibility exists that the observations of Imamoglu et al (41) were the result of growing children with serum baseline levels of 67.4±70.6/ ng/ml. It is during this rapid growth period that Zn needs are highest and supplementation may have the potential to influence both plasma IGF-I and BMC gains.

Summary

In summary, though 50-85% of variance in peak bone mass is genetically determined (51), nutrition can have an important impact on an individual’s ability to achieve their genetic potential for peak BMC accrual. Prevention is key because 90% of adult bone mass is achieved by the end of adolescence. Several strategies have been aimed at improving bone mineral accrual during childhood, most notably physical activity and calcium supplementation. There has been less focus on the effects of trace minerals on bone. Zn is known to play a key role in bone metabolism, as data in mice and cell culture models indicate that Zn deficiency leads to impaired skeletal growth (7, 37, 52-54). Zn supplementation also has the potential to enhance bone
mineralization and development as shown in both animal and human adult models (9, 43, 44, 47). Moreover, if improved bone turnover responses to Zn supplementation are shown, the mechanism may be mediated through IGF-I. However, this novel application has only recently been observed in healthy, non-deficient, populations. Zn supplementation could lead to important public health implications by serving as an effective and inexpensive strategy for improving bone strength in adolescents and reducing the burden of fractures in the elderly population.
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CHAPTER 3

ZINC SUPPLEMENTATION IMPROVES BONE FORMATION IN YOUNG ADOLESCENT GIRLS

Zinc Supplementation Improves Bone Formation in Young Adolescent Girls


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Running head: Effects of zinc supplementation on bone

Definitions: Zinc (Zn), control (CON), procollagen type 1 amino propeptide (P1NP), osteocalcin (OC), deoxypyridinoline (DPD), pyridinoline (PYD), carboxyterminal telopeptide region of type 1 collagen (ICTP), insulin-like growth factor-I (IGF-I)

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Abstract

**Background:** Both animal and human studies show that beyond correcting skeletal and growth impairments under deficiency conditions, supplementation with zinc (Zn) may have a bone health-promoting role.

**Objective:** The purpose of this study was to determine if Zn supplementation (23 mg/d Zn sulfate) over 4 weeks altered plasma Zn, markers of bone turnover [bone formation: osteocalcin (OC), procollagen type 1 amino propeptide (P1NP); bone resorption: carboxyterminal telopeptide region of type 1 collagen (ICTP), pyridinoline (PYR), deoxypyridinoline (DPD)] and insulin-like growth factor-I (IGF-I) in white and black females (9 to 11 years of age). A secondary aim was to determine if there was a race x treatment interaction among these variables.

**Design:** Females in the early stages of puberty were randomized into a Zn supplementation group (ZN; n=75) or placebo group (CON; n=72), in a double-blind, placebo-controlled trial. Plasma Zn was determined using atomic absorption spectrophotometry. OC, P1NP and ICTP were assessed using radioimmunoassay (RIA). PYR and DPD were determined using high-performance liquid chromatography (HPLC) and IGF-I was determined by enzyme-linked immunosorbent assay (ELISA).

**Results:** Over 4 weeks, plasma Zn and P1NP increased significantly more in the ZN vs. CON group (P<0.05). There were no group differences in the other biomarkers (all P>0.05). No differences were observed by race.

**Conclusions:** Findings from this study provide preliminary evidence that Zn may be a viable nutritional strategy for improving bone strength in young females. A long-term clinical bone trial
is warranted to more definitively assess the potential for supplemental Zn to improve bone strength and to explore potential mechanisms involved.

**Key words:** Zinc (Zn), adolescence, biomarkers of bone turnover, P1NP, ICTP, IGF-I, supplementation
Introduction

Zinc (Zn) is a trace mineral found in many tissues of the body, with 20% stored in the skeleton (1) and is essential for bone development and maintenance (2). In cell culture, Zn has been shown to have a stimulatory effect on bone formation and mineralization and an inhibitory effect on bone resorption (2-4), although the mechanisms for these effects are unclear. Studies using various animal models have shown improvements in bone mineralization and strength following Zn supplementation (5-7). For example, Rhesus monkeys fed low levels of dietary Zn (2 ug/g diet) had slowed skeletal growth and development that was most apparent toward the end of the skeletal growth spurt (6). Ovesen et al (7) demonstrated that Zn supplementation in growing male rats improved bone strength via biomechanical testing over 4 weeks.

Human studies have demonstrated significant relationships between dietary Zn and bone. For instance, Zn intake has shown to be positively correlated to areal BMD (aBMD) and other measures of bone geometry, size and strength in both adults and children (8-11). Trials in adults demonstrate that daily Zn supplementation (50 mg/d) in healthy men over 12 weeks significantly increased markers of bone formation, particularly bone specific alkaline phosphatase (BSAP) activity (an index of total osteoblast activity) (12). In children and adolescents, most Zn supplementation trials have been conducted in children with short stature. Imamoglu et al (13) observed that daily Zn supplementation of 50 mg elemental Zn over a period of 6 weeks in healthy pre-pubertal children, ages 7.7 ± 2.6 y, with idiopathic short stature increased bone formation markers [osteocalcin (OC)] and serum Zn.

The influence of Zn on bone formation may be via its action on circulating insulin-like growth factor-I (IGF-I). IGF-I is synthesized by bone cells and is just one of a network of local...
growth factors and systemic hormones that mediate the process of bone remodeling (14, 15). Zn may stimulate the production of IGF-I, and ultimately lead to improved bone mass. Zn supplementation has been shown to increase circulating IGF-I concentrations in both animal and human models, but only in children of short stature (13, 16).

To date, only one Zn supplementation trial has been conducted in healthy, normal stature, non-deficient populations with bone outcomes. Clark et al (17) supplemented healthy adolescent non-Hispanic white females, ages 12.2 ± 0.3 y, with 15 mg elemental Zn/d (as citrate; ~0.3 mg/kg body weight) over 6 weeks. Serum Zn increased vs. placebo (P<0.001), but no significant differences were found in bone formation markers, OC or deoxypyridinoline (DPD), following supplementation.

The purpose of this study was to determine if Zn supplementation (9 mg/d) over 4 wks altered plasma Zn, markers of bone turnover and IGF-I in early pubertal white and black females (ages 9-11 y). A secondary aim was to determine if there was a race x treatment interaction among these variables. We hypothesized that early pubertal females receiving 9 mg Zn/d over 4 weeks vs. placebo would have greater increases in markers of bone formation and no change in resorption markers. A second hypothesis was that plasma IGF-I would increase at greater rates in children receiving 9 mg/d Zn for 4 weeks vs. placebo. Our third hypothesis was that the biochemical responses to Zn supplementation would vary by race, demonstrating less of an effect in non-Hispanic black participants.
Subjects and methods

Study design and participants

Healthy white and black females, ages 9-11 y (N=147) were recruited for this 4-wk, randomized, double-blind, placebo controlled, clinical trial (Figure 1). Participants were recruited from the Athens-Clarke County area through flyers and announcements. Telephone pre-screening was used to determine eligibility. Exclusion criteria were determined by self-report and included menses, known bone disease or disease known to influence bone metabolism, growth disorders, Zn malabsorption (e.g., acrodermatitis enteropathica), the use of medications that may influence bone metabolism (e.g., corticosteroids) and unwillingness to provide a blood sample. Subjects were not to be taking any vitamin, mineral or herbal supplements for 4 weeks prior to enrolling in the study. Subjects agreed to not alter dietary intake during their participation. Inclusion criteria included being female, of non-Hispanic white or black race, and healthy (defined as absence of disease or illness requiring medications) (18). After completing a telephone screen, potential subjects were mailed a sexual maturation stage self-assessment form to complete and mail back to the laboratory. Sexual maturity status was based on stages of breast development (19). Subjects were given photographs and written explanations of each stage to assist them with completing questionnaires. Stage 1 indicates the pre-pubertal state, stages 2/3 represent early/mid-puberty and stages 4/5 represent later-puberty. For this study we included only those females in stages 2 or 3. All methods and procedures were approved by the University of Georgia Institutional Review Board on Human Subjects. All participants were notified of the possibility of adverse events with 9 mg of Zn/d.
**Dietary intake**

Dietary intake was assessed at baseline and at the end of the 4-week study to monitor dietary Zn and energy intakes, as well as other nutrients known to influence bone. To prevent large differences in dietary Zn intakes between subjects, participants were discouraged from consuming foods containing more than the current recommended dietary allowance (RDA) of 8 mg Zn/d. Participants were provided with a list of foods that are commonly consumed and are high in Zn. Dietary intake was assessed using a 3-d diet record. Each subject and their parent received instructions on completing the 3-d diet record at home. Diet records were analyzed using The Food Processor SQL version 9.7.3 (ESHA Research, Salem, OR). Single measure intra-class correlation (ICC) coefficients were conducted from a pilot study in female children 6-10 y of age (n=10), whose 3-d diet records were completed twice in a 2-week period, and are calculated for energy (R=0.47), Ca (R=0.71) and vitamin D (R=0.94).

**Physical activity**

To assure that changes in bone biomarkers or growth factors were not the result of acute changes in level or mode of physical activity, physical activity was assessed using the 7-d Physical Activity Recall (PAR) questionnaire that includes the type, duration, and intensity of activity. The 7-d PAR questionnaire was interviewer administered at baseline and 4 weeks by the same researcher. The PAR has been validated in children (20).

**Supplements**

Tablets containing 9 mg elemental Zn in the form of Zn sulfate (23 mg) and placebo pills were provided by Vesta Pharmaceuticals, Inc. (Indianapolis, IN) in pre-packaged supplement bottles, labeled with product code numbers. Subjects were instructed to consume one tablet per day. The manufacturer packaged 4-week supplies and labeled the bottles with ID letters. Using
pre-assigned codes, subjects were assigned the next sequential ID upon enrollment and were provided with the corresponding packet of supplements. Covance Laboratories (Madison, WI) verified the Zn content of the supplements (8.7 mg). The 9 mg Zn supplement in the proposed project is considered safe and provides a level of Zn that would be expected to elicit positive changes in bone turnover. Compliance was determined by pill count, and defined as greater than 80% of participants completing the supplement regimen over 4 weeks.

**Anthropometry**

Height and body weight were recorded at baseline and at 4-week. Weight was measured to the nearest 0.1 kg using an electronic scale (Seca Bella 840, Columbia, MD). Height was measured using a wall-mounted stadiometer to the nearest 0.10 cm (Novel Products Inc., Rockton, IL). All measurements were taken twice at each visit and the averages were used for analysis. In our laboratory, one-way random effects model, ICC coefficients were computed for anthropometric procedures in females, 6-10 y of age (N=10), measured by the same individual twice in a 2-week period. The average measure ICC (R-value) and test-retest CVs (%), respectively for body weight were calculated to be 0.99 and 1.4%. Individual anthropometric values were plotted to determine BMI-for-age percentiles (18).

**Body composition**

Total body fat mass, fat-free soft tissue, percent body fat, and bone mineral content (BMC) were determined using dual energy X-ray absorptiometry (Hologic, model DPA/QDR-1;SN 9374). For consistency, the same technician positioned the subjects, conducted the scans and performed the analyses. Single measure ICCs were calculated in females 5-8 y of age (N=10) scanned twice in our laboratory during a 7-d period for BMC of the total body, lumbar spine, proximal femur and forearm (all R≥0.98). Test-retest measurements using DXA
demonstrated the following CVs for BMC of the lumbar spine (1.3%), total proximal femur (1.6%) and forearm (2.1%).

Plasma zinc, serum bone biomarkers and IGF-I

All samples were stored and frozen at -70°C until analyses. Fasting blood was drawn at baseline and at 4 wk. Samples were assayed for plasma Zn, serum bone biomarkers; [bone formation: osteocalcin (OC), type 1 amino propeptide (P1NP); bone resorption: carboxyterminal telopeptide region of type 1 collagen (ICTP), pyridinoline (PYD) and deoxypyridinoline (DPD)], and serum IGF-I.

Plasma Zn was assessed by atomic absorption spectrophotometry using a Perkin Elmer Analyst 400 (Shelton, CT). Accuracy was verified by assessing the Zn content of a standard obtained from the United States National Institute of Standards and Technology (Gaithersburg, MD). Standards from the National Bureau of Standards were run in conjunction with samples to determine day-to-day variation. In order to monitor the safety of the supplements, ESOD activity and erythrocyte Cu, Zn superoxide activity will be assessed using the method of Marklund and Marklund (21).

P1NP, a marker of bone formation, and ICTP, a marker of bone resorption, were both measured in serum and assayed in duplicate using RIA (Orion Diagnostica, Espoo, Finland). Intra- and inter-assay CVs in our lab were 2.8% and 5.6%, respectively, for P1NP and 3.4% and 5.9%, respectively, for ICTP. OC was measured by RIA and the inter- and intra-assay CVs are 10% and 5%, respectively. BSAP was measured by ELISA (Quidel Corporation; Metra, San Diego, CA). The commercial kit has a detection sensitivity of 0.7 U/L and a reported inter- and intra-assay CV less than 8% and 6%, respectively. Urinary PYD and DPD were assessed by HPLC.
The inter-assay CV for measurement of PYD and DPD is 3.8% and 5.9%, respectively. Second void urinary assays were used and corrected for dilution by creatinine concentration.

Plasma IGF-I was assessed using recombinant human IGF-I quantitative sandwich immunoassay technique (ELISA; R&D Systems). The plasma samples were pretreated with: A) an acidic dissociation solution and B) a buffered protein with blue dye and preservatives; lyophilized, to release IGF-I from binding proteins. The inter- and intra-assay coefficients of variation are 7.5-8.3% and 3.5-4.3%, respectively (22).

Statistical analyses

Analyses were performed with SPSS software (v 20.0; SPSS Inc, Chicago, IL) on an intent-to-treat basis. Two-way (race by treatment) analyses of variance were used to determine baseline differences between the groups in the response variables of primary interest as well as the potential effect-modifying variables (bone turnover markers, growth factors, weight, height, and calcium intake). Responses of the dependent variables to the intervention (Zn or placebo) were assessed by measuring the gain score over the study period for each subject and modeling these scores with a two-way analysis of variance model. From this model, effects of the intervention (Zn vs. placebo) in each race by treatment group were examined by estimating each group’s mean response and testing whether these means are zero. In addition, main effects of race, treatment and their interaction were tested to determine how the mean gain scores differed across the experimental groups. Finally, the gain scores were reanalyzed using a two-way analysis of covariance model controlling for those variables by which the treatment groups differed at baseline. A $P$ level of $< 0.05$ was used to denote statistical significance.
Results

Baseline characteristics of participants are presented in Table 1. There were no differences in baseline characteristics between Zn and placebo groups. By race, however, mean values for age, OC, PYD/C and DPD/C were higher in whites vs. blacks at baseline, while FFST, total body BMC, plasma Zn, ceruloplasmin, IGF-I and P1NP were higher in blacks vs. whites. Dietary Zn intakes were not different between groups; however mean intakes for both groups were lower than the RDA for this age range (8 mg/d). At baseline, approximately 50% of the participants were classified as either overweight/obese according to their BMI-for-age percentile (n=29 overweight; n=44 obese).

A total of 147 participants were enrolled and 143 completed the study, maintaining a 97% retention rate. The supplement compliance rate was 60.7%, which was assessed by questionnaire and pill count, and was tallied by the same researcher.

The effects of Zn supplementation on plasma Zn, bone biomarkers and IGF-I are reported in Figure 2. Mean plasma Zn and P1NP concentrations increased significantly over 4 weeks in the Zn supplemented group vs. placebo groups, but other biomarkers and IGF-I were not altered (P<0.05). After controlling for race, P1NP and plasma Zn remained significant for a time x treatment interaction (P=0.036, P=0.001, respectively). There was a trend for a treatment x race interaction such that the circulating IGF-I response was greater in black vs. white participants over the 4 weeks (P=0.08).

Discussion

This was only the second study to examine Zn supplementation in healthy females, but the first to take into consideration race (non-Hispanic white and black). The primary finding
from the study was that the bone formation marker, P1NP, increased significantly following 4-weeks of supplementation with 9 mg/d of elemental Zn.

The only other study to examine Zn supplementation, Clarke et al (17), administered 15 mg/d elemental Zn for 6 weeks to white adolescent females, and observed no effect of Zn on the bone formation marker OC. OC is incorporated into the bone matrix and is released into the circulation during bone resorption, and is synthesized by osteoblasts during formation (23). Thus, it may be a better marker of overall bone turnover rather than a specific biomarker of bone formation (24). In contrast, P1NP is a more specific marker of bone formation and has recently garnered considerable interest in research. Because we observed an increase in P1NP following supplementation, P1NP may be more sensitive to the effects of supplementation, particularly during periods of growth (25). The fact that we observed increases in bone formation with 9 mg/d of elemental Zn, combined with typical dietary Zn intakes, may suggest that the current Zn RDA for children in this age group is not optimal for bone health.

Although a sensitive and specific biomarker of Zn status has yet to be identified, plasma (or serum) Zn concentrations are responsive to Zn supplementation and are the most widely used biomarker for Zn studies (26). In this study, we observed significant increases in plasma Zn following supplementation, confirming that our participants were compliant with taking the supplement. Our compliance rate was 85%. Our results are consistent with Clark et al (17), who also observed increases in serum Zn after supplementation. Previous studies have reported increases in plasma concentrations, but with higher doses of Zn (ranging from 10-20 mg/d) (27).

Similar to Clark et al (17), we found no significant changes in plasma IGF-I with Zn supplementation. Zn supplementation has been shown to increase serum IGF-I in children of short-stature and in Zn deficient children (13, 16). In the study by Hamza et al (16), participants
were younger and had lower IGF-I concentrations at baseline compared to our study. Interestingly, we observed a trend for a treatment x race interaction favoring a greater IGF-I response to Zn supplementation in blacks vs. whites (P=0.08). Black children have higher serum concentrations of IGF-I compared with white children (28). The trend for the treatment x race interaction should be explored further. White children had lower mean plasma Zn values than black children, the plasma Zn concentrations at baseline and after 4 weeks in the placebo group were <10.7 umol/L, the cutoff for a population that is Zn deficient (29). This may be related to adiposity because the mean weight of the black participants was greater than the white participants (48 kg vs. 46 kg). The black participants also exhibited higher levels of the bone formation marker, P1NP, but lower levels of OC. Other trials have observed higher levels of OC in black participants of similar age (30). However, higher levels of P1NP may be related to earlier bone accrual in white females vs. black females (31), or increased bone turnover in black females (32). The lower levels of OC may reflect bone turnover, rather than formation, as previously stated. The fact that PYD/C and DPD/C were higher in whites is also surprising as concentrations of these markers are correlated with growth velocity, and black females attain their peak height velocity earlier than white females (31, 33).

It is unlikely that the lack of changes in other bone biomarkers or IGF-I was due to the study duration of 4 weeks. There have been many other supplementation studies that have observed changes in bone biomarkers after 4 weeks and even less (34-38). There have also been animal model, cell culture and human studies that have observed changes in IGF-I concentrations after 4 weeks (39-42). Therefore, we determined that 4 weeks would be enough time to elicit changes in biomarkers of bone turnover and IGF-I in early adolescent females.
In summary, we found that Zn supplementation increased bone formation in non-Hispanic white and black females. Findings from this study provide preliminary evidence that Zn may be a viable nutritional strategy for improving bone strength in young females. A long-term clinical bone trial is warranted to more definitively assess the potential for supplemental Zn to improve bone strength and to explore potential mechanisms involved.

Acknowledgements

RDL, EML, NKP, AG, SAS, KHD, CMI were responsible for the study concept and design. EML and NKP conducted the statistical analysis. AG was responsible for the plasma Zn assays; SAS was responsible for the bone resorption marker assays; KHD and CMI were responsible for the IGF-I assays; NKP and PJB was responsible for the P1NP assays. PKB was responsible for writing and editing the manuscript. All authors contributed to the revision of the manuscript. RDL, EML, AG, VC were responsible for the interpretation of the data and drafting of the manuscript. This research was supported by the NIH (RCT; R03 HD54630).
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Table 1 Baseline descriptive characteristics of participants (9 mg)

<table>
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<tr>
<th>Variable</th>
<th>Control (n=72)</th>
<th>Zinc (n=75)</th>
<th>Zinc vs. Control</th>
<th>White vs. Black</th>
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</thead>
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<tr>
<td>Age, y</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Race (W/B)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40/32</td>
<td>40/35</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Tanner Stage, I-5</td>
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<td>2.3 ± 0.5</td>
<td>0.664</td>
<td>0.874</td>
</tr>
<tr>
<td>Height, cm</td>
<td>148.6 ± 6.6</td>
<td>148.1 ± 6.8</td>
<td>0.684</td>
<td>0.144</td>
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<tr>
<td>Weight, kg</td>
<td>47.3 ± 10.9</td>
<td>46.8 ± 11.6</td>
<td>0.790</td>
<td>0.305</td>
</tr>
<tr>
<td>BMI-for-age percentile, %</td>
<td>75.8 ± 26.0</td>
<td>74.4 ± 26.3</td>
<td>0.755</td>
<td>0.449</td>
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<tr>
<td>FFST mass, kg</td>
<td>31.4 ± 5.1</td>
<td>30.8 ± 5.1</td>
<td>0.463</td>
<td>0.053 B &gt; W</td>
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<tr>
<td>Fat mass, kg</td>
<td>15.4 ± 7.0</td>
<td>15.4 ± 7.6</td>
<td>0.977</td>
<td>0.759</td>
</tr>
<tr>
<td>Percent fat, %</td>
<td>30.5 ± 8.1</td>
<td>30.9 ± 8.1</td>
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<td>0.338</td>
</tr>
<tr>
<td>Total body BMC, g</td>
<td>1337 ± 223</td>
<td>1303 ± 228</td>
<td>0.365</td>
<td>0.012 B &gt; W</td>
</tr>
<tr>
<td>Plasma zinc, µg/dl</td>
<td>12.1 ± 3.3</td>
<td>12.0 ± 3.2</td>
<td>0.864</td>
<td>0.001 B &gt; W</td>
</tr>
<tr>
<td>Dietary Zn, mg/dl</td>
<td>4.7 ± 2.3</td>
<td>5.0 ± 2.7</td>
<td>0.482</td>
<td>0.143</td>
</tr>
<tr>
<td>IGF-I, µg/dl</td>
<td>400.1 ± 257.6</td>
<td>414.9 ± 233.1</td>
<td>0.715</td>
<td>0.001 B &gt; W</td>
</tr>
<tr>
<td>P1NP, µg/l</td>
<td>708.9 ± 242.7</td>
<td>703.7 ± 188.1</td>
<td>0.986</td>
<td>0.007 B &gt; W</td>
</tr>
<tr>
<td>Osteocalcin, ng/ml</td>
<td>33.3 ± 9.1</td>
<td>32.0 ± 9.2</td>
<td>0.397</td>
<td>0.001 W &gt; B</td>
</tr>
<tr>
<td>ICTP, µg/l</td>
<td>24.7 ± 6.5</td>
<td>24.2 ± 6.6</td>
<td>0.729</td>
<td>0.103</td>
</tr>
<tr>
<td>PYD/C, nmol/mmol</td>
<td>98.6 ± 40.1</td>
<td>101.6 ± 39.1</td>
<td>0.641</td>
<td>0.001 W &gt; B</td>
</tr>
<tr>
<td>DPD/C, nmol/mmol</td>
<td>29.6 ± 11.0</td>
<td>29.6 ± 9.7</td>
<td>0.986</td>
<td>0.001 W &gt; B</td>
</tr>
</tbody>
</table>

Values are means ± SD. B (blacks), BMC (bone mineral content), BMI (body mass index), DPD/C (dioxypyridinoline/creatinine), FFST (fat free soft tissue), ICTP (carboxyterminal telopeptide of type 1 collagen), IGF-I (insulin like growth factor-I), P1NP (procollagen type 1 amino propeptide), PYD/C (pyridinoline/creatinine), W (whites).

<sup>a</sup>Test of significance between groups were based on two-way ANOVA

<sup>b</sup>Test of significance between groups were based on chi-square test
Figure 1. Recruitment flow chart

- **Total Assessed for Eligibility**: 253
- **Excluded**: 44
- **SUBJECTS ENROLLED**: 147
- **Allocated to Zn Group (n=75)**
  - **Lost to follow-up (n=1)**
  - **Lost to adverse event (n=1)**
  - **Completed the trial (n=73)**
- **Allocated to Placebo Group (n=72)**
  - **Lost to follow-up (n=2)**
  - **Completed the trial (n=70)**
- **Subjects Retained**: 143
- **Declined**: 59

**Lost to follow-up**

1. **SUBJECTS ENROLLED**: 147
2. **Excluded**: 44
3. **Allocated to Zn Group (n=75)**
   - **Lost to follow-up (n=1)**
   - **Lost to adverse event (n=1)**
   - **Completed the trial (n=73)**
4. **Allocated to Placebo Group (n=72)**
   - **Lost to follow-up (n=2)**
   - **Completed the trial (n=70)**
Figure 2.

ZN (Zn), OC (osteocalcin), P1NP (procollagen type 1 amino propeptide), ICTP (carboxyterminal telopeptide of type 1 collagen), PYD (pyridinoline), DPD (dioxypyridinoline), IGF-I (insulin-like growth factor I)
CHAPTER 4

SUMMARY AND CONCLUSIONS

Adolescence is an important time to strengthen bone and potentially prevent future risk of fractures and osteoporosis. Several strategies have been employed aimed at improving bone mineral accrual and strength during childhood, most notably physical activity and calcium supplementation. There has been less focus on the effects of trace minerals on bone. Zinc (Zn) is known to play a key role in bone metabolism. In cell culture, Zn has been shown to have a stimulatory effect on bone formation and mineralization and an inhibitory effect on bone resorption (1-3). Animal models have demonstrated how Zn supplementation enhances bone mineralization and strength (4-6). Supplementation trials conducted in humans have shown Zn increasing markers of bone formation (7-9). This action may be through insulin like growth factor-I (IGF-I), however this relationship is still being researched.

Only one published Zn supplementation trial has been conducted in healthy, normal stature, non-deficient populations, and the researchers found that after 6 weeks of supplementation with 15 mg/day Zn, markers of bone formation and IGF-I were unchanged (9). However, no study has been conducted in both black and white races. We conducted a RCT in early-pubertal females, 9 - 11 years of age, of both non-Hispanic white and black race (see Chapter 3). The primary finding from this 4-week study was that supplementation with 9 mg/day of elemental Zn significantly increased plasma Zn and the bone formation marker procollagen type I amino-terminal propeptide (P1NP). Plasma IGF-I concentrations increased 6% more in the Zn vs. placebo group, but the increase was not statistically significant (p=0.19). Supplementation with 9 mg/day elemental Zn provides approximately 0.2 mg Zn/kg body weight. Other studies that observed increases in IGF-I in children with short stature used a higher Zn dose, 0.6 mg Zn/kg body weight (8). A higher dose of Zn may elicit optimal responses.
in markers of bone turnover and IGF-I. For that reason a second study was initiated with a higher
dose of Zn (24 mg/day), which would provide 0.67 mg Zn/kg body weight for a female, 10.5
years of age, with a body weight of 36 kg (50th percentile BMI/age).

To determine if a higher Zn dose would elicit changes in bone biomarkers and IGF-I
concentrations, healthy, non-Hispanic white, early adolescent females were recruited for a pilot
study and were supplemented with either 24 mg/day elemental Zn/day (n=20) or placebo (n=18)
for 4 weeks. The sample size was determined using an α-level of 0.05 for power of at least 80%
to detect statistical differences in 4-week changes in bone biomarkers and IGF-I in response to
Zn or placebo treatment. Similar to the previous study, 4 weeks of supplementation significantly
increased plasma Zn after controlling for outliers (p<0.05). However, unlike the previous study
P1NP was not significantly increased. Mean baseline P1NP concentrations in the pilot study
were 805 µg/L in the Zn supplemented group, compared to the 703 µg/L in the first study. The
participants with higher baseline P1NP concentrations may not have responded to Zn in the same
way as those with lower concentrations, such as in the previous study. Another possibility is that
the girls in the pilot study, while similar in sexual maturation, were slightly older. Although our
participants were enrolled according to sexual maturation stages (only including those in 2 or 3),
these criteria does pose limitations. If a participant reports being in stage 2, she may actually be
closer to entering stage 3. Therefore, more accurate measures should be used, such as serum
hormone levels. When girls are approaching maturity, hormones (e.g., estrogen and IGF-I)
increase and predominantly influence (10). Around the age of 10 years, hormonal changes occur
more rapidly and likely begin to overshadow the influence of micronutrient intakes on skeletal
growth. Similarly, the potential for micronutrient intakes to influence bone may vary during
critical phases of bone apposition throughout maturation (10). Later in puberty estrogen begins to
influence bone structure over IGF-I. Therefore, it may have been beneficial to measure serum estrogen levels in our participants.

Consistent with the previous study, in which we administered 9 mg Zn/day to adolescent females, we did not observe an increase in IGF-I in the Zn supplemented group. Clark et al (9) found that circulating IGF-I was unchanged after healthy adolescent white females received 15 mg Zn/day for 6 weeks. Imamoglu et al (8) observed increases in IGF-I (p<0.001) in children ages 7.7 ± 2.6 years, after 6 weeks of Zn supplementation (50 mg/day; 0.6 mg Zn/kg body weight). The researchers may have had more significant results because at baseline the IGF-I levels were lower in their participants compared to our participants, who were also older. Because IGF-I levels peak in puberty it is hard to distinguish if the increase is due to Zn or the stage of maturation of our participants. This variability may indicate a weak association between Zn and its role in non-deficient, healthy, normal stature adolescents.

In conclusion, 9 mg of elemental Zn/day significantly increased plasma Zn and P1NP concentrations in early adolescent females, 9-11 years of age, after 4 weeks of supplementation regardless of race. Supplementation with 24 mg/d of elemental Zn over 4 weeks increased plasma Zn levels, but did not significantly increase P1NP or IGF-I, in a similar population. Therefore, a higher dose of Zn may not be optimal for healthy adolescents in promoting bone formation and bone mineral accrual; however, future studies should include healthy adolescent females, to clarify this relationship.
References


phosphatase, osteocalcin and growth in prepubertal children with idiopathic short stature.


Appendix A: VERBAL CONSENT SCRIPT (9 mg)
Verbal consent script (pilot):

I am ____________ from the University of Georgia-Athens, from the Department of Foods and Nutrition. I am conducting research entitled the EFFICACY OF SUPPLEMENTAL ZINC FOR IMPROVING BONE STRENGTH IN EARLY PUBERTAL CHILDREN and would like to know more about whether zinc supplements improve bone health in young females. This interview should only take 15 minutes.

The purpose of the research is to see whether zinc supplements taken each day for one month will improve blood and urine markers of bone health. To determine if your child qualifies, I will conduct a telephone screening questionnaire with you. If your child meets our criteria, you will first be asked to complete a sexual maturation questionnaire that I will mail to you right after our phone conversation. The sexual maturation questionnaire will have several drawings of sexual maturation stages. Your child will simply circle the picture that best represents her level of maturation. If your child is in the stage of sexual maturation that we are looking for, you and your child will come to our laboratory on the University of Georgia campus so that we may measure your child’s height, sitting height and leg length. This will allow us to calculate how far away your child is from her growth spurt. If your child does not qualify for the study, she will receive $10 for coming to our laboratory for these measurements. If your child is within the range we are looking for, your child will be invited to participate in this study, which lasts one month. Your child will be placed in either the zinc group or the placebo group. Participants in the zinc group will take 24mg of zinc sulfate per day for one month. This level is a safe level of zinc. Participants in the placebo group will take a pill identical in appearance to the zinc pill each day for one month.

If your child decides to participate in our study, the following procedures will take place. Your child will come to our laboratory for testing twice: once at the beginning and once at the end (at one month). For the first and last testing sessions, the following procedures will be done: your child will fill out consent forms and questionnaires about nutrition, health, bone, physical activity, sun exposure, and demographics; give a fasting blood sample and a urine sample; be measured for height and weight; be given two different types of bone scans; and be given a take-home 3-day diet record. You will be asked to mail the diet record back to our laboratory in an envelope we will provide. These testing sessions will last about 1½ hours. For the 1-month testing session, your child will complete all of the things I listed except the bone scans.

We will count the pills to document how many pills your child has taken. We will also give you a calendar to help keep track of each day. Your child will receive $25 for the baseline testing session, and $50 at the end of the study for a total of $75 for the entire study.

Do you have any questions?

Let me assure you that any information you and your child provide will be kept strictly confidential. In final research products I will disguise your identity by only presenting aggregate
Your child’s participation in taking zinc supplements and providing me with information on your health is completely voluntary and you may discontinue our interaction at any time or skip any question you don’t want to answer. If your child does not qualify or decides to not participate after responding to some or all of the questions, her information will be destroyed.

Do you agree to complete the screening questionnaire for your child?

[If yes, continue with telephone screening questionnaire]

[If not, say “Thank you for your time, goodbye.”]

The contact information for the researcher conducting this project is:

Emma Laing
279 Dawson Hall
University of Georgia
706-542-4918
APPENDIX B: TELEPHONE SCREENING QUESTIONNAIRE (9 mg)
UGA ZINC STUDY

Telephone Screening Questionnaire

This interview should take approximately ten minutes:

Date: _____________ Time: _____________ Screen completed by: ________________

1. A. Has your child started her menstrual cycles? YES ___ NO ___

1. B. How would you describe your child’s ethnicity/race?

**Ethnicity:**
- Hispanic or Latino
- Non-Hispanic or Latino

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

*Parents of participants may indicate one or more of the following (mixed racial heritage should be indicated by selecting more than one category):*

2. We would also like to know the ethnicity/race of the child’s biological parents. How do you describe your child’s mother and father?

<table>
<thead>
<tr>
<th>Child’s Mother</th>
<th>Child’s Father</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity:</strong></td>
<td><strong>Ethnicity:</strong></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>Hispanic or Latino</td>
</tr>
<tr>
<td>Non-Hispanic or Latino</td>
<td>Non-Hispanic or Latino</td>
</tr>
</tbody>
</table>

**Race:**
- American Indian or Alaska Native
- American Indian or Alaska Native
Asian
Black or African American
Native Hawaiian/ Pacific Islander
White

3. How old is your child? ____________ Years; DOB: ______/____/____

4. What grade does she attend in school? __________

5. How tall is your child? _____ft____ in How much does she weigh? __________ lbs.

   Calculate BMI and plot on CDC growth chart – must be between 5th and 85th percentile BMI for age and sex to qualify.

6. Has your child lost or gained weight in the past 3 months? YES _____ NO _____
   If yes, how much? ____________ lbs

7. Has your child ever been diagnosed with any of the following diseases/conditions?

   Bone Disease YES ____ NO ____
   Diabetes YES ____ NO ____
   High Blood Pressure YES ____ NO ____
   High Cholesterol YES ____ NO ____
   Renal Disease or Kidney Stones YES ____ NO ____
   Cerebral Palsy YES ____ NO ____
   Intestinal Malabsorption YES ____ NO ____
   Juvenile Rheumatoid Arthritis YES ____ NO ____
   Growth Disorders YES ____ NO ____
   Thyroid Disease YES ____ NO ____
   Zinc Malabsorption ((e.g. acrodermatitis enteropathica) YES ____ NO ____
   Psychological Illness YES ____ NO ____

8. Is your child currently taking any medications? YES ________ NO ________
   If yes, what medication(s)? ____________________________________________

   (check approved/non-approved medication list: check specifically for Adderall, Ritalin, and steroid medications)

   When did your child begin taking above medication(s)? ____________________
9. Is your child taking an herbal, vitamin or mineral supplement? YES ____ NO ____

If yes, how much and how often? ____________________________________________

If yes, would your child be willing to stop taking the supplement? YES ____ NO ____

(child would be eligible to enroll in the study after a 4-week washout period)

10. In this study, all participants must provide blood and urine samples 2 times (at the start and after 3-4 weeks). Is your child willing to do this? YES ____ NO _____

11. Before initiation of this study, we will ask your child to give a self-assessment of sexual maturation. We will send you the form for your child to complete. Would your child be willing to fill out a self-assessment of sexual maturation form and mail it back in a self-addressed envelope we will provide? YES _____ NO _____

12. If your child meets our criteria for sexual maturation, then he/she will come to:

   a) our laboratory to have his/her height, weight, sitting height, and leg length measured to calculate how far away he/she is from her peak time of growth *OR only if pre-determined: b) community center in subjects local area: ____________

   Your child will receive $10 for her time. Would your child be willing to come to a) our laboratory or b) (above location) _________ for these measurements? YES______ NO______

If the caller is still interested, explain more about the study and why we are doing it and collect the following information:

Parent’s name: ____________________________________________________________

Child’s name: ____________________________________________________________

Address: __________________________________________________________________

Zip Code: __________________________________________________________________

Daytime Phone Number: _________________________________ (home or work?)

Email Address: ______________________________________________

Is it okay to call in the evening? If yes, evening phone: ______________________

66
How did you hear about the study? ____________________________________________

If selected to participate, what mornings during the week would you be available for testing?
M____ T____ W_____ Th____ F_____ S ___

“This is the end of our telephone screening. We will review this and determine your child’s eligibility for the study. We will get back to you within one week to let you know the status of her eligibility. Do you have any additional questions for me?”

Make sure the potential volunteer has contact numbers for future questions.

If child is eligible based on the telephone screen, notify parent that we will be sending them the maturation questionnaire and a few consent forms by mail within the next week. Once mailed, we will make a follow-up phone call to check status of maturation questionnaire and child’s eligibility.
APPENDIX C: PARENTAL CONSENT/ASSENT FORMS (9 mg)
PARENTAL PERMISSION FORM- Pilot Study

I, __________, agree to give permission for my child, __________, to participate in the research titled "EFFICACY OF SUPPLEMENTAL ZINC FOR IMPROVING BONE STRENGTH IN EARLY PUBERTAL CHILDREN," which is being conducted by Drs. Richard Lewis and Emma Laing of the Department of Foods and Nutrition at the University of Georgia. Dr. Lewis may be reached in room 279 Dawson Hall at 542-4901. I understand that the participation of my child is completely voluntary. I can refuse to grant permission or withdraw permission at any time without penalty or loss of benefits to which my child is otherwise entitled. I can have the results of the participation, to the extent that which it can be identified as my child's, returned to me, removed from the research records, or destroyed.

The following points have been explained to me:

I) The reason for the research is to study the impact of zinc supplementation on growth factors and markers of bone turnover in children. The benefits that my child and I can expect from participation are the assessment of diet, maturation, growth, bone health (bone mineral density), and body composition (percentage of body fat and nonfat tissue). Additionally, if my child does not qualify for the study but completes a screening session, she will receive a monetary payment of $10. Once enrolled in the study, my child will receive $25 for completion of baseline testing and another $50 at the completion of the 1-month study. Payments will be distributed only if all testing sessions are completed for a given time point and supplements are taken as directed. In order to process the payment for my child's participation, the researchers need to collect my child's name and mailing address on a separate payment form. This completed form will be sent to the Department of Foods and Nutrition business office and then to the UGA Business Office. The researchers have been informed that these offices will keep my child's information private, but may have to release her name and the payment amount to the IRS, if ever asked. The researchers connected with this study have gone to great lengths to protect my child's private information and will keep this confidential in their locked files. However, they are not responsible once her name and mailing address leave their office/laboratory for processing of payment. Refusal to participate in this study will involve no penalty. All measurements are being used for research imposes only, not medical purposes. However, if abnormalities are found in any measure, I and/or my child will be notified and referred to an appropriate health care professional.

2) The procedures are as follows:

a) Prior to enrolling in the study, my child will be mailed a sexual maturation self-assessment form for her to complete at home and mail back to the Bone and Body Composition Laboratory (BBCL). My child will compare her own appearance to drawings representative of each sexual maturation stage and circle the drawing she most closely resembles. If my child meets the criteria for inclusion for sexual maturation, she will be scheduled to attend a screening session during which her height, sitting height and leg length will be recorded. If my child meets the criteria for inclusion for these growth measures, she will be enrolled in the study and scheduled to attend Session I (described below).

b) Session I of testing will be conducted at two different time points [at the beginning of the study and after one month].
c) My child will fast the night before Session 1. On the day of testing, my child and I will arrive in the BBCL in Dawson Hall or a determined safe location in the community (community center, health department, school, clinic, etc) at the scheduled time. Prior to any testing or participation, a permission form will be read to me and an assent form will be read to my child. After which, the researcher and I will sign the permission form and my child will sign the assent form. During the reading of the permission and assent forms, my child and I will be briefed and familiarized with the testing procedures that will be used during the study (15 minutes). My child and I will be given the opportunity to reread the permission and assent forms and ask any questions that we may have about the study. Prior to any testing, my child and I will be walked through all procedures and reminded that we are free to withdraw without penalty at any time.

My child will provide her second morning urine sample in a private restroom. A trained phlebotomist will then draw approximately 20 mL of blood from my child's arm, after which she will be given a snack (15-20 minutes). My child's blood and urine will be analyzed for compounds that reflect how her bones are responding to the supplements, and if she has certain genetic factors that may affect this response. Any unused portions of the blood that is collected will be discarded after 10 years. If a blood sample cannot be obtained after two attempts, no further attempts will be made. My child and I will complete a general information/health questionnaire, sun exposure questionnaire, and a seven-day recall physical activity recall (approximately 15 minutes). We will also be given a three-day diet record to be mailed back to the BBCL in a stamped, self-addressed envelope provided by the researcher. Session 1 will require approximately 45 minutes.

d) Session 2 of testing will be conducted at one time point [at the beginning of the study only]. After completion of the questionnaires, my child's height, sitting height, leg length, and weight, as well as my height will be measured. She will also have her bone mineral density and body composition measured using a non-invasive bone scanning machine. If testing occurs at locations other than the BBCL, the bone and body composition scans are optional, and my child will be offered the opportunity to travel to the BBCL at a time during the study for the bone scan measures, if desired. These measurements will require approximately 30 minutes, which includes a small break in between each scan (four scans total). My child will also have her bone structure analyzed using a different kind of bone scanner (a pQCT machine). This measurement will require about 20 minutes. I understand that a trained laboratory technician under the supervision of Dr. Richard D. Lewis will conduct all measurements. Because our current knowledge of the risk of DXA to the unborn child is limited, prior to conducting the bone scans, my daughter will sign a consent form developed for use with the DXA and pQCT that asks if she is currently pregnant or believes she may be pregnant. If my daughter is pregnant, she will be told that she cannot participate because the X-rays from the DXA pose a risk to the fetus. If my child expresses any doubts regarding pregnancy, a pregnancy test will be provided to complete in the privacy of her own home prior to DXA testing. If pregnancy test is refused or if determined to be pregnant, my daughter may maintain confidentiality by electing not to disclose the pregnancy test results to the research group, but must voluntarily withdraw from the study. Refusal will be documented. If my daughter and I elect to notify the research group of the pregnancy, she/we will receive a referral to her pediatrician or primary care physician.

Information from all analyses will be stored in locked filing cabinets. My child and I will be
instructed on the proper use of the provided supplements. We agree to follow the instructions on the label of the supplements. I understand that the supplement is either 24 mg of zinc or a placebo (a substance of no medical value), neither of which is expected to cause harm to my child if taken properly. If supplementation should cause any noticeable, negative side effects (i.e. Nausea, stomachache, or dizziness), my child may opt to either continue the study without taking supplements, or may discontinue the study completely. Session 2 will require approximately 1½ hours.

3) The discomfort or stresses that may be faced during this research are minor physical discomfort from blood draws and minor psychological discomfort from the questions about my child's diet or medical history. To minimize this stress, participants will be interviewed in private rooms. If undue discomfort occurs, my child has the right to discontinue the testing at any time.

4) I understand that one of the foreseen risks to my child is discomfort during the blood draw. I understand that if a blood sample cannot be obtained after two attempts, no further attempts will be made. I understand that another foreseen risk to my child is exposure to a small amount of radiation when assessing body composition and bone status with the bone scanning machines. The scans for the entire study will give a total radiation dose of 165.75 microsieverts (μSv). This dose is very small, as radiation doses from an adult chest X-ray ranges from 500 to 800 μSv and environmental background radiation per week totals 35 μSv. Thus the total radiation exposure for the study is 21-33% of standard chest X-rays. In the event that information from any scan is lost or unusable, no additional scans will be performed.

### Radiation Doses Per Testing Session:

<table>
<thead>
<tr>
<th>Testing Session</th>
<th>DXA</th>
<th>μSv</th>
<th>pQCT</th>
<th>μSv</th>
<th>Times</th>
<th>Session Conducted</th>
<th>Total Radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>165</td>
<td>0.75</td>
<td>μSv</td>
<td>1</td>
<td>165.75</td>
<td>μSv</td>
<td></td>
</tr>
</tbody>
</table>

5) The results of my participation and that of my child will be confidential and will not be released in any identifiable form without my child's prior permission and mine unless required by law. My signature on this form authorizes that use of my data and my child's data in group analyses which may be prepared for public dissemination, without breaching my own or my child's confidentiality. To accomplish this, my child will be assigned a four digit subject participation code, which will be used on all data collected during my child's participation in this research. A master list with my child's name and corresponding code number will be kept separate from testing data and locked at all times.

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

My child was given the opportunity to complete a simple mine test for pregnancy: (Check one): YES NO

Signature Date_________________________

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I refuse for my child to take the pregnancy test: (Check one): YES___  NO

__________________________  ____________________________
Signature                  Date

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

Richard Lewis/Emma Laing
Name of Researcher
Telephone: 542 4901
Email: rlewis@fcs.uga.edu

__________________________  ____________________________
Name of Parent or Guardian  Signature                  Date

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

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

University of Georgia
Institutional Review Board
Approved:  I-1  09
Expires:  09-0-0

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Assent Form (Child) Pilot Study

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

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

I will take my zinc or placebo supplement every day according to the directions. I will bring my unused supplements to the researcher after 1 month so that she may count how many I missed.

Too much zinc in the diet can cause stomach aches, dizziness and/or nausea. If I feel any of these symptoms, I will report them to my parent and the researcher, and I may either choose to continue to take the supplements, or to discontinue the study. I will also be asked to answer questions about how the supplements are affecting me.

Before entering the study:
- I will receive a sexual maturation self-assessment form in the mail that I will complete in private at home. I will compare my own appearance to drawings of growth stages and circle the drawing that looks most like me.
- I will have my height measured against a wall and my weight measured on a scale.
- If I complete these measures listed above but do not qualify for the study, I will receive $10.
- If any of these procedures cause me to be uncomfortable, I may skip those procedures and any information about me will not be shared with anyone else.

At the beginning of the study and 1 month later:
- A trained nurse will take a blood sample from my arm.
- I will provide a urine sample in a private bathroom.
- I will have my height measured against a wall and my weight measured on a scale.
- My parent and I will write down what I eat during two weekdays and one weekend day.
- I will answer questions about my physical activity.
- If I complete these measures listed above, I will receive $25 at the beginning of the study and $50 after 1 month.
- I may experience hunger before the blood and urine collection, but I will receive a snack once the tests have been completed.
- I may experience a bruise under my skin after the blood draw, which should disappear within a few days.
- If any of these procedures or questions asked of me cause me to be uncomfortable, I may skip those procedures/ questions and any information about me will not be shared with anyone else.

If the equipment is available, at the beginning of the study, I will have pictures taken of my bones. During one set of pictures I will lie on a table for approximately 30 minutes. I will take short breaks between the different pictures that are taken. During another set of pictures, I will place my leg in the circular part of a machine for about 10 minutes. During the last set of pictures, I will place my arm on a box for about 5 minutes. These pictures provide a small
amount of radiation, similar to the X-ray pictures taken at the dentist's office. If any of these procedures or questions cause me to be uncomfortable, I may skip those procedures/questions and any information about me will not be shared with anyone else. If the equipment is unavailable, I have the option to travel to the Bone & Body Composition Lab in Athens, Georgia to have a picture of my bones at another time during the study. Before I have a picture of my bones taken, I will be asked if I am pregnant. If I am not sure, I will be given a pregnancy test. If I am pregnant or refuse the pregnancy test, I will not participate in the study. If I have any questions or concerns, I can always call the researcher, Dr. Richard Lewis at the following number: 542-4901.

Sincerely,

Emma Laing, PhD, RD, LD
Department of Foods and
Nutrition University of Georgia
279 Dawson Hall

__________________________  _______________________
Signature                  Date

I was given the opportunity to complete a simple urine test for pregnancy: (Check one): YES     NO

__________________________  _______________________
Signature                  Date

I refuse to take the pregnancy test: (Check one): YES     NO

__________________________  _______________________
Signature                  Date

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

__________________________  _______________________
Signature                  Date

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

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

University of Georgia Institutional Review Board

Approved: ________; Expires: ________

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APPENDIX D: DXA CONSENT FORM (9 mg)
Consent Form for the Use of the Hologic Delphi A X-Ray Bone Densitometer and XCT 2000 pQCT

Are you pregnant or do you think you might be pregnant? YES NO
*If yes, please do not participate in this study using the Delphi A bone densitometer and the XCT 2000 pQCT.

I, __________ am hereby giving my consent to be used for research conducted by Dr. Richard D; Lewis, University of Georgia, Foods and Nutrition Department, 279 Dawson Hall.

I understand that by giving my consent I am agreeing to be scanned on the Hologic Bone Delphi A X-Ray Densitometer and on the XCT 2000 peripheral Quantitative Computer Tomography machine. Both of these instruments use a low dose x-ray to determine bone mineral density and body composition.

I understand that the Hologic Delphi A X-RAY Bone Densitometer uses a very low level of x-ray and that under most operating conditions, the entrance dose to the patient is 0.5mRem-10mRem. This equals about 3% to 30% of the exposure of a standard chest x-ray and is of no danger to me.

I understand that the XCT 2000 pQCT uses a very low level of x-ray and that under most operating conditions, the maximum entrance dose to the patient is less than 1 mRem.

I understand that The University of Georgia is responsible for my safety during my participation in this study. However, any illness or injury not related to this study is not the responsibility of the investigator or the University of Georgia.

I understand that my participation is entirely voluntary. I can withdraw my consent at any time without penalty and have the results of my participation returned to me, removed from records or destroyed.

______________________________
Signature of Investigator

______________________________
Signature of Participant

______________________________
Date

______________________________
Date
APPENDIX E: HIGH ZINC FOODS TO AVOID (9 mg)
High Zinc Foods to Avoid:

- Mollusks (Oysters, Scallops, Mussels, Clams)
- Crustaceans (Crabs, Shrimp, Crawfish)
- Baked Beans
- Kellogg’s Product 19
- Kellogg’s Complete Wheat Bran Flakes
- General Mills Total Raisin Bran
- General Mills Total Corn Flakes
- General Mills Whole Grain Total
- General Mills Frosted Wheaties
- General Mills Wheaties

Look for zinc listed here. Avoid food items with zinc levels above 8 milligrams (mg) per serving. Avoid foods that have more than 50% of the Daily Value.
APPENDIX F: 3-DAY DIET RECORD (9 mg)
DIRECTIONS FOR KEEPING A 3-DAY DIET DIARY

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

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

2. Write down the first foods you ate for that day. Write down:
   - The time of day you ate the food(s).
   - Each food that you ate.
   - How the food was prepared (baked, boiled, fried, microwaved).
   - How much you ate (cup, 1/2 cup, pieces, tablespoons, teaspoons).

3. It is important to describe each food you eat in detail. For example:
   - Write down brand names for each food you ate if you know them.
   - Write down the type of milk (whole, 2%, or skim) and bread (white, wheat, etc).
   - Write down if the food was fresh, frozen, or canned.
   - If you ate a casserole or a salad, write down the foods there were in it and amounts.
   - If you add things like butter, jelly, sugar, honey, or cream to foods or beverages, please write them down with the amounts used.

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

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

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

7. About how many glasses of water do you drink each day? ____________________
DAY 1 OF THE DIET DIARY

ID: ___________________________________________ CHECKED BY: _______________________________________

DATE: ________________________________________ DAY OF THE WEEK: ____________________________

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

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

________________________________________________________________________________________

________________________________________________________________________________________

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

<table>
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<tr>
<th>Time Eaten</th>
<th>Foods Eaten</th>
<th>Preparation Methods</th>
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DAY 2 OF THE DIET DIARY

ID: _______________________________ CHECKED BY: ___________________________

DATE: ___________________________ DAY OF THE WEEK: ________________

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

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

______________________________________________________________________________

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

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

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

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APPENDIX G: 7-DAY PHYSICAL ACTIVITY RECALL (9 mg)
Subject Code No._____
Date ____________

7-DAY PHYSICAL ACTIVITY RECALL QUESTIONNAIRE

1. On the average, how many hours did you sleep each night during the last 5 weekday nights (Sunday-Thursday)? Record to nearest quarter-hour.

   Hours: _____________   Minutes: _____________

2. On the average, how many hours did you sleep each night last Friday and Saturday nights?

   Hours: _____________   Minutes: _____________

3. First let’s consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them? Please tell me to the nearest half-hour.

   Hours: _____________   Minutes: _____________

4. Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)

   Hours: _____________   Minutes: _____________

5. Now let’s look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them? Please tell me to the nearest half-hour.

   Hours: _____________   Minutes: _____________

6. Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)

   Hours: _____________   Minutes: _____________

7. Now let’s look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour.

   Hours: _____________   Minutes: _____________

8. Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do? (Can you think of other sport, job, or household activities that would fit in this category?)

   Hours: _____________   Minutes: _____________
Physical Activity List

Moderate Activities
Occupational Tasks:
9. Delivering mail or patrolling on foot
10. House painting
11. Truck driving (making deliveries – lifting and carrying light objects)

Household activities:
1. Raking the lawn
2. Sweeping and mopping
3. Mowing the lawn with a power mower
4. Cleaning windows

Sports Activities (Actual playing time)
1. Volleyball
2. Ping pong
3. Brisk walking for pleasure or to work (3 mph or 20 min/mile)
4. Golf-walking and pulling or carrying clubs
5. Calisthenic exercises

Hard Activities
Occupational Tasks:
1. Heavy carpentry
2. Construction work – doing physical labor

Household Tasks:
1. Scrubbing floors

Sports Activities (Actual playing time):
1. Doubles tennis
2. Disco, Square, or Folk dancing

Very Hard Activity
Occupational Tasks:
1. Very Hard physical labor – digging or chopping with heavy tools
2. Carrying heavy loads, such as bricks or lumber

Sports Activities (Actual playing time):
1. Jogging or swimming
2. Singles tennis
3. Racquetball
4. Soccer
5. Aerobics
6. Stair climbing
7. Weight training
8. Gymnastics
7-DAY PHYSICAL ACTIVITY RECALL

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time Spent</th>
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Worksheet for Calculating Daily Energy Expenditure

1. Add up all the hours of sleep and naps you had.  
2. Multiply the total number of hours of sleep and naps (line 1) by 1  
   \[ \times 1 = \]
3. Add up the total number of hours spent in moderate activity.  
4. Multiply the hours spent in moderate activity (line 3) by 4.  
   \[ \times 4 = \]
5. Add up the total number of hours spent in hard activity.  
6. Multiply the hours spent in hard activity (line 5) by 6.  
   \[ \times 6 = \]
7. Add up the total number of hours spent in very hard activity.  
8. Multiply the hours spent in very hard activity (line 7) by 10.  
   \[ \times 10 = \]

9. Add up the figures in lines 1, 3, 5, and 7.  
   \[ 1 + 3 + 5 + 7 = \]
10. Hours spent in light activity is equal to 24 hours minus the hours in lines 1, 3, 5, and 7.  
    \[ 24 - (1 + 3 + 5 + 7) = \]
11. Multiply the figure in line 10 by 1.5.  
    \[ \times 1.5 = \]
12. Add up the figures in lines 2, 4, 6, 8, and 11.  
    \[ (2 + 4 + 6 + 8 + 11) = \]
13. The figure you arrived at in line 12 is the total kilocalories per kilogram of body weight expended per day.  
    \[ \text{(kcal kg}^{-1} \text{ day}^{-1}) = \]
14. To calculate the total number of calories you expended in one day, multiply your total body weight in kilograms) weight in pounds \[ \text{by the figure in line 13. Body weight (kg) X kcal kg}^{-1} \text{ day}^{-1} = \text{total calories expended =} \]

The following are some average kcal kg\(^{-1}\) day\(^{-1}\) for individuals of different ages:

<table>
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<tr>
<th>Age Group</th>
<th>17-19 years</th>
<th>20-29 years</th>
<th>30-39 years</th>
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<tr>
<td>Male</td>
<td>44</td>
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<td>Female</td>
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<th>Age Group</th>
<th>40-49 years</th>
<th>50-59 years</th>
<th>60-69 years</th>
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APPENDIX H: ANTHROPOMETRIC RECORDING SHEET (9 mg)
UGA ZINC STUDY

Participant Information Sheet
Anthropometrics/DXA

Subject ID: ___________ Visit Date:________

Race/Ethnicity: ________ DOB: Month _____ Day _____ Year ______

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

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

BMI: __________

LEG LENGTH ______ SITTING HEIGHT ______

Waist Circumference: ______

LENGTH OF RADIUS (mm) __________ x 0.66 = _______ R or L? ______
LENGTH OF TIBIA (mm) __________ x 0.66 = _______ R or L? ______

MOM’S HEIGHT _____ DAD’S HEIGHT _____ SELF-REPORT? YES NO _____

PREDICTED HEIGHT __________ % PREDICTED HEIGHT __________

---

DXA operator use

- [ ] WB
- [ ] Hip
- [ ] Non-Dominant Limb: R  L
- [ ] AP Spline
- [ ] Radius

Scan date: __________ Complied by: __________
initials of operator

---

pQCT operator use

- [ ] Radius
- [ ] Tibia
- [ ] Non-Dominant Limb: R  L

Scan date: __________ Complied by: __________
initials of operator
APPENDIX I: DEMOGRAPHIC QUESTIONNAIRE (9 mg)
GENERAL INFORMATION QUESTIONNAIRE

Demographic Data:

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

1. What is your date of birth? Month ____ Day _______ Year ______
2. What is your age? Years _______ Months ________________
3. Gender: (Circle One) Female Male
4. What is your grade in school? ________________
5. How do you describe yourself? (Circle One or More: Mixed racial heritage should be indicated by checking more than one category)

   Ethnicity: Hispanic or Latino
   Non-Hispanic or Latino

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

6. Do you live with your parents? (Circle One) YES  NO
   6a. If no, with whom do you live? ______________________________

7. Do you have any brothers or sisters? (Circle One) YES  NO
   7a. If yes, list ages of: _______ Years (Brother) _______ Years (Sister)
        Years (Brother) _______ Years (Sister)
        Years (Brother) _______ Years (Sister)
   7b. If yes, do they participate in sports? (Circle One) YES NO
   7c. If yes, list the sport and gender of sibling. Sport ________________ 
       (Brother or Sister)
       Sport ________________ (Brother or Sister)
       Sport (Brother or Sister) Sport (Brother or Sister)

8. Do you have a twin brother or sister? (Circle One) YES  NO
9. What is your parents' income? (Circle One) 
   Less than $9,999
   $10,000 - $19,999
   $20,000 - $29,999
   $30,000 - $39,999
   $40,000 - $49,999
   $50,000 - $59,999
   $60,000 - $69,999
   $70,000 - $79,999
   $80,000 - $89,999
   $90,000 - $99,999
   Over $100,000

10. What is your mother's occupation? ____________________________

11. What is your father's occupation? ____________________________
GENERAL INFORMATION QUESTIONNAIRE

Health Data

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

1. Have you gained or lost any weight (≥ 10 pounds) in the last 3 months? (Circle One) YES NO
   1a. If yes, how much? +____ pounds OR -_________ pounds

2. Have you had any height changes in the past 3 months? (Circle One) YES NO
   2a. If yes, how much? ___________ feet ___________ inches

3. How would you rate your present health? (Circle One) Poor Fair Good Excellent

4. Have you started your menstrual cycles? (Circle One) YES NO
   If so, what date?

5. Do you have any diseases or illnesses? (Circle One) YES NO
   5a. If yes, what diseases? ________________________________

6. Are you taking any medications either prescribed by a doctor or over-the-counter (self-prescribed)? (Circle One) YES NO
   6a. If yes, what medications? _ Amount per day _________
       _ Amount per day _________
       _ Amount per day _________

Those were some difficult questions to answer because the questions were so private. I want to assure you again that I am the only person who knows how you answered these questions. Thank you for being so honest with your answers.
GENERAL INFORMATION
QUESTIONNAIRE

Nutrition Data:

These next questions are about your diet and eating habits. Try to think about how you eat.

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

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

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

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

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

6. Have you ever eaten a large amount of food and then vomited to get rid of the food? (Circle One) YES NO
   6a. If yes, how old were you? ____________ years _______ months
       ____________ years _______ months

7. Were you breastfed as an infant? (Circle One) YES NO
   7a. If yes, for how many months? _______________

Thank you for answering all of those questions. You did really well, and I appreciate you being so truthful with your answers.
GENERAL INFORMATION QUESTIONNAIRE

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

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

2. Do you have any health problems that limit your activity? (Circle One)  
   - YES
   - NO
   2a. If yes, what health problem? ______________________________

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

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

Bone Health Data:
The next questions have to do with your bones and your family’s bones.

1. Does anyone in your family (including your parent’s, grandparents, aunts, uncles, cousins) have osteoporosis or “humpback”? (Circle One) YES NO
   1a. If yes, who? __________________________

2. Has anyone in your family (including your parents, grandparents, aunts, uncles, cousins) had a hip or wrist fracture? (Circle One) YES NO
   2a. If yes, who? __________________________

3. Have you ever had a bone fracture or broken bone? (Circle One) YES NO
   3a. If yes, which bone(s)? __________________________
   3b. If yes, how old were you? ________ years _________ months

4. Have you ever been told by a doctor that you have bone disease? (Circle One) YES NO
   4a. If yes, what disease? __________________________
   4b. If yes, how old were you? ________ years _________ months
APPENDIX J: SUN EXPOSURE QUESTIONNAIRE (9 mg)
Your Name: ____________________________________________

(Once your vitamin D results and this questionnaire have been matched, your name will be removed from the records)

Date: ____ / ____ / 2007

SUN EXPOSURE QUESTIONNAIRE

Thank you for agreeing to take part in this study. It is very important that you answer all the questions. If you have any difficulties, feel free to discuss them with the Researcher(s).

If you have any problems or queries please do not hesitate to contact:

• Dr. Richard Lewis at 706-542-4901 or rlewis@fcs.uga.edu
• Dr. Emma Laing at 706-583-0040 or emonk@uga.edu

All of the information that you provide will remain strictly confidential

This project is funded through the National Institutes of Health, Grant Number: xxxxxx
SECTION 1: PERSONAL DETAILS

Firstly we would like to ask you some questions about your background:

1. What gender are you? *(Please tick the appropriate box)*  
   □ Male  □ Female

2. What is your date of birth?  
   □ □ □ (b) current age: □ □ years

3. In what country were you born?  
   ______________________

4. IF YOU WERE NOT BORN IN THE USA  
   In what year did you come to live in the USA?  
   □ □ □ □ □ □ □ □

5. We are interested in your parents’ **ETHNIC ORIGIN** (that is, the place where most of their ancestors came from) and the **COUNTRY THEY WERE BORN IN**

<table>
<thead>
<tr>
<th>Country of Birth</th>
<th>Ethnic Origin (Please tick the appropriate box)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>North European</td>
</tr>
<tr>
<td>Mother</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td></td>
</tr>
</tbody>
</table>

6. Please tick the box below which best describes your after-school activities:

□ Mainly indoors
Half indoors and half outdoors
Mainly outdoors

SECTION 2: YOUR SKIN AND THE SUN

The questions in this section are about your skin and how it reacts to the sun.

7. We are interested in whether your skin BURNS.
   SUPPOSE your skin is exposed to strong sunlight for the first time in summer, WITH NO PROTECTION such as sunscreen or shade. If you stayed in the sun for 30 minutes would your skin...
   □ Never □ Rarely □ Sometimes □ Mostly □ Always
   burn burn burn burn burn

8. We are also interested in whether your skin TANS.
   IMAGINE you spend several weeks at the beach in strong sunlight, without any protection such as sunscreen or clothing. What would your skin be like? (Please tick the appropriate box)
   □ Very brown & □ Moderately □ Slightly □ Not tanned
   Deeply tanned tanned tanned at all

SECTION 3: TIME SPENT IN THE SUN & SUN-PROTECTION

9. During the PAST MONTH, how much time did you usually spend outside each day between sunrise & sunset on:
   a. typical WEEKDAYS (Monday - Friday)? □ Hours
   b. typical WEEKENDS (Saturday and Sunday)? □ Hours

10. Is the PATTERN of sun exposure described above in questions 10 fairly TYPICAL for you at this time of year?
   □ Yes (IF YES, Please go to Question 14)
   □ No IF NO, do you usually spend □ MORE or □ LESS time in the sun?
11. If you answered NO to question 11, please tell us WHY your pattern of sun exposure has been DIFFERENT over the PAST MONTH? (e.g. indoors more because you were sick, or outdoors more because you were on holidays, or working outdoors in a new job etc).

____________________________________________________________________________________
____________________________________________________________________________________

12. This question asks about the SUN PROTECTION you may have used in the PAST MONTH.

<table>
<thead>
<tr>
<th>During THE PAST MONTH: when outside in the sun did you...</th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Usually</th>
<th>Almost always</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wear a hat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wear sunscreen?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wear a shirt with long sleeves?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wear long trousers or clothing that covers all or most of your legs?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13. If you have used sunscreen in the PAST MONTH, what is the sun-protection factor (SPF) of the sunscreen that you have been using? (e.g. SPF 30+) SPF?

14. Over the PAST MONTH, when you have used sunscreen, HOW OFTEN have you applied it?

   □ once /day   □ 2 times /day   □ 3 or more times/day   □ Other: ____________

15. Over the PAST MONTH, when you used sunscreen, WHERE did you usually apply it?

   (Please shade the parts of the body where you usually applied sunscreen).
*Thank you very much for taking the time to participate in the study*
APPENDIX K: COMPLIANCE QUESTIONNAIRE (9 mg)
Compliance Questionnaire

1) In your opinion, how frequently have you been taking your supplement:
   a. Daily
   b. Almost every day
   c. 3-5 times a week
   d. 1-2 times a week
   e. Not regularly

2) Is it easy for you to remember to take your supplement (circle one)? Yes  No

3) Is there any way that we could help make it easier for you to remember to take your supplement?

4) Do you have any comments or complaints about being in the study you would like to share with me?

5) Since you began our study, have you experienced any of the following more frequently than before you began the study (circle one)?
   a. Nausea/vomiting  Yes  No
   b. Loss of appetite  Yes  No
   c. Abdominal cramping  Yes  No
   d. Diarrhea  Yes  No
   e. Headaches  Yes  No

(If the participant answers “Yes” to any of these questions continue to #6, if not, go to #7)
6) Could you explain when you experienced (complaint from # 5)? How frequently has this occurred since the beginning of the study?

________________________________________________________________________

________________________________________________________________________

7) Since you began our study, have you experienced any of the following more frequently than before you began the study (circle one)?
   a. Fatigue  Yes  No
   b. Confusion Yes  No
   c. Colds or infections Yes  No
   d. Other Yes  No

8) Could you explain when you experienced (complaint from # 7)? How frequently has this occurred since the beginning of the study?

________________________________________________________________________

________________________________________________________________________

9) Are you enjoying/have you enjoyed being in the study?_____ Do you have anything else you would like to discuss?

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
APPENDIX L: PILL COUNT FORM (9 mg)
Pill Count Form

Subject ID#: ____________
Interviewer: ____________
Date of Interview: ____________

Date________________________?

Number of study pills (zinc or placebo) left in the bottle:______________

Any complaint of side effects?   Yes   No

If yes, please report:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
APPENDIX M: ADVERSE EVENT FORM (9 mg)
Response to Supplementation Questionnaire

Subject ID#: ____________
Interviewer: ____________
Date of Interview: _________

How is your overall health?

How do you feel about the supplements you have been taking?
APPENDIX N: VERBAL SCRIPT (PAST AND NEW PARTICIPANTS) (24 mg)
Verbal Permission Script (Phase 1):

Hello, this is Valerie Chertin calling from the UGA Bone Clinic and I am working under the direction of Dr. Richard Lewis in the Department of Foods and Nutrition. Your daughter participated in our GAPI vitamin D study in 2009/10/11 (or our Zinc and Bone Study 20 07/08/09/10). I am calling today to let you know that we are currently in the recruitment phase for a new supplementation study. Although your child who participated in the prior study is not eligible to participate in this study, a younger sibling or a friend or relative may qualify. Would you be interested in further information about this study?

{If NO}: Thank you very much for your time.

{If YES}: Thank you for your interest. Do you have a child who may qualify or are you requesting information for another party?

{If for another party}: We would greatly appreciate you passing our contact information along to friends or family who may have a qualifying white daughter between the ages of 9-13 years. (Give our contact information here).

{If for own child}: Thank you. I would like to take a few minutes to tell you more about the study and determine whether your child may be qualified to participate. I do need to make you aware that there is no monetary compensation for this phone interview; it should take about 15 minutes. There is no direct benefit for participating in this phone interview. The researcher hopes that the completed research project will benefit society by providing information on the ability of zinc to improve overall bone health and participants in the study may also learn about nutrition and bone health.

The purpose of the research is to see whether zinc supplements taken each day for one month will improve blood markers of bone health. Before enrolling in the study, we'll need to determine if your child qualifies. To do this, I will ask you some questions about your child and her health. If your child meets our criteria, you will be mailed a pubertal maturation form that has breast stage drawings. Your child will use this form to assess the stage of maturation she is in. Please mail the form back to our laboratory in the provided stamped self-addressed envelope. If your child is within the range we are looking for, your child will be invited to participate in this study, which is on the University of Georgia campus. Your child will be asked to come to our laboratory for testing twice: once at the beginning of the study and once at the end (after one month). You and your child will fill out questionnaires; your child will give a fasting blood sample; be measured for height and weight; be instructed on the completion of a computerized diet record, which can be done during the appointment or at home; be given two different types of bone scans. The bone scans are a kind of x-ray and will involve a small amount of radiation. These testing sessions will last about 1 1/2 hours. Your child will be placed in either the zinc group or the placebo group. Participants in the zinc group will take 24 mg of zinc sulfate per day for one month. This level is a safe level of zinc. Participants in the placebo group will take a pill identical in appearance to the zinc pill each day for one month. We will count the pills to document how many pills your child has taken. Your child will receive $25 for the baseline testing session, and $25 at the end of the study for a total of $50 for the entire study.

I want to assure you that any information that can identify you or your child that I receive from you today will be strictly confidential and will be kept under lock and key. Participation is voluntary; you can refuse to answer any questions, or stop this phone interview at anytime without penalty or loss of benefits to which you are otherwise entitled. If your child does not qualify for the study, the screening data collected over the telephone will be immediately destroyed. If you or your child decides to not participate after responding to some or all of the questions, her information will be destroyed.

There is a possibility that some of the questions you will be asked concerning diet, physical activity, history of menstruation status, body image, and eating disorders may make you uncomfortable or distressed; if so, please let me know. You don't have to answer these questions if you don't want to.
Do you have any questions?

Do you agree to complete the screening questionnaire for your child? [If yes, continue with telephone screening questionnaire] [If not, say “Thank you for your time, goodbye.”]

The contact information for the researcher conducting this project is: Valerie Chertin
279 Dawson Hall
University of Georgia
706-542-4918

Additional questions or problems regarding your child’s rights as a research participant should be addressed to The Chairperson of the Institutional Review Board of University of Georgia, Telephone (706) 542-3199; E-Mail Address IRB@uga.edu.
Verbal Permission Script (Phase 1):

Thank you for your interest in our research study. My name is______ from the University of Georgia-Athens, and I am working under the direction of Dr. Richard Lewis in the Department of Foods and Nutrition. The purpose of the study is to determine the effects of Zinc supplementation on bone health and growth in young females. Do you think you might be interested in this study?

{If NO}: Thank you very much for your time.

{If YES}: Thank you. I would like to take a few minutes to tell you more about the study and determine whether your child may be qualified to participate. I do need to make you aware that there is no monetary compensation for this phone interview; it should take about 15 minutes. There is no direct benefit for participating in this phone interview. The researcher hopes that the completed research project will benefit society by providing information on the ability of zinc to improve overall bone health and participants in the study may also learn about nutrition and bone health.

The purpose of the research is to see whether zinc supplements taken each day for one month will improve blood markers of bone health. Before enrolling in the study, we’ll need to determine if your child qualifies. To do this, I will ask you some questions about your child and her health. If your child meets our criteria, you will be mailed a pubertal maturation form that has breast stage drawings. Your child will use this form to assess the stage of maturation she is in. Please mail the form back to our laboratory in the provided stamped self-addressed envelope. If your child is within the range we are looking for, your child will be invited to participate in this study, which is on the University of Georgia campus. Your child will be asked to come to our laboratory for testing twice: once at the beginning of the study and once at the end (after one month). You and your child will fill out questionnaires; your child will give a fasting blood sample; be measured for height and weight; be instructed on the completion of a computerized diet record, which can be done during the appointment or at home; be given two different types of bone scans. The bone scans are a kind of x-ray and will involve a small amount of radiation. These testing sessions will last about 1½ hours. Your child will be placed in either the zinc group or the placebo group. Participants in the zinc group will take 24 mg of zinc sulfate per day for one month. This level is a safe level of zinc. Participants in the placebo group will take a pill identical in appearance to the zinc pill each day for one month. We will count the pills to document how many pills your child has taken. Your child will receive $25 for the baseline testing session, and $25 at the end of the study for a total of $50 for the entire study.

I want to assure you that any information that can identify you or your child that I receive from you today will be strictly confidential and will be kept under lock and key. Participation is voluntary; you can refuse to answer any questions, or stop this phone interview at anytime without penalty or loss of benefits to which you are otherwise entitled. If your child does not qualify for the study, the screening data collected over the telephone will be immediately destroyed. If you or your child decides to not participate after responding to some or all of the questions, her information will be destroyed.

There is a possibility that some of the questions you will be asked concerning diet, physical activity, history of menstruation status, body image, and eating disorders may make you uncomfortable or distressed; if so, please let me know. You don’t have to answer these questions if you don’t want to.

Do you have any questions?

Do you agree to complete the screening questionnaire for your child? [If yes, continue with telephone screening questionnaire]
[If not, say “Thank you for your time, goodbye.”]

The contact information for the researcher conducting this project is:
Valerie Chertin  
279 Dawson Hall  
University of Georgia  
706-542-4918

Additional questions or problems regarding your child’s rights as a research participant should be addressed to The Chairperson of the Institutional Review Board of University of Georgia, Telephone (706) 542-3199; E-Mail Address IRB@uga.edu.
UGA ZINC STUDY
Telephone Screening Questionnaire (Phase 1)

This interview should take approximately ten minutes:

Date: ______________ Time: ____________ Screen completed by: ____________________

1. A. Has your child started her menstrual cycles? YES ____________ NO ______

1. B. How would you describe your child’s ethnicity/race?

**Ethnicity:**
- Hispanic or Latino
- Non-Hispanic or Latino

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

*Parents of participants may indicate one or more of the following (mixed racial heritage should be indicated by selecting more than one category):*

2. We would also like to know the ethnicity/race of the child’s biological parents and grandparents. How do you describe your child’s mother and father?

<table>
<thead>
<tr>
<th><strong>Child’s Mother</strong></th>
<th><strong>Child’s Father</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity:</strong></td>
<td><strong>Ethnicity:</strong></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>Hispanic or Latino</td>
</tr>
<tr>
<td>Non-Hispanic or Latino</td>
<td>Non-Hispanic or Latino</td>
</tr>
<tr>
<td><strong>Race:</strong></td>
<td><strong>Race:</strong></td>
</tr>
<tr>
<td>American Indian or Alaska Native</td>
<td>American Indian or Alaska Native</td>
</tr>
<tr>
<td>Asian</td>
<td>Asian</td>
</tr>
<tr>
<td>Black or African American</td>
<td>Black or African American</td>
</tr>
<tr>
<td>Native Hawaiian/ Pacific Islander</td>
<td>Hawaiian/other Islander</td>
</tr>
<tr>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>any combination of the above</td>
<td>any combination of the above</td>
</tr>
</tbody>
</table>

How do you describe your child’s paternal grandmother and grandfather?

<table>
<thead>
<tr>
<th><strong>Child’s Paternal Grandmother</strong></th>
<th><strong>Child’s Paternal Grandfather</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity:</strong></td>
<td><strong>Ethnicity:</strong></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>Hispanic or Latino</td>
</tr>
<tr>
<td>Non-Hispanic or Latino</td>
<td>Non-Hispanic or Latino</td>
</tr>
</tbody>
</table>
Race: American Indian or Alaska Native  American Indian or Alaska Native  American Indian or Alaska Native  Asian  Asian  Black or African American  Black or African American  Native Hawaiian/ Pacific Islander  Native Hawaiian/other Islander  White  White  any combination of the above  any combination of the above

How do you describe your child’s maternal grandmother and grandfather?

<table>
<thead>
<tr>
<th>Child's Maternal Grandmother</th>
<th>Child’s Maternal Grandfather</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity:</td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>Hispanic or Latino</td>
</tr>
<tr>
<td>Non-Hispanic or Latino</td>
<td>Non-Hispanic or Latino</td>
</tr>
<tr>
<td>Race:</td>
<td></td>
</tr>
<tr>
<td>American Indian or Alaska Native</td>
<td>American Indian or Alaska Native</td>
</tr>
<tr>
<td>Asian</td>
<td>Asian</td>
</tr>
<tr>
<td>Black or African American</td>
<td>Black or African American Native</td>
</tr>
<tr>
<td>Hawaiian/ Pacific Islander</td>
<td>Native Hawaiian/other Islander</td>
</tr>
<tr>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>any combination of the above</td>
<td>any combination of the above</td>
</tr>
</tbody>
</table>

3. How old is your child? ________________ Years; DOB: ___ / ___ / ___

4. What grade does she attend in school? ________________

5. How tall is your child? ______ ft _____ in  How much does she weigh? ________________ lbs.

_Calculate BMI and plot on CDC growth chart – must be between 5th and 85th percentile BMI for age and sex to qualify._

6. Has your child lost or gained weight in the past 3 months? YES ________________

   If yes, how much? ________________ lbs

7. Has your child ever been diagnosed with any of the following diseases/conditions?

<table>
<thead>
<tr>
<th>Disease</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal Disease or Kidney Stones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral Palsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal Malabsorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile Rheumatoid Arthritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychological Illness</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Is your child currently taking any medications? YES __________ NO

   If yes, what medication(s)?
9. Is your child taking an herbal, vitamin or mineral supplement? YES ____ NO

If yes, how much and how often?

______________________________

If yes, would your child be willing to stop taking the supplement? YES ____ NO ____ (child would be eligible to enroll in the study after a 4-week washout period)

10. In this study, all participants must provide blood samples 2 times (at the start and after 4 weeks). Your child will fast the night before testing each appointment. On the day of testing, you and your child will arrive in the BBCL in Dawson Hall at the scheduled time. A trained phlebotomist will then draw approximately 32 mL (~2 Tbsp) of blood from your child’s arm, after which she will be given a snack (15-20 minutes). Your child’s blood will be analyzed for compounds that reflect how her bones are responding to the supplements, and if she has certain genetic factors that may affect this response.

Is your child willing to do this? YES ____ NO ____

11. Before initiation of this study, we will ask your child to give a self-assessment of pubertal maturation. We will send you the form for your child to complete. Would your child be willing to fill out a self-assessment of sexual maturation form and mail it back in a self-addressed envelope we will provide? YES ______ NO ______

If the caller is still interested, explain more about the study and why we are doing it and collect the following information:

Parent’s name: _______________________________________________

Child’s name: ________________________________________________

Address: ____________________________________________________

Zip Code: ___________________________________________________

Daytime Phone Number: _________________________________ (home or work?)

Email Address: ________________________________

Is it okay to call in the evening? If yes, evening phone:

__________________________

How did you hear about the study?

______________________________________________
If selected to participate, what mornings during the week would you be available to come to the UGA Bone and Body Composition Lab, located in Dawson Hall, for testing? M_____ T_____ W_____ Th_____ F_____ S _____

“This is the end of our telephone screening. We will review this and determine your child’s eligibility for the study. We will get back to you as soon as possible to let you know the status of her eligibility. Do you have any additional questions for me?”

Make sure the potential volunteer has contact numbers for future questions.

If child is eligible based on the telephone screen, notify parent that we will be sending them the consent forms by mail or email. Once mailed, we will make a follow-up phone call to schedule a time for the hand-wrist scan to determine child’s eligibility.
APPENDIX P: PARENTAL CONSENT/ASSENT FORMS (24 mg)
PARENTAL PERMISSION AND CONSENT FORM- Phase 1

Efficacy of Supplemental Zinc for Improving Bone Strength in Early Pubertal Children

I, __________, agree to give permission for my child, __________, to participate in the research titled "EFFICACY OF SUPPLEMENTAL ZINC FOR IMPROVING BONE STRENGTH IN EARLY PUBERTAL CHILDREN," which is being conducted by Dr. Richard Lewis of the Department of Foods and Nutrition at the University of Georgia. Dr. Lewis may be reached in room 279 Dawson Hall at (706) 542-4901. Participation in this research is voluntary. I can refuse to grant permission or withdraw permission and my child can refuse to take part or stop taking part at any time without penalty or loss of benefits to which my child is otherwise entitled. If my child and I decide to discontinue or withdraw from the study, the information/data collected from or about my child up to the point of withdrawal will be kept as part of the study and may continue to be analyzed, unless I ask to have information that can be identified as my child's returned to me, removed from the research records, or destroyed.

The following points have been explained to me:

1) Purpose: The reason for the research is to study the impact of zinc supplementation on growth and bone health in children.

2) Duration: My child's participation will last 1-month with approximately 4 hours of testing, divided across 2 appointments.

3) Procedures:

a) My child and I will be briefed and familiarized with the testing procedures that will be used during the study (15 minutes). My child and I will be given the opportunity to ask any questions that we may have about the study.

b) Session 1 will be conducted at 2 different time points (at the beginning of the study and after 1 month): As agreed to during the screening phone interview and instructed when scheduling this session, my child has fasted last night and will fast the night before the last appointment. A trained phlebotomist will draw approximately 32 mL (2 blood will be analyzed for compounds that reflect how her bones are responding to the supplements, and if she has certain genetic factors that may affect this response. An example may include the genotyping of IGF-I genes. My child's samples will be coded for anonymity to ensure confidentiality. In the event of an unexpected breach of confidentiality, a recent federal law (Genetic Information Non-Discrimination Act or GINA) will help protect my child from health insurance or employment discrimination based on genetic information obtained about her through research such as this. These samples will be analyzed in association with this study. Samples may also be analyzed at a future date in conjunction with research also related to factors affecting bone strength and growth. Any unused portions of the blood that is collected will be discarded after 10 years post completion of the study, or on the child's 18-year birthdate, whichever occurs first.

I agree to the storage and use of my child's coded serum samples in future research as described above. (Check one) **YES **NO **
My child and I will complete a general information/health questionnaire, sun exposure questionnaire, and a seven-day recall physical activity recall (approximately 15 minutes). We will also be given instructions to complete an online diet record with an individualized access code provided by the researcher. We will complete 3 non-consecutive days, including a weekend day. If we do not have access to a computer to complete this at home, we will be given a hard copy of the questionnaire to complete that will later be entered by a UGA researcher. Session 1 will require approximately 45 minutes.

c) Session 2 of testing will be conducted at one time point (at the beginning of the study only): After completion of the questionnaires, my child's height, sitting height, leg length, and weight, as well as my height will be measured. My child will also have her bone mineral density and body composition measured using non-invasive bone- and muscle-scanning machines called DXA and pQCT. These measurements will require approximately 50 minutes, which includes a small break in between each scan. My child will receive a total of 5 DXA scans and 6 pQCT scans at their baseline appointment. Some scans will focus on certain body parts like her hand and wrist and others will be performed on her whole body. A trained laboratory technician under the supervision of Dr. Richard Lewis will conduct all measurements. Session 2 will require approximately 1½ hours.


d) My child and I will be instructed on the proper use of the provided supplements. We agree to follow the instructions on the label of the supplements. I understand that the supplement is either 24 mg of zinc or a placebo (a substance of no medical value), neither of which is expected to cause harm to my child if taken properly. If supplementation should cause any noticeable, negative side effects (i.e., nausea, stomachache or dizziness), my child may opt to either continue the study without taking supplements, or may discontinue the study completely. During the course of the study, neither the subjects nor the researcher will be aware of who gets zinc and who gets the placebo. The assignment to the zinc or placebo group is random which means that your child has a 50/50 chance of ending up in either group. Once all participants have completed the study and all of the planned assays and statistical analysis have been completed, the supplement code will be unmasked and subjects will be notified of those details via US mail.

4) Compensation in Case of Injury: The researchers will exercise all reasonable care to protect my child from harm as a result of her participation. In the event of an injury, as an immediate and direct result of my child's participation, the researchers' sole responsibility is to arrange transportation for her to an appropriate facility if additional care is needed. In the event that my child suffers a research-related injury, my child's medical expenses will be my responsibility or that of my third-party payer, although I am not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research. In the event of a research-related injury, I should seek medical help and then immediately contact Dr. Lewis at (706) 542-4901. Participants should notify the researcher immediately in the case of any change to health status, including new or discontinued use of any medication.

5) Benefits: The benefits that my child and I may expect from participation are the assessment of diet, maturation, growth, bone health (bone mineral density), and body composition
(percentage of body fat and nonfat tissue). In addition, participants will receive a group counseling session with a registered dietitian to help interpret the individual information the participants obtain from the study. Moreover, if the researcher's hypothesis is correct, dietary intervention with zinc will be a cost effective approach to improving bone health during adolescence, reducing the risk of osteoporotic fractures, and therefore beneficial to participants and others. All measurements are being used for research purposes only, not medical purposes. However, if abnormalities are found in any measure, my child and I will be notified and referred to an appropriate healthcare professional.

6) Confidentiality: Every effort will be taken to protect my child's identity. No individually-identifiable information about my child, or provided by me or my child during the research, will be shared with others, except if necessary to protect my child's rights or welfare (for example, if my child is injured and needs emergency care), or if required by law. The telephone screening form, which contains information that can identify my child and me, will be stored in locked filing cabinets. My child's participation results, which will include an assigned participant number, my child's assent form and my permission form, will not be stored together. A separate list will be the only document linking my child's name and participant number, and therefore will be kept along with the permission forms in a locked file drawer, and accessed only by Dr. Lewis and his immediate research team. This list will be destroyed ten years from the date all subjects have completed their participation. All other documents that were used to collect data will only include my child's participant number. My child will not be identified in any report or publication of this study. The final dataset will be stripped of any of my child's individual identifiers.

7) Financial Costs of this Research: There is no cost to me or my child to participate in this research; however, I must provide or arrange my child's transportation to and from the sessions, all of which are in on-campus facilities. Driving directions will be provided.

8) Risks and Discomforts of Questionnaires: The discomforts or stresses that my child may face during this research include psychological discomfort from the disclosure of information concerning sexual maturation, diet, physical activity and history of menstruation status. In addition, my child may be asked sensitive questions about body image and eating patterns. However, my child may skip any question that may be distressing. To minimize this stress, participants will be interviewed in private rooms. If undue discomfort occurs, my child has the right to discontinue the testing at any time.

9) Risks and Discomforts of Blood Draw: One of the foreseen risks to my child is discomfort during the blood draw. The risks of drawing blood from the arm include the unlikely possibilities of a small bruise or localized infection, bleeding, and fainting. These risks will be reduced in the following ways: blood will be drawn only by a qualified and experienced phlebotomist who will follow standard sterile techniques, who will observe subjects after the needle is withdrawn, and who will apply pressure to the blood-draw site. I understand that if a blood sample cannot be obtained after two attempts, no further attempts will be made.

10) Risks and Discomforts of Bone-Scanning Machines: I understand that another foreseen risk to my child is exposure to a small amount of radiation during the bone (or DXA and
pQCT) scans. The DXA and pQCT scans performed will total 180 f.\text{Sv} of radiation. For comparison, natural and man-made background exposure is approximately 122f.\text{Sv} per week (source, US EPA). The total amount of possible radiation exposure in the study is far less than the 500 to 800 f.\text{Sv} of radiation received from an adult chest X-ray. Alternatively, a round-trip airline flight from Athens, GA to Athens, Greece would be approximately 180f.\text{Sv} of exposure, equal to the total amount of radiation exposure in the study. Considering these comparisons, it is reasonable to assess the risk of harm from the amount of radiation exposure for subjects versus non-subjects as minimal. In the event that information from any scan is lost or unusable, no additional scans will be performed. A copy of my child's DXA scans will be provided to me, but I understand that the researchers are not medical doctors. The DXA results will be explained to my child and me, and may be clinically relevant, but for diagnosis and health questions, I should consult a qualified physician.

11) Risk and Discomforts of Supplementation: I understand that as a participant in this study, my child will be required to take a supplement pill every day for 28 days. This supplement pill will consist of either a placebo (in the form of microcrystalline cellulose) or 23 mg elemental zinc (in the form of 66 mg zinc sulfate). This is a double-blinded study, so that during the time my child is enrolled in the study neither the researcher nor I will know which supplement my child is taking. Once all participants have completed the study, I will be notified as to whether my child took the zinc or the placebo. It is ideal for participants to swallow the pill at approximately the same time each day with a meal or a large snack. If my child has difficulty swallowing the pill, I can place the pill into a small amount of peanut butter, yogurt, pudding or applesauce to ease ingestion. I understand that the Recommended Dietary Allowance (RDA), or the average daily level of intake sufficient to meet the nutrient requirements of a child between the ages of 9 to 13 years is 8 mg of elemental zinc. I also understand that the Tolerable Upper Intake Level (UL), or maximum daily intake that is unlikely to cause adverse health effects is 23mg of elemental zinc. These intake values were determined by the Food and Nutrition Board at the Institute of Medicine of the National Academies. Possible adverse effects of high zinc intake can include: nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches. I understand that if supplementation causes my child to experience noticeable, negative side effects, I should alert the researchers by calling Dr. Lewis at 706-542-4901. At that time, she may opt to either continue the study without taking supplements, or may discontinue the study.

12) Additional Risks for Pregnant Females: Because our current knowledge of the risk of X-ray to the unborn child is limited, prior to conducting the bone and muscle scans, my child will sign an assent form developed for use with these machines that asks if she is currently pregnant or believes she may be pregnant. Being a part of this study while pregnant may expose the unborn child to a yet undiscovered risk; therefore, pregnant females or those who suspect they could be pregnant will be excluded from the study. If my child expresses any doubts regarding her pregnancy status, a pregnancy test will be provided to her, which she may complete in a private location prior to undergoing DXA or pQCT scanning. If the pregnancy test is positive, my child and I may maintain confidentiality by electing not to disclose any information to the research group, but my child must voluntarily decline to undergo the DXA and pQCT procedures. If I elect to notify the research group of my child's pregnancy, my child and I will receive information about and referral to a primary care physician. By signing this form, parents of females of childbearing potential are certifying to the best of their knowledge
that their child is not pregnant. My refusal for my child to take the pregnancy test will also be documented below.

13) Incentive: Once enrolled in the study, my child will receive $25 for completion of baseline testing and another $25 at the completion of the 1-month study. Payments will be distributed only if all testing sessions are completed for a given time point and supplements are taken as directed. In order to process the payment for my child's participation, the researchers need to collect my child's name and mailing address on a separate payment form. This completed form will be sent to the Department of Foods and Nutrition business office and then to the UGA Business Office. The researchers have been informed that these offices will keep my child's information private, but may have to release her name and the payment amount to the IRS, if ever asked. The researchers connected with this study have gone to great lengths to protect my child's private information and will keep this confidential in their locked files. However, they are not responsible once my child's name and mailing address leave their office/laboratory for payment processing.

I certify that my child is not pregnant, or trying to become pregnant.
(Check one):
Yes/NO

My child was given the opportunity to complete a simple urine test for pregnancy.
(Check one): YES___ N

I understand the risks described above, and refuse for my child to take the pregnancy test.
(Check one): YES___ NO

14) Clinical Trial Information: A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify your child. At most, the Web site will include a summary of the results. ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. You can search this Web site at any time.

15) Permission for Future Contact: By signing my initials here,________, I agree to allow the investigators of this study to contact me in the future to request my/my child's participation in future studies. I understand that at that time, I/my child may refuse any further participation with no negative consequences. The best way to locate me/my child in the future is:

Address____________________________________
Telephone Number(s): _________________________
Email: __________________________

16) Questions: My child and I have the opportunity to ask, and to have answered, any questions we may have about this research. If my child and I have other questions, either now or during the course of the project, or if a research-related injury occurs, Dr. Lewis can be reached at (706) 542-4901.
I have read the information provided above. My questions have been answered to my satisfaction, and I voluntarily agree to participate and to allow my child to participate in this study. After it is signed I understand that I will receive a copy of this permission form.

Richard Lewis
Name of Researcher
Telephone: 542-4901
Email: rlewis@fcs.uga.edu

Name of Parent or Guardian
Signature

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

Additional questions or problems regarding your child's rights as a research participant should be addressed to The Chairperson, Institutional Review Board, University of Georgia, 629 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu.
CHILD ASSENT FORM Pilot- Phase 1

Efficacy of Supplemental Zinc for Improving Bone Strength in Early Pubertal Children

I, - - - - - - - - - **agree** to take part in a research study about bone health and growth.

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

I will take my zinc or placebo supplement every day according to the directions. I will bring my unused supplements to the researcher after 1 month so that she may count how many I missed.

Too much zinc in the diet can cause stomachaches, dizziness and/or nausea. *If* I feel any of these symptoms, I will report them to my parent and the researcher, and I may either choose to continue to take the supplements, or to discontinue the study. I will also be asked to answer questions about how the supplements are affecting me.

Before entering the study:

  *Y* I will look at drawings and choose the one that looks most like my body.
  *Y* I will have my height measured against a wall and my weight measured on a scale.
  *Y* If any of these procedures cause me to be uncomfortable, I may skip those procedures and any information about me will not be shared with anyone else.

At the beginning of the study and 1 month later:

  *Y* A trained nurse or phlebotomist will take a blood sample from my arm.
  *Y* I will have my height measured against a wall and my weight measured on a scale.
  *Y* My parent and I will answer questions about what I eat and about my physical activity.
  *Y* If I complete these measures listed above, I will receive $25 at the beginning of the study and $25 after 1 month.
  *Y* I may experience hunger before the blood collection, but I will receive a snack once the tests have been completed.
  *Y* I may experience a bruise under my skin after the blood draw, which should disappear within a few days.

At the beginning of the study, I will have pictures taken of my bones. During one set of pictures I will lie on a table for approximately 30 minutes. I will take short breaks between the different pictures that are taken. During another set of pictures, I will place my leg in the circular part of a machine for about 10 minutes. During the last set of pictures, I will place my arm on a box for about 5 minutes. These pictures provide a small amount of radiation, similar to the X-ray pictures taken at the dentist's office. If any of these procedures or questions causes me to be uncomfortable, I may skip those procedures/questions and any information about me will not be shared with anyone else. Before I have a picture of my bones taken, I will be asked if I am pregnant. *If* I am not sure, I will be given a pregnancy test. *If* I am pregnant or refuse the pregnancy test, I will not participate in the study. I am not pregnant, or trying to become pregnant.
(Check one):  **YES**    NO

I was given the opportunity to complete a simple urine test for pregnancy:
(Check one):  **YES**    NO

I refuse to take the pregnancy test:
(Check one):  **YES**    NO

If I have any questions or concerns, I can always call the researcher, Dr. Richard Lewis, at the following phone number: (706) 542-4901.

Richard Lewis, **PhD, RD**
Department of Foods and Nutrition University of Georgia
279 Dawson Hall

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

______________________________
Signature of the Participant/Date

______________________________
Signature of the Researcher/Date

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

**Additional questions or problems regarding your rights as a research participant should be addressed to:** The Chairperson, Institutional Review Board, University of Georgia, 629 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone: (706) 542-3199; E-Mail Address: IRB@uga.edu.
APPENDIX Q: HIGH ZINC FOODS TO AVOID (24 mg)
High Zinc Foods/Products to Avoid:

- Mollusks (Oysters, Scallops, Mussels, Clams)
- Crustaceans (Crabs, Shrimp, Crawfish)
- Baked Beans
- Kellogg’s Product 19
- Kellogg’s Complete Wheat Bran Flakes
- General Mills Total Raisin Bran
- General Mills Total Corn Flakes
- General Mills Whole Grain Total
- General Mills Frosted Wheaties
- General Mills Wheaties

Also be aware that some over the counter cold remedies or immune function supplements contain zinc. Please check all labels. Ex., Airborne products, Zicam, et al.

![Nutrition Facts](image)

\[\text{Ingredients: Whole grain wheat, wheat bran, sugar, brown sugar, syrup, contains 2% or less of salt, milk flavoring.} \]

\[\text{Vitamins and Minerals: Vitamin C (sodium ascorbate, ascorbic acid), vitamin A palmitate, niacinamide, reduced iron, zinc oxide, calcium pantothenate, vitamin B6 (pyridoxine hydrochloride), vitamin D, vitamin B12 (cyanocobalamin) vitamin E (alpha tocopherol acetate), folic acid, vitamin B6.}\]

CONTAINS WHEAT INGREDIENTS.
APPENDIX R: ASA 24-HR RECALL INSTRUCTION SHEET (24 mg)
ZINC 2012 Study Directions

- Remember to **take your pill every day** with food

- Bring back red folder and the blister pack with remaining pills

- Your follow-up appointment is scheduled for:

- **Instructions for Online Diet Recall** *
  - Use this web address to access the online diet recall
    https://asa24.westat.com/
  - Log in using the following User ID: _____ and Password: _____
  - Complete 1st record for 2 week days and 1 weekend day, between _______ and _______
  - Complete 2nd record for 2 week days and 1 weekend day, between _______ and _______
  - Notes/Issues related to diet recall:
    __________________________________________________________
    __________________________________________________________
    __________________________________________________________
    __________________________________________________________

*If applicable, it might be helpful to have your child's school menu in front of you to help recall meals and snacks consumed at school.
APPENDIX S: HEALTH HISTORY QUESTIONNAIRE (24 mg)
Supplemental Zinc in Early Adolescence

Health History Questionnaire

Surgery/Medication/Fracture History

1. Have you had any major medical procedures, surgeries and/or injuries in your lifetime?
   YES or NO; circle one
   □ If yes, please provide the details of each procedure, surgery or injury.

2. Have you ever gone through an extended period of time where you were bedridden or immobilized?
   YES or NO; circle one
   □ If yes, briefly explain the circumstances.
   □ If yes, how old were you and how long did this immobilization last?

3. Are you currently taking any medications either prescribed by a doctor or over-the-counter (self-prescribed)? YES or NO; circle one
   □ If yes, please list medications in the chart below.

<table>
<thead>
<tr>
<th>Name of medication</th>
<th>Dose amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

4. Has any member of your family been diagnosed with a medical condition related to obesity or osteoporosis? YES or NO; circle one
   □ If yes, briefly explain the circumstances.

5. Have you ever experienced a skeletal fracture in your lifetime? YES or NO; circle one
   □ If yes, list any fractures in your lifetime in the table below.

<table>
<thead>
<tr>
<th>Fracture Site</th>
<th>Cause</th>
<th>Age at the time of Fracture</th>
</tr>
</thead>
</table>
Other History

1. How would you rate your present health? _____Poor_____Fair_____Good_____Excellent

2. Do you smoke cigarettes now? YES or NO; circle one
   □ If yes, on the average, about how many cigarettes a day do you smoke now?
     □1-5, □6-14, □15-24, □25-35, □35 or more

3. Have you ever smoked cigarettes? YES or NO; circle one
   □ If yes, how long ago did you smoke? _______years
   □ If yes, on average, about how many cigarettes a day did you smoke?
     □1-5, □6-14, □15-24, □25-35, □35 or more

4. (If Female) Have you started your menstrual cycles? YES or NO; circle one
   □ If yes, at what age did you start your menstrual cycles? _________________
   □ If yes, are your menstrual cycles regular? YES or NO; circle one
     □ If no, how many cycles do you have per year?
       □≥9 cycles/year □between 4-8 cycles/year □≤3 cycles/year
     □ If no, long have your cycles been irregular? ________year(s)

5. (If Female) Are you currently taking birth control? YES or NO; circle one
   □ If yes, please describe in table below.

<table>
<thead>
<tr>
<th>Name of Birth Control</th>
<th>Dose</th>
<th>Start Date of Birth Control</th>
<th>End Date of Birth Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

   □ If yes, how old were you when you began using birth control pills? ________________
   □ If yes, how long have you been using birth control pills? ________________

6. (If Female) Have you ever stopped using birth control pills? YES or NO; circle one
   □ If yes, please describe in table below.

<table>
<thead>
<tr>
<th>Name of Birth Control</th>
<th>Start Date of Birth Control</th>
<th>End Date of Birth Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
7. Are you taking any nutritional supplements? YES or NO; circle one
   □ If yes, please describe in table below.

<table>
<thead>
<tr>
<th>Name of supplement</th>
<th>Dose amount</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
</tbody>
</table>

8. Are you currently dieting or on a special type of weight loss program (Weight Watchers, Atkins, self-regulating diet, etc.) YES or NO; circle one
   □ If yes, what is the name of the program? __________________________
   □ If yes, how long have you been dieting/on the program? ____________

9. Do you have any health problems that limit your physical activity? YES or NO; circle one
   □ If yes, briefly explain the circumstances.

   ___________________________________________________________

10. How many hours, on average, do you spend watching TV, or on the computer? ________ per day.
APPENDIX T: PHYSICAL ACTIVITY QUESTIONNAIRE (24 mg)
Physical Activity Questionnaire (Ages 8-14)

Subject ID #:______________________________  Age:___________

Sex:  M_____  F_______  Grade:___________

Teacher:______________________________

We are trying to find out about your level of physical activity from the last 7 days (in the last week). This includes sports or dance that make you sweat or make your legs feel tired, or games that make you breathe hard, like tag, skipping, running, climbing, and others.

Remember:
1. There are no right and wrong answers — this is not a test.
2. Please answer all the questions as honestly and accurately as you can — this is very important.

1. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark only one circle per row.)

<table>
<thead>
<tr>
<th>Activity</th>
<th>No</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7 times or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skipping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rowing/canoeing</td>
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<tr>
<td>In-line skating</td>
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<td></td>
</tr>
<tr>
<td>Tag</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking for exercise</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bicycling</td>
<td></td>
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<tr>
<td>Jogging or running</td>
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<tr>
<td>Aerobics</td>
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</tr>
<tr>
<td>Swimming</td>
<td></td>
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<tr>
<td>Baseball, softball</td>
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<tr>
<td>Dance</td>
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<td></td>
</tr>
<tr>
<td>Football</td>
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<tr>
<td>Badminton</td>
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<tr>
<td>Skateboarding</td>
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<tr>
<td>Soccer</td>
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<tr>
<td>Street hockey</td>
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<tr>
<td>Volleyball</td>
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<tr>
<td>Floor hockey</td>
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</tr>
<tr>
<td>Basketball</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ice skating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-country skiing</td>
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<tr>
<td>Ice hockey/hockey</td>
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<tr>
<td>Other:</td>
<td></td>
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</tbody>
</table>

_________________________________________  __________  __________  __________  __________
2. In the last 7 days, during your physical education (PE) classes, how often were you very active (playing hard, running, jumping, throwing)? (Check one only.)
   I don’t do PE ..................................... □ □
   Hardly ever ..................................... □ □
   Sometimes ..................................... □ □
   Quite often ..................................... □ □
   Always ......................................... □

3. In the last 7 days, what did you do most of the time at recess? (Check one only.)
   Sat down (talking, reading, doing schoolwork) ...........□
   Stood around or walked around .......................... □
   Ran or played a little bit ................................ □
   Ran around and played quite a bit ....................... □
   Ran and played hard most of the time ................. □

4. In the last 7 days, what did you normally do at lunch (besides eating lunch)? (Check one only.)
   Sat down (talking, reading, doing schoolwork) ...........□ □
   Stood around or walked around .......................... □ □
   Ran or played a little bit ................................ □ □
   Ran around and played quite a bit ....................... □ □
   Ran and played hard most of the time ................. □ □

5. In the last 7 days, on how many days right after school, did you do sports, dance, or play games in which you were very active? (Check one only.)
   None ........................................... □
   1 time last week ................................ □
   2 or 3 times last week ......................... □
   4 times last week .......................... □
   5 times last week ......................... □

6. In the last 7 days, on how many evenings did you do sports, dance, or play games in which you were very active? (Check one only.)
   None ........................................... □
   1 time last week ................................ □
   2 or 3 times last week ......................... □
   4 or 5 times last week ......................... □
   6 or 7 times last week ......................... □
7. **On the last weekend** how many times did you do sports, dance, or play games in which you were very active? (Check one only.)

   - None ........................................... □
   - 1 time ........................................ □
   - 2—3 times .................................... □
   - 4—5 times .................................... □
   - 6 or more times ............................ □

8. **Which one** of these following describes you best for the last 7 days? Read all five statements before deciding on the one answer that describes you.

   A. Almost all of my free time was spent doing things that involve little physical effort ...................................................... ... ... ... □
   B. Sometimes (1—2 times last week) did physical things in my free time (e.g., played sports, went running, swimming, biking, did aerobics) ...................................................... □
   C. Often (3—4 times last week) did physical things in my free time ...................................................... □ □
   D. Quite often (5—6 times last week) did physical things in my free time ...................................................... □ □
   E. Very often (7 or more times last week) did physical things in my free time ...................................................... □

9. Mark how often you did physical activity (like playing sports, games, doing dance, or any other physical activity) for each day last week.

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Little bit</th>
<th>Medium</th>
<th>Often</th>
<th>Very often</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
</tr>
<tr>
<td>Tuesday</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
</tr>
<tr>
<td>Wednesday</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
</tr>
<tr>
<td>Thursday</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
</tr>
<tr>
<td>Friday</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
</tr>
<tr>
<td>Saturday</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
</tr>
<tr>
<td>Sunday</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
<td>①</td>
</tr>
</tbody>
</table>

10. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

    - Yes ................................................................................... ①
    - No .................................................................................... ①

   If Yes, what prevented you?  ........................................................................
APPENDIX U: ANTHROPOMETRIC RECORDING SHEET (24 mg)
Supplemental Zinc in Early Adolescence

**Participant Information Sheet**

**Anthropometrics/DXA/pQCT**

<table>
<thead>
<tr>
<th>Subject ID:</th>
<th>Visit Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race/Ethnicity:</td>
<td>Sex:</td>
</tr>
<tr>
<td>DOB: Month</td>
<td>Day</td>
</tr>
<tr>
<td>Year</td>
<td></td>
</tr>
<tr>
<td>Weight (kg):</td>
<td>Measure 1 Measure 2</td>
</tr>
<tr>
<td></td>
<td>Average of 1 and 2</td>
</tr>
<tr>
<td>Height (cm):</td>
<td>Measure 1 Measure 2</td>
</tr>
<tr>
<td></td>
<td>Average of 1 and 2</td>
</tr>
<tr>
<td>Sitting Ht (cm):</td>
<td>Measure 1 Measure 2</td>
</tr>
<tr>
<td></td>
<td>Average of 1 and 2</td>
</tr>
<tr>
<td>Waist Cir (cm):</td>
<td>Measure 1 Measure 2</td>
</tr>
<tr>
<td></td>
<td>Average of 1 and 2</td>
</tr>
<tr>
<td>BMI (g/cm²):</td>
<td></td>
</tr>
<tr>
<td>Maturity Offset (years):</td>
<td></td>
</tr>
</tbody>
</table>

Non-Dominant Arm: R L circle one
Non-Dominant Leg:  R L circle one

---

**DXA operator use**

- WB
- Hip
- Non-Dominant Limb: R L
- AP Spine
- Radius

Scan date: 
Completed by: 

**pQCT operator use**

- Radius
- Tibia
- Non-Dominant Limb: R L

Scan date: 
Completed by: 

Initials of operator
APPENDIX V: PILL COUNT FORM (24 mg)
Pill Count Form

Subject ID#: __________
Interviewer: __________
Date of Interview: ______

Date: ____________________

Number of study pills (zinc or placebo) left: ________________

Any complaint of side effects? Yes  No

If yes, please report:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
APPENDIX W: ADVERSE EVENT FORM (24 mg)
Response to Supplementation Questionnaire

Subject ID#: ____________
Interviewer: ______________
Date of Interview: ________

How is your overall health?

How do you feel about the supplements you have been taking?
APPENDIX X: FLOW CHART FOR RECRUITMENT (24 mg)
24 mg Recruitment Data

Excluded 27

Total Assessed for Eligibility 86

Declined 20

SUBJECTS ENROLLED 39

Allocated to Zinc group (n=21)

Adverse event (n=1)

Completed the trial (n=20)

Withdrawn due to blood draw (n=1)

Allocated to Placebo group (n=18)

Completed the trial (n=18)

SUBJECTS RETAINED 38
APPENDIX Y: TABLE OF BASELINE DESCRIPTIVE CHARACTERISTICS (24mg)
### Baseline Descriptive Characteristics of Participants (24 mg)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=18)</th>
<th>Zn (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>±SD</td>
</tr>
<tr>
<td>Age, y</td>
<td>11.0</td>
<td>1.38</td>
</tr>
<tr>
<td>Tanner Stage, 1-5</td>
<td>2.11</td>
<td>0.323</td>
</tr>
<tr>
<td>Height, cm</td>
<td>145</td>
<td>11.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>37.0</td>
<td>7.96</td>
</tr>
<tr>
<td>BMI-for-age percentile, %</td>
<td>47.0</td>
<td>25.1</td>
</tr>
<tr>
<td>FFST mass, kg</td>
<td>27.2</td>
<td>5.62</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>9.90</td>
<td>3.01</td>
</tr>
<tr>
<td>Percent fat, %</td>
<td>26.6</td>
<td>4.11</td>
</tr>
<tr>
<td>Total body BMC, g</td>
<td>1227</td>
<td>238</td>
</tr>
<tr>
<td>Total body BMD, g/cm²</td>
<td>0.853</td>
<td>0.0806</td>
</tr>
<tr>
<td>Dietary Zn, mg/dl</td>
<td>8.03</td>
<td>1.35</td>
</tr>
<tr>
<td>Plasma zinc, ug/dl</td>
<td>9.03</td>
<td>4.52</td>
</tr>
<tr>
<td>IGF-I, ug/dl</td>
<td>325</td>
<td>144</td>
</tr>
<tr>
<td>P1NP, ug/l</td>
<td>809</td>
<td>280</td>
</tr>
<tr>
<td>ICTP, μg/l</td>
<td>41.1</td>
<td>8.66</td>
</tr>
</tbody>
</table>

*p < 0.05 Zn group significantly than controls
APPENDIX Z: PERCENT CHANGE OVER 4 WEEKS GRAPH (24 mg)
Zn (zinc), P1NP (procollagen type 1 amino-terminal propeptide), ICTP (cross-linked carboxyterminal telopeptide of type I collagen), IGF-I (insulin like growth factor-I)

*p<0.05 after removing outliers

**p<0.05 after removing outliers and controlling for TB BMD