DEHYDROEPIANDROSTERONE SULFATE, GROWTH AND PLASMA ZINC IN ADOLESCENT MALES

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Dedicated to the memory of

my father

Dr. Charles Richard Bender

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AND PLASMA ZINC IN ADOLESCENT

MALES

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CHAPTER I

INTRODUCTION

Growth rates during adolescence are second only to that during fetal development and early infancy. Yet information on which recommended nutrient intakes are based stems from isolated studies with few subjects. Little information exists concerning the relationships between growth, maturation, and blood lipids which may affect cardiovascular health later in life. Likewise, only a few studies have been conducted which investigated healthy adolescents and mineral status.

Significance of the Problem

In 1986 the United States Department of Agriculture (USDA) reported to Congress a comprehensive plan for human nutrition research (1). Included in this plan was increased information on nutritional requirements for adolescents. A special need for knowledge about trace mineral needs in adolescent males was stated due to their rapid growth rate.

While USDA emphasized lipids in aging as another concern for nutrition research, the concern for cardiovascular health also existed for adolescents. If there were a relationship between blood lipids in

adolescents and lipid levels in these individuals as they aged, then those at risk needed to be identified early.

Mineral interactions are known to exist. For example, iron and zinc competition was identified in several studies and clearly demonstrated by Solomons (2,3). Copper and zinc may also compete for absorption and/or utilization. Since these minerals may affect lipid metabolism, it seemed important to investigate plasma lipids in respect to maturation and trace minerals.

The essentiality of zinc for animals was demonstrated in the rat in 1934 (4). Essentiality has since been confirmed in many other species. However, due to the wide distribution of the element, zinc was not considered likely to be lacking in the diet of humans. Nevertheless, zinc deficiency was first suspected in humans in Iran in 1961 (5). This deficiency was later confirmed in controlled studies in Egypt (6,7). These severe zinc deficiencies were characterized by dwarfism, severe anemia, hypogonadism, hepatosplenomegaly, mental lethargy and geophagia. The initial studies of zinc deficiency in Iran and Egypt included only male subjects; however, zinc deficiency was later discovered in females as well (8). It is possible, however, that males are more subject to zinc deficiency than females since zinc seems to be preferentially sequestered in the male genital tract while it is not in the female genital tract. In addition studies in other parts of the world have

shown that indices suggestive of possible zinc deficiency are more common in male children than in female (9-11).

Zinc is known to be required for DNA and RNA synthesis and, thus, is involved in growth. Since little has been reported on zinc status in adolescent males, there is a need to determine not only zinc status but also maturation and growth in this age group. There is no single biochemical measure of zinc status which is currently accepted as indicative of body stores. Nevertheless, plasma zinc is commonly reported by those researching zinc status and mineral interactions. Therefore, there is a need to investigate the relationships between growth, maturation and plasma zinc.

This study described the nutritional status of adolescent males with emphasis on plasma zinc and indicators of maturation and growth. Plasma cholesterol and hemoglobin levels were examined in relationship to growth, maturation and plasma zinc. Special emphasis was given to identifying markers of maturation status and to relationships between anthropometric measurements and maturation status.

Objectives

The following research objectives were developed for the proposed study:

1. to determine the nutritional status of adolescent males based on age;

2. to determine the growth achievement of adolescent males in relation to maturation status;
3. to determine the relationship between plasma zinc and measures of maturation;
4. to determine the relationship between maturation and plasma lipids; and
5. to determine the relationship between zinc intakes and plasma zinc, growth, maturation, and blood lipids.

Hypotheses

The following hypotheses were developed for this study:

1. there will be no statistically significant differences by age in nutritional status measures among healthy male adolescents;
2. there will be no statistically significant differences in growth measures relative to maturational status among healthy male adolescents;
3. there will be no statistically significant differences in plasma zinc based on maturation among healthy male adolescents;
4. there will be no statistically significant differences in plasma lipids based on maturation among healthy male adolescents; and
5. there will be no statistically significant relationship between dietary zinc and measures of

plasma zinc, growth, maturation and plasma lipids among healthy male adolescents.

Limitations

This study is not generalizable since the subjects were volunteers, males only, and restricted in geographical area. The number of subjects was a further limitation based on time constraints of the researcher and economic factors. Measurement of plasma zinc was limited by available methodology.

Definitions

The following terms were defined for the purposes of this study:

1. Dehydroepiandrosterone sulfate - an adrenal androgenic hormone which has been shown to increase progressively with age and maturation and which has little diurnal variation.
2. Anthropometric measurements - physical measurements of the body. In this study these include height, weight, midarm circumference, and triceps skinfolds.
3. Health - freedom from disease both acute and chronic.
4. Geophagia - the practice of eating clay.

CHAPTER II

REVIEW OF LITERATURE

This chapter includes a brief review of nutritional assessment methodology focusing on the adolescent. Nutritional needs for maturation and growth are discussed. Zinc, a mineral known to be essential for maturation, is emphasized. A secondary emphasis is the status of blood lipids in adolescents related to growth and maturation. This concern existed due to possible relationships to cardiovascular disease later in life. A brief review of dietary intake methodology is included.

Measures of Nutritional Status

Nutritional status assessment in the complete sense includes measures of all factors which may affect the biological functioning of the individual. These measures would necessarily include anthropometric measures of growth and body composition, clinical examination for overt signs of nutritional deficiency or excess, biochemical measures of the nutrient or nutrients of concern, as well as measures of food consumption. Only in studies in which several investigators are collaborating can all of these components of nutritional assessment be undertaken due to time,

expertise and financial limitations. Thus, in each study some portions may not be addressed.

Adolescence is a time of many physiological changes. Hormonal changes occur affecting growth and development. These changes are not instantaneous nor do they occur at a uniform time in all individuals. Therefore, in assessing individuals during adolescence, maturation must also be considered.

Growth in Adolescence

During adolescence growth rates for height vary greatly. Therefore, using growth curves not associated with maturation status can classify youth incorrectly. Growth curves have been developed for North American children which not only consider those with average maturation rates but also those in whom maturation occurs early or late (12). For males these growth and height velocity charts were based on typical growth of individuals from 11 to 19 years. Prior to age eleven National Center for Health Statistics (NCHS) values were used. Data were grafted from NCHS values for prepubertal height, height at adulthood, and height at peak height velocity to the slope and height of the typical adolescent male. Rather than use previously published curve fitted data, these values were based on those empirically derived. The peak height velocity charts were useful in longitudinal studies and for clinicians but did not provide information on maturation without accurate height

measurement from previous years. Tanner maturation standards (described in Appendix A) were used in these charts so that those individuals at the 97 centile for height are "early maturers" and those below the third centile are "late maturers."

Baumgartner et al. (13) developed incremental growth tables based on longitudinal data from the Fels Research Institute. The sample size was small and localized consisting of approximately 818 subjects in southwestern Ohio. They produced tables from age six-months in intervals of six months for weight and stature increases without assessment of maturation status. Values were given for growth rates based on percentiles and $\pm$ 1 or 2 standard deviations of the mean. These data were useful in the clinical context for determining satisfactory growth rates during childhood. They did not provide information on actual heights by percentiles.

Growth and Puberty

Mills et al. (14) studied the nutritional status and growth of 78 boys from age 6 months to 14 years. Both three day food records and anthropometric measures were taken at ages 1, 3, 6, 9, 12, and 14 years. Stage of puberty at age 14 was not related to intake of any dietary factor at any age. However, those with more developed maturation as measured by Tanner stages were heavier as early as age six months than those who matured later. At many ages skinfolds

were also positively related to more advanced maturation, but significance of the relationship existed at only a few points. Muscle mass development as estimated by calculation of cross-sectional muscle area in seven of the nine time periods was also significantly related to timing of puberty. Weight was a better predictor of pubertal stage than was height. In this study muscle mass was the strongest predictor of pubescence. These researchers hypothesized, because early weight, lean body mass, and pubic hair development were particularly strongly related, that an adrenal hormonal factor might be responsible for the timing of puberty.

Wilson et al. (15) also developed growth curves for adolescents based on data collected during the third cycle of the Health Examination Survey (1966-1967). These curves used actual heights attained, age and a sexual maturity index (SMI) to set the pattern of curves for those maturing at average times. For a clinician or researcher to use the curves, however, both pubic hair and genital development must be assessed. These curves could then be used to predict adult height.

Body Composition

Cronk and Roche (16) developed race and sex-specific reference values for triceps and subscapular skinfolds and weight/stature for both males and females from ages 6 to 50.9 years. The data for these references were derived from

the first National Health and Nutrition Examination Survey (NHANES-I). Using these data, the researchers concluded that the best single predictor of percent body fatness for males ages 6 to 18 was subscapular skinfolds. Nevertheless, these researchers recommended that in boys for whom there is need to know body fatness both triceps and subscapular fatfolds be measured and the weight/stature squared (W/S^2) should be calculated as well.

Siervogel et al. (17) compared data from the Denver Growth Study and the Melbourne Growth Study to determine which measurements were the best predictors of body fatness. The Denver data consisted of skinfold measurements from 10 body sites as well as radiographically determined subcutaneous fat while the Melbourne data had only skinfold values. Both of these were serial studies so that values for the same child might appear in more than one age group. These researchers found that abdominal and triceps skinfolds predicted body fat to approximately the same degree in prepubertal males. Prepubertal age was arbitrarily defined as 4 - 12 years. In the pubertal males, defined as ages 13 - 16, pectoral and triceps skinfolds predicted body fatness to the same degree. Thus, in males through age 16 triceps were a satisfactory, accessible and easily measured skinfold which predicted body fatness with a correlation from r=0.69 to r=0.79.

When the correlations of each site were calculated with each other site, Siervogel and colleagues (17) found that

site to site variation was great during the prepubertal years in both sexes. However, as the individual approached puberty the subcutaneous fat layers tended to become more evenly distributed. That is, if the skinfold thickness were small at one site the tendency was to be small at all sites. This similarity of fat thickness throughout the body tended to disappear after puberty and into the mid-twenties.

In analyzing to determine the relationship between body fatness and the available anthropometric measures, Siervogel and coworkers (17) found that the skinfolds were strongly correlated to weight but were more strongly related to W/S^2. There were no significant correlations between skinfolds and height. Based on their data from Denver and Melbourne, these researchers stated that triceps was the single best measurement site in boys through age 16. They cautioned that age and gender should be considered when selecting a site to be representative of body fatness.

Frishancho (18) also used NHANES-I data to determine norms for upper limb fat and muscle areas. Tables were developed using data from white subjects only. These norms do not represent the values that could be classified as desirable, but rather these values were the means and distributions of values found in the national probability sample. These values indicated that with increasing age the increase in upper arm fat was more clearly shown when fat was expressed as fat area rather than skinfold thickness. Frisancho suggested that both triceps and arm circumference

measures be converted into fat area and muscle area for a more adequate description of an individual's energy and protein reserves.

Hemoglobin in Adolescents

Although growth curves have been developed for heights and weights of children for some time, Dallman and Siimes (19) recently developed percentile curves for hemoglobin levels in white infants and children from samples totaling 2314 children collected in the United States (3 groups) and Finland (2 groups). All these children lived near sea level and in all but one group additional hematological data were collected to eliminate children with evidence of iron deficiency, thalassemia minor and/or hemoglobinopathy. The curves include the third, fiftieth, and ninety-seventh percentiles of non-indigent subjects from birth through age sixteen. Tabular data were also presented on the median (50th percentile) and lower limits (3rd percentile) by age and gender groups. These curves and table showed a gradual increase in hemoglobin concentration throughout childhood.

Daniel (20) has reported changes in hematocrit percent related to pubertal changes. The subjects were adolescents 11 to 20 years from low income families. Hematocrit was related to Tanner sexual maturity staging but not to chronological age in males. This relationship was not found in females. After maturation stage two a significant increase in hematocrit occurred which remained linear

throughout the ages studied. For black males the slope remained lower throughout the maturity stages covered and did not have the flat portion found in whites between stages one and two.

Iron nutriture data from NHANES-II were summarized by an expert group from the Life Sciences Research Office of FASEB (21). The conclusion reached from these analyses was that there currently was no single indicator which was diagnostic of iron deficiency. However, they found evidence of changes in iron status indicators based on age particularly in infancy, childhood and adolescence. Several models using biochemical indicators of tissue iron were developed to predict iron status.

This group also compared the association between low hemoglobin concentration and abnormal values for the various iron status indicators in whites. They found an increase in occurrence of low hemoglobin values in individuals identified as having abnormal serum ferritin, mean corpuscular volume, transferrin saturation and erythrocyte protoporphyrin. The three iron status models developed required two of three indicator values to be abnormal before identification of the individual as iron deficient. When comparing those identified by the iron status models, they found few individuals had a severe enough deficiency to be reflected in low hemoglobin concentrations. Thus hemoglobin alone missed those individuals with marginal deficiency or inadequate stores of iron.

Conclusions concerning the iron status of the healthy non-institutionalized population in the United States included that males aged 11 to 14 years had a high prevalence of iron deficiency indicators compared to all other age and gender groups including females of the same ages. They also concluded that there were discrepancies for all three of the models in estimating the prevalence of iron deficiency. In adolescent males occurrence of iron deficiency was predicted to range from 3.5% to 12.1% depending on the model used. This variation between models supported the need for more than one biochemical indicator for defining iron status of individuals or populations.

There are changes in adolescence that affect need for dietary iron which if unmet may result in iron deficiency. Dallman (22) reported in a review article that, in order to maintain a constant hemoglobin concentration, male adolescents must increase their net body iron level by 300 mg in the year of peak growth. Additional iron was reported to be necessary because the concentration of hemoglobin also increases between 5 and 10 g/L during these years.

Others have reported increased hemoglobin levels during the adolescent years. Seoane et al. (23) studied hemoglobin, hematocrit, and serum ferritin levels in 574 Canadian adolescents 10 to 18 years old related to dietary iron intake from three day food records. No abnormal hemoglobin or hematocrit values were found. The group (two

year age interval groups) means for serum ferritin were all within the normal levels but were low. The hemoglobin concentration increased significantly ($p<0.01$) from ages 10 to 18 years in males. No relationship was found between dietary iron intakes and hemoglobin levels. The low serum ferritin concentrations suggest that iron deficiency existed in the adolescents, but this was not discussed.

As part of the Bogalusa Heart Study, Nicklas et al. (24) examined hemoglobin concentration and erythropoietic nutrients in adolescents and preadolescents in a racially mixed community. Duplicate 24 hour dietary recall records were used to assess the nutrient intakes of these children. They found higher hemoglobin concentrations in males 15 years old than in 10 year old males. Using regression analysis they found that individual dietary components (heme iron, non-heme iron, copper, zinc, folacin, and vitamins C, B_6, B_{12}, and E) contributed little to the prediction of hemoglobin levels.

Looker et al. (25) examined dietary intakes and iron status indicators of vitamin-mineral supplement users and nonusers in individuals 1 - 19 years. The data source was NHANES-II. Only individuals using supplements daily were classified as users; nonusers were individuals never using supplements. Occasional users were excluded from the data subset. Data did not include the amount of iron in the supplements. No significant differences were found between users and nonusers for hemoglobin, mean corpuscular volume,

erythrocyte protoporphrin, or transferrin saturation in males 11 - 19 years. They also reported few dietary differences between users and non users.

From these reports it seemed that adolescent males may be a commonly overlooked group for insufficiency of iron intake. There appeared to be increases in hemoglobin concentration occurring with puberty and little relationship of reported nutrient or supplement intakes and measures of iron status.

Hormonal Changes and Puberty

Physiological changes occur in maturation before the actual occurrence of puberty. Among these changes are increases in secretion of adrenal hormones. This period is defined as adrenarche. While the adrenals secrete several hormones, dehydroepiandrosterone (DHA) and dehydroepiandrosterone sulfate (DHEAS) have been described as the most useful markers because the adrenals account for over 90% of the adult plasma levels of these hormones (26). The increase in DHEAS is seen as early as age 7, much earlier than increases in gonadal hormones.

Katz et al. (27) examined 208 Black adolescent males who were participating in the Philadelphia Blood Pressure Project. There were 5 age groups nearly evenly divided from age 12 to 16. They tested the hypothesis that earlier maturation was associated with increased adrenal androgens (measured only as DHEAS) independent of gonadal maturation

(measured by testosterone level). They found that levels of DHEAS were consistently associated with adiposity regardless of stage of gonadal maturation. A consistent and significant pattern of increased DHEAS was associated with an increased level of adiposity throughout adolescence. A significant relationship was also found between DHEAS and bone age. They concluded that a prepubertal increase in DHEAS accompanied by excess adiposity were associated with earlier onset of sexual maturation.

Katz et al. (28) also examined the relationship between levels of DHEAS and blood pressure as part of the Philadelphia Blood Pressure Project. When the subjects were grouped into tertiles of DHEAS by each age group, the high DHEAS groups (DHEAS concentration greater than one standard deviation above the mean concentration for each age) were found to be significantly different from the lower two tertiles in skeletal age, weight, height, and W/S^2. There was no difference in blood pressure based on DHEAS level in males. Blood pressure variations in males were associated with the interaction of W/S^2 and serum DHEAS.

Lee and Migeon (29) conducted a longitudinal study correlating the concentrations of eight steroid hormones in males with other physiological events associated with puberty, including age. In the group of subjects were 11 boys who had no signs of puberty as well as 43 boys in various stages of puberty. Physical examinations and serum studies were conducted at intervals of six months. Data

from the boys followed throughout puberty showed that DHEAS rose only after Tanner's stage one of sexual maturation both for genital and pubic hair staging and that significant changes in DHEAS concentration occurred between stage one and two. These changes occurred when DHEAS rose from 500 to 750 ng/mL (1.3 - 2.0 umol/L). Comparisons of the pubertal events of genital development and appearance of pubic and axillary hair with the various hormones studied showed that DHEAS levels increased with the appearance of both axillary hair and growth of the genitals. In this study, the increase in serum concentration of DHEAS was associated with the onset of puberty.

Hopper and Yen (30) also studied concentrations of DHEAS during puberty. Their data showed a rise in DHEAS levels in males beginning at age eight with a two-fold increase in the level between eight and adulthood. However, their data do not include any observations of development of secondary sex characteristics.

Babalola and Ellis (31) developed reference charts for serum DHEAS in a normal pediatric population. They used 274 children between the ages of 1 month to 18.7 years who were hospitalized for minor surgery. Of these 274 children 131 were males. Only those who were clinically normal were included in the study. From the patients' records, Tanner pubertal staging was determined when possible. The data were presented in figures which were estimated to include 95% of the population. There was wide variation in serum

levels of DHEAS in all of the Tanner stages, but there was no overlap between Tanner stage one and two or between stage three and four in mean values and one standard error of the mean. Considerable variation was seen not only with maturation but also with chronological age; values <3 umol/L were seen in children 8 - 9 years of age and were considered normal. However, during the prepuberty years of 10 - 12 values of <6 umol/L were listed as normal. Furthermore, between the ages of 13 to 16 normal ranges were 3 - 13 umol/L. Apparently, variation occurred in maturation and chronological age as identified by the adrenal androgen DHEAS.

Forti et al. (32) studied the peripheral and testicular levels of testosterone and DHEAS in 29 boys being treated for undescended testis, inguinal hernia, or variciole. Of these, 18 were determined to be prepubertal. The subjects were grouped into two groups -- either prepubertal (Tanner stage 1) or pubertal (Tanner stage 2-4). Significant differences were found between the two groups for DHEAS but no differences were seen within groups for spermatic or peripheral levels of either hormone. Testosterone levels were significantly higher in spermatic serum than in peripheral in both groups with the levels being significantly higher in the pubertal group than in the prepubertal group. There was no relationship between spermatic testosterone and DHEAS in either group. Their

data neither supported nor ruled out the possibility of testicular production of DHEAS.

Reiter et al. (33) studied secretion of DHEAS from infancy through adolescence. Preadolescent children were grouped into 2-year chronological age intervals. DHEAS levels rose significantly within the 8-10 age group and the 10-12 age group and again for the 12-14 age group. A significant increase was again found for the 14-16 age group. When the data were evaluated according to skeletal maturation, the same distinctions were found. This group did not find significant differences between males and females for DHEAS although the mean values for males tended to be higher after age 10 than those values in females. This study included children with precocious adrenarche; the levels of DHEAS found in these children compared with the normal children support the hypothesis that DHEAS was involved in initiating the growth of pubic and axillary hair. In the patients with chronic adrenocorticotrophic hormone (ACTH) deficiency, there was no observable secretion of DHEAS. When ACTH was administered in pharmacologic doses, DHEAS levels reached normal prepubertal and pubertal levels within four hours. These results supported the hypothesis that secretion of DHEAS is influenced by ACTH.

Hormone Status and Measures of Zinc Status

Severe zinc deficiency is known to cause delayed sexual maturation, however, the effects of marginal deprivation during the adolescent growth spurt are not known. Golub et al. (34) used Rhesus monkeys as a model to study mild zinc deprivation during adolescence because these monkeys show similarities with humans in regard to the adolescent growth spurt and to the physiological changes surrounding it. Ten male monkeys from dams fed either zinc deficient or control diets from conception to weaning were evaluated for growth, sexual maturation, testosterone level, food intake, plasma zinc and red cell zinc as well as presence of signs of more severe zinc deficiency such as alopecia and dermatitis. Between weaning and adolescence these monkeys were fed ad libitum either a zinc deficient or control diet. At 30 months and continuing until 42 months the animals were pair fed with the amount of diet supplied to controls based on the amount consumed by the experimental animals. Diets were formulated to contain 4 mg Zn/kg for the experimental group and 100 mg/kg for the controls. The effects of zinc deficiency on growth were significant delays in onset of weight gain and sitting height and decrease in subcutaneous fat. Sexual maturation did not differ in the groups although there was great individual variation. Sexual maturation in monkeys is thought to occur between four and five years and did not occur in this group by the end of the study at 42

months. Plasma zinc levels were significantly lower in the test group at the beginning of the study than in the controls, but by the end of the study decreases had occurred in the control group leading to no significant differences in the groups at 42 months. Red blood cell zinc declined about 30% in both groups between months 30 and 36 leading to a significantly greater decline in the experimental group from ages 33 to 42 months. Because the animals were pair fed, differences that might occur due to amount of food consumed were eliminated. As has been documented elsewhere, plasma zinc levels tended to decline as the growth spurt progressed.

Castro-Magana et al. (35) studied serum zinc and response to methyltestosterone administration in 369 boys with constitutional growth delay or familial short stature. Before treatment with methyltestosterone subjects with constitutional growth delay had significantly lower serum zinc levels than those boys with familial short stature. After treatment serum and hair zinc levels increased; the increase in those with constitutional growth delay was highly significant ($p<0.001$).

When Collipp et al. (36) subsequently treated two children, who were deficient in growth hormone and who had low hair zinc concentrations, with zinc supplements, both children had significant increases in growth which persisted at least two years. The zinc doses consisted of 220 mg $ZnSO_4$ per day for two years. No growth hormone was given,

but after the oral zinc treatment the hormone levels were normal. While this report was based on only two children, one male and one female, it suggested the need for investigation of zinc status in growth deficient children before further treatment.

This research team also treated a group of children between the ages of 7 and 13 who had low hair zinc and short stature but no other apparent abnormality (37). All had been of normal birth weight but present bone age was at least 2 years less than their chronological age. One group was treated with 50 mg of elemental zinc per day for two months followed by 50 mg per week for ten months. The other group received 100 mg of elemental zinc daily for one year. No reference was made to verification of compliance. Following treatment the children were again divided into two groups--those who grew more than 6 cm/yr and those who grew less than 6 cm/yr. The children who grew more than 6 cm/yr had significant increases in hair, serum, and urine zinc within 2 months of treatment while those who grew less did not. In both groups of children hormone levels of serum testosterone, somatomedin C and growth hormone at insulin peak increased following zinc supplementation. There was a different response in children who took the two different size doses of zinc; those taking the 50 mg dose grew less and had lower hair zinc levels than those receiving the 100 mg dose. In addition, the high zinc dose group also had lower serum copper levels after treatment than did those

receiving the 50 mg zinc dose. While no overt signs of copper deficiency were observed, the report did not extend beyond the one year supplementation trial. Again, interrelationships between hormones involved in growth and zinc were shown; the mechanisms for those interrelationships were not revealed.

Cheruvanky et al. (38) reported the results of an intervention trial in children with growth hormone deficiency treated with zinc supplements. They compared hair, serum, and urinary zinc with growth rate. These children were treated with growth hormone for three months with no zinc supplements followed by the hormone therapy with the addition of 50 mg zinc per day. Hormone treatment alone increased hair zinc levels and decreased urinary zinc significantly but there was no significant change in serum zinc. Zinc administration in addition to the hormone therapy significantly increased growth velocity over the velocity from hormone therapy alone. No report of effect of supplemental zinc on serum zinc was given. These researchers concluded that androgens were involved in the metabolism of zinc and that the effect of these anabolic hormones was mediated through their action on zinc metabolism.

Zinc Deficiency in Children

Zinc deficiency and marginal zinc deficiency have been reported both in the United States and in other countries.

Classical studies eliciting the characteristics of zinc deficiency originated in the Middle East. The first such report was from the Nemazee Hospital in Iran (5). Prasad et al. reported what appeared to be undernourished males with no sign of intestinal parasites nor blood loss whose symptoms included severe iron deficiency anemia, hepatosplenomegaly, short stature and hypogonadism. Iron supplements corrected the anemia and decreased the liver enlargement; this treatment also resulted in beginning of development of the genitalia. The common characteristics of the eleven subjects' diets were that they consisted primarily of unleavened wheat bread with little meat or dairy products and fruits and vegetables occasionally in the growing season. The diets were notably deficient in animal protein. Geophagia of long duration was noted in all but one subject; that subject may also have consumed clay but was not asked. Serum zinc was determined in only one subject; the value was low--71 ug/dL. While zinc deficiency was not confirmed in this early study, it was hypothesized to be the factor responsible for the symptoms.

In 1963 Prasad et al. (6) published a report on further studies on males with the syndrome of hepatosplenomegaly, anemia, dwarfism and hypogonadism. The subjects for this report were from villages near Cairo, Egypt. Biochemical tests led to the conclusion that these subjects were zinc deficient. This group was not treated with zinc supplements to confirm the diagnosis.

In Iran, Eminians and colleagues (39) reported a syndrome similar to nutritional dwarfism with other associated symptoms of anemia, hepatosplenomegaly, and growth retardation. The purpose of this study was to seek preadolescent children who had the hypothesized symptoms of zinc deficiency. Hospitalized youth with the symptoms of anemia, hepatomegaly, splenomegaly or growth retardation not explained by other diseases comprised the sample. Biochemically, these children had lower plasma zinc, hair zinc, and red blood cell zinc than normal subjects in the same village and other suburban children. While their specific diets were not studied, they were known to contain high levels of phytates and fiber which bind zinc. Clinical evidence of zinc deficiency was found more often in males than in females and more often in village males than urban males.

Zinc deficiency was later confirmed in females by Halsted and associates (8). In this study male and female patients were randomly assigned to one of two groups: placebo and a nutritious diet or zinc supplemented and the good diet. A third group was formed from those receiving the placebo by replacing the placebo with the zinc supplement after six months of initial treatment and continuing the diet. Those who were anemic also received an iron supplement, which was tested to be low in zinc, for the length of time required to relieve the anemia.

The two female subjects who were included had symptoms similar to those of the males including sexual immaturity. Thus, zinc deficiency is known to affect both males and females but seems to be more prevalent in males.
The good diet and treatment for anemia resulted in slow growth increases but no change in sexual immaturity. Within six weeks of adding zinc supplements (27 mg elemental zinc) overt signs of puberty were present. In addition, growth rate markedly increased with the zinc supplements.

Because zinc is preferentially sequestered in the male genital tract but not in the female, it has been suggested that either a greater need for or a need for greater retention of zinc existed in males. Several studies have found signs of overt or marginal zinc deficiency with greater frequency in males than in females (9-11,39).

In the United States marginal zinc deficiency has been observed in young children. In 1972 Hambidge et al. (40) reported that some apparently normal healthy young children had very low levels of zinc in hair (<70 ppm). Nine of these ten children also were below the tenth percentile for height and/or weight. Five of the six children when tested for taste acuity had significantly higher taste thresholds than did the controls with hair zinc at normal adult levels (>70ppm). Retesting following one to three months of treatment with 1-2 mg $ZnSO_4$/kg body weight resulted in normal taste acuity and substantial increases in hair zinc levels.

This first report of suspected zinc deficiency in young apparently healthy children in the United States led to further investigations in children both in impoverished and middle income families. In the mid 1970's Hambidge et al. (41) selected children enrolled in a Head Start program who were below the tenth percentile on Iowa Growth Grids. Hair and plasma were analyzed for zinc and compared with children from middle income homes attending a private preschool. The subjects were not fasting when blood was drawn but were at least 2 hours postprandial. Plasma zinc was low (<68 ug/dL) in about one third of the Head Start subjects but only low in 3.8% of the middle income children. No supplementation studies were done with these children, but the authors concluded that poor zinc nutrition might be common in low income children.

Because low income children, many of whom were of Mexican descent, were shown to have symptoms characteristic of zinc deficiency, Chase et al. (42) studied the relationship between vitamin A and zinc concentrations in Mexican-American migrant children who were growth retarded. Growth retardation determined as less than the third percentile in either height, weight or head circumference was the criterion for participation. Fasting blood samples were obtained for determination of serum vitamin A and serum zinc as well as other biochemical measures. No significant correlations were found among serum vitamin A, serum zinc, hair zinc or height. However, mean values for serum vitamin

A, zinc and hair zinc were lower in the migrant children than in the middle income controls from a previous study. A later study (11) paired low income Mexican-American preschool children for a 12 month zinc supplementation study. All subjects and controls were below the tenth percentile for height for age. Selection was further limited by requiring a dietary intake of <2/3 of the RDA for zinc, hair zinc <105 ug/g, or plasma zinc <68 ug/dL. Fifty-seven subjects were thus initially recruited for the study. Experimental subjects received a total of 10 mg zinc per day in divided doses while the controls received placebos indistinguishable from the zinc supplements. Results showed significant increases in height velocity in the experimental group primarily as a result of the increases in the males. Mean plasma zinc did not increase in the experimental group but significant increases in hair zinc were found in both the experimental and control groups. There were no significant correlations between hair zinc and plasma zinc. Because only the male children had significant increases in height when given zinc supplements, the possibility of higher zinc needs for males than for females was supported.

Before zinc fortification of infant formulas was a common practice, Walravens and Hambidge (9) investigated the effect on growth, hair zinc and plasma zinc of a zinc fortified formula. The study design was a double blind control protocol with the unsupplemented formula containing 1.8 mg/L zinc and the supplemented formula containing

5.8 mg/L. Results were significantly different by six months; the supplemented males were significantly longer and heavier than the control males. No significant differences were found between supplemented and control females. At three months both male and female supplemented infants had significantly higher plasma zinc than the controls, but by six months the difference was significant only for male infants. No significant differences were found between the groups in hair zinc concentration during the study.

Smit Vanderkooy and Gibson (43) recently reported food consumption patterns of Canadian preschool children in whom zinc and growth status were assessed. Average intakes of protein, calcium, iron, and zinc for both males and females were above the Canadian Recommended Nutrient Intakes; there were significant correlations between dietary zinc and both energy and protein intakes. For males only, significant correlations were found between hair zinc and height for age percentiles; those with hair zinc <70 ug/g were significantly shorter than those with hair zinc >70 ug/g. Boys with either low hair zinc or retarded growth (group A) consumed significantly more calcium than did the other males; no differences were seen for energy, protein or any other nutrients between these groups. However, the boys in group A consumed significantly less meat, fish, and poultry and more milk and dairy products and, therefore, consumed zinc from different food groups than did those boys with either higher hair zinc or greater growth achievement.

Gibson et al. (44) later examined the effect of zinc supplementation on young boys (mean age 76.7 months) who were at or below the 15th percentile for height and whose parents were >25th percentile for height. Based on the earlier study which showed evidence of zinc deficiency more commonly in males, this group documented the suspected zinc deficiency in a double-blind pair-matched 12 month study. All subjects (n=60) were Caucasian, apparently healthy, and full term with appropriate weight at birth. Subjects were tested, measured, and hair and blood samples were taken at the beginning, at 6 months and at the 12 month completion. All blood samples were obtained from fasting subjects; compliance in consuming the 10 mg zinc supplement or placebo was monitored at 6-week intervals. Weighed 5 day food records were kept at the beginning and end of the study. Three day records were kept at midpoint. Vitamin-mineral supplements were recorded although parents discontinued the use of iron supplements during the study. There were no significant differences at 6 months or 12 months between the supplemented and placebo groups for any of the measures examined: changes in weight for age, height for age, weight for height Z scores, taste acuity, hair zinc, serum zinc or copper, or ceruloplasmin. When the subjects were grouped according to initial hair zinc levels, no differences were seen at initial screening except that mean weight of the boys with low hair zinc was significantly less than those with "normal" hair zinc when height was considered a

covariate. No significant differences were seen in dietary intakes although those with low hair zinc tended to consume less energy nutrients and more calcium and phosphorus. Initial mean intakes of calcium, iron, zinc and protein were >100% of the Canadian Recommended Nutrient Intakes with the exception of calcium in the high hair zinc group. Those boys with low hair zinc had significantly higher initial mean recognition thresholds for salt than did boys with high hair zinc although at the end of the study there were no significant changes in median taste thresholds for the low or high hair zinc groups according to zinc supplementation. By the end of the study boys with initial low hair zinc who received the zinc supplement had significantly greater changes in height for age Z scores than those boys receiving the placebo. Results showed that only boys with low height percentiles, low hair zinc and hypogeusia responded significantly to the effect of zinc supplementation by increases in height for age.

Hambidge et al. (45) used a zinc fortified breakfast cereal to study the effects of zinc supplementation in 93 middle income children enrolled in a private preschool and kindergarten in Denver. A specially formulated zinc supplemented breakfast cereal was given to families in the experimental group; control group families received cereals as well which were unsupplemented. Supplementation was sufficient to provide 25% RDA for zinc per one ounce serving (3.75 mg zinc as zinc oxide). At the initial assessment

boys had significantly higher mean plasma zinc and parotid salivary zinc but significantly lower hair zinc than did the girls. Changes occurred in both control and experimental subjects over the course of the school year for plasma zinc and hair zinc, but analysis of changes in experimental subjects showed that there were statistically significant changes in plasma zinc (girls alone and both sexes) and hair zinc (girls). Children in the experimental group had a smaller decline in plasma zinc and a greater increase in hair zinc than did the controls. At the end of the study period there were no significant differences in plasma zinc between zinc supplemented girls and the mean values for young adult women in that laboratory. But for males, both the supplemented and control boys had significantly lower plasma zinc levels than were found in healthy young adult males. Mean daily growth increments tended to be greater in the supplemented children but not significantly different from the controls. The amount of zinc added to the children's diets was small averaging only 2.57 mg/day. This amount may not have been sufficient to see statistically significant differences in the two groups. Growth rates tended to be greater in the supplemented group, and had they received more zinc the difference might have been significant.

Interestingly, all of these studies in preschool and very early elementary children have documented only mild zinc deficiency characterized by short stature, low hair

zinc levels and in some cases hypogeusia. Questions arise concerning the outcome of these children during the rapid growth spurt of adolescence had their mild deficiency not been documented and treated. Would these children be those who mature late or who continue to be short for age?

School age children were screened for zinc deficiency in several studies in both the United States and other countries. Many of these studies found incidences of possible and/or definite zinc deficiency (10,46,47).

Heinersdorff and Taylor (10) examined the growth and hair zinc of British children between the ages of 10 and 11 to document the existence of marginal zinc deficiency in British children by standards which had been used previously--growth retardation and low hair zinc. Hair zinc levels were significantly lower in males than in females although only 3.7% of the sample had low hair zinc. Fourteen of the 219 participants were below the 10th percentile for height and 19 were below this percentile for weight, but none of these had hair zinc <90 ug/g. There were no statistically significant relationships between hair zinc, height, weight, birth rank, socioeconomic status, or food consumption practices. No measures of maturation were taken and no other biochemical measures were reported. It was assumed that this was a healthy population with normal zinc status.

A group of 110 Yugoslavian children between the ages of 9 and 12 were studied by Buzina et al. (46) for taste acuity

and zinc nutritional status. Taste acuity was determined by the three drop forced choice method, and zinc status was measured by hair and plasma zinc. The results showed that children who had greater height for age also had higher plasma and hair zinc levels than did the children who were shorter for age. Regression analysis revealed significant correlations between hair and plasma zinc and relative body weight as well as between these biochemical measures and upper arm circumference. No significant relationship was found between plasma zinc and taste acuity although the prevalence of plasma zinc <70 ug/dL was greater in the group with hypogeusia. The hypogeusia found in some of these children was mild and did not have a significant effect on growth; three of the four children who were below 90% of the standard of weight for age had moderate to a more severe degree of taste dysfunction.

From these studies of children there appeared to be a relationship between growth, taste acuity and zinc status. Hair zinc may not be a good measure of zinc status because the level is known to change with differences in seasonal growth rates. There may also be problems in using absolute amounts of plasma zinc as a measure of zinc status during growth years.

Butrimovitz and Purdy (47) reported a study of apparently healthy children in the Baltimore, MD, area in which non-fasting plasma zinc levels followed a curvilinear pattern increasing early in childhood and decreasing during

the ages in which one would expect puberty to occur. No measures of maturation were taken, however. These declines occurred in both females and males although the decline was approximately two years earlier in females than in males. They suggested that while the decline in males was not sharp, the extended growth period of adolescent males was reflected in the continual decline of plasma zinc from about age 13 to 18.

In 1985, fasting serum zinc levels were reported for youth for whom measures of sexual maturation also existed (48). The sample consisted on 205 adolescents between the ages of 11 and 19 with 95% between 12 and 16. Sexual maturation was determined at the time of clinical visits according to Tanner stages. Mean serum zinc for males with the adult rating of sexual maturity (stage 5) was significantly higher than for the males in all other stages. There were no differences in serum zinc between the other four stages in males. No data were collected on growth achievement or other measures of zinc status. No distinctions could be made between mild deficiency and normal physiological changes.

Biochemical Defects in Zinc Deficiency

In animals low zinc diets affected growth and appetite with such rapidity that these functions decreased before general tissue depletion of zinc occurred. Such results suggested that the primary site of zinc action was in

constituents of tissues with high zinc turnover rates; further, the action of zinc must be in those enzymes in which the zinc was freely exchangeable between the enzyme and the other macromolecules which require zinc for action (49).

Studies of tissue homogenates of rats and pigs showed that zinc content and RNA to DNA ratios were significantly decreased in liver, kidney, pancreas, and bone in zinc deficient animals when compared to pair-fed controls (49). Tissue homogenates had significantly less protein and RNA but not less DNA than did the controls. Specifically, the activities of zinc dependent enzymes in these tissues were reduced: alcohol dehydrogenase in liver, kidney, and bone; aldolase in liver and kidney, alkaline phosphatase in kidney and bone; carboxypeptidase in pancreas; and lactic dehydrogenase in bone. Adding zinc to the tissue homogenate did not increase enzyme activity which suggested the mineral was a structural part of the enzyme or was tightly bound. Tissue changes were not observed for the iron dependent enzymes in these tissues suggesting that zinc deficiency affected only the zinc dependent enzymes.

It was important to note that activities of zinc dependent enzymes needed to be determined on those tissues sensitive to zinc depletion (49). Not all zinc dependent enzymes were similarly affected due to the varying affinity for zinc of these enzymes and to the turnover rate of the cells. Prasad et at. (49) reviewed studies by Sandstead and

Rinaldi that suggested a role for zinc in cell division; Sandstead and Rinaldi had previously found reduced thymidine incorporation in the nuclei of zinc deficient tissues while the DNA content of the tissue remained unchanged.

Tipton et. al.(50) found the tissues in humans with the greatest amount of zinc in decreasing order were: retina, choriod, prostate, kidney, muscle and bone. The enzymes and tissues identified by Prasad et al.(49) as important for determining the effects of zinc deficiency were not available for human studies. Because these tissues are not accessible, routine screening examinations require that other tissues be studied.

Factors Affecting Zinc Homeostasis

Effects of Phytate and Fiber

With the typical diets of Middle Eastern subjects known to contain large amounts of fiber and phytates, Reinhold (51) conducted studies to determine the bioavailability of zinc. The effect of phytate on zinc availability was only at or near the sites of absorption and was dependent upon the pH of the gastrointestinal tract. Calcium was a synergistic factor involved as a complexing factor in the precipitation of zinc by phytate. When Reinhold and associates added leaven to the wholemeal bread, they found the solubility and absorption of the zinc present increased. While the leavening agent may increase solubility, they believed that

a factor other than the phytate was responsible for the poor absorption of the abundant zinc in the village bread.

Later Reinhold and co-workers (51) found that the zinc-fiber bond in wholemeal bread was in a proportional relationship so that as the proportion of zinc increased the amount of zinc bound to fiber decreased. They stated the binding curve was suggestive of an adsorption relationship. Studies of village bread indicated a zinc binding capacity of 3 mg/g fiber. Although phytate in wholemeal could be degraded, the fiber could not. Therefore, this adsorption might be particularly important in zinc deficient subjects who consumed large amounts of dietary fiber.

The binding of zinc by phytate and bran, among other substances, was pH dependent. The solubility of zinc was low at neutral pH and increased with decreasing pH particularly at pH less than 6.0. This solubility-pH relationship occurred when zinc hydroxide was mixed with various solids indicating that the solubility was a property of zinc itself and not a property of gastric contents (51).

In a controlled study with eight men housed for four to eight months and consuming diets similar to those of typical middle class American men, Sandstead et al. (52) studied the effects of increasing the fiber content of the diet by the addition of 26 grams of either soft white wheat bran or dried milled corn bran. Net total fiber was more than twice as high in the corn bran as in the wheat bran. Neither of these fiber sources affected zinc balance in the amounts

fed. This study as well as one conducted by Sandstrom et al. (53) showed that when fiber was added to the typical Western diet no deleterious effects were seen in zinc balance.

Drews et al. (54) studied the effect of fiber added to the diets of adolescent males. Eight subjects participated while living at home but consuming only the diet in the laboratory. The study was short--only 21 days: two days for nitrogen depletion, a three day nitrogen adjustment period which was followed by four experimental periods during which a basal diet was fed or basal plus 14.2 grams of hemicellulose, cellulose or pectin. Dietary zinc averaged 11 grams per day. Dry milk solids provided the only source of animal protein. Results indicated that hemicellulose significantly increased fecal zinc excretion compared with the other fiber sources and with the basal diet. There were no significant differences in the other two fiber sources and the basal diet. Serum zinc did not change over the course of the study. However, the study was short and may not have allowed changes to be detected. While this report suggested that fiber might affect zinc absorption, the diet was very limited and contained no animal flesh sources of zinc.

Effects of Folic Acid

Both zinc and folic acid are essential nutrients for cell division and replication. There appeared to be a

relationship between these nutrients which affected the absorption of zinc in a study conducted by Milne et al. (55). They found that supplemental folic acid decreased zinc retention. Both urinary and fecal zinc excretion were increased during the control and zinc depletion stages of the research when 400 ug of pteroylglutamic acid was given every other day.

Examination of plasma zinc levels during the experiment showed that plasma was sensitive to levels of zinc in the diet. With low dietary zinc and folic acid supplementation plasma zinc levels fell to a greater extent than with low dietary zinc and no supplementation. In addition, those subjects not receiving folic acid supplements had a more rapid rise in plasma zinc during repletion than those who received supplements. They stated that the observed decrease in both plasma and urinary zinc could result from folic acid inhibiting both the absorption and retention of zinc. Folate forms complexes with heavy metals; in this study the monopteroylglutamate presumably formed insoluble salts with zinc.

To investigate the mechanism of the inhibition of zinc absorption by folate, Ghislan et al. (56) designed in vivo and in vitro experiments using male rats; charcoal binding studies were also conducted to determine if the inhibition were due to the formation of zinc-folate complexes. Results from both the in vivo and in vitro studies were that zinc and folic acid inhibit the absorption of each other. The

effect of folate was in the gut because parenteral administration of folate had no effect on zinc absorption. Using cannulated adult rats, perfusions which contained only 200 ug folic acid significantly reduced zinc absorption. The charcoal binding study results revealed that the inhibiting action of folic acid on zinc and vice versa was not due to a folic acid:zinc bound molecule. Altering the pH of the solutions resulted in Zn:folate binding at pH 2.0 but the bond reversed when the pH was raised to 6.0. If similar binding took place in the living organism, the bond should be broken at normal intestinal pH. Thus, the effect of folic acid on zinc absorption was suggested to be at the membrane level. The results of these two studies caused questions to be raised on the effects of the use of folic acid supplements by free-living persons consuming marginal zinc diets.

Effects of Iron

Solomons and Jacob (2) studied the effects on zinc absorption of adding heme and nonheme iron using a rise in plasma zinc as the measure of zinc absorption. Thirty-one adults participated in one or more of 46 absorption tests. Fasting blood samples were drawn before each testing period. After ingestion of a mixed test dose of 25 mg inorganic zinc (as 110 mg $ZnSO_4 \cdot 7H_2O$) and nonheme iron in doses of 25, 50 or 75 mg (as $FeSO_4 \cdot 7H_2O$), blood was drawn hourly. The inclusion of nonheme iron significantly decreased the plasma

zinc compared to plasma zinc when only zinc was given. When measuring the rise in plasma zinc after the test dose as the indicator of the effect of nonheme iron, there was a decrease in the rise of plasma zinc. That is, nonheme iron resulted in decreased absorption of zinc. These differences were significant over the hours of the study except for the 1:1 ratio of iron and zinc. Subsequent trials with nonheme iron demonstrated that nonheme iron did not affect zinc absorption when the zinc was in the "organic" form from raw oysters. Slightly different procedures were required when heme iron was studied due to the low solubility of heme iron. The heme was consumed directly into the mouth as a powder providing 75 mg elemental iron. The powder was immediately followed by the solution containing the zinc. Heme iron did not affect the absorption of zinc even at the 3:1 ratio in which it was given. The methods used in this study showed changes in absorption rate only at quite high doses. While the doses were high, the iron:zinc ratios were kept constant at 1:1, 2:1, or 3:1. They suggested that when studying zinc status one should be cognizant of supplemental nonheme iron which may affect zinc status.

In a further study Solomons et al. (3) studied the differences in absorption of ferric and ferrous iron and the effect which ascorbic acid had on the absorption of inorganic zinc. Ferrous iron reduced the absorption of zinc significantly at hours 1, 2, and 3 of the test while ferric iron did so only at hour 3 in the 2:1 mixture of iron:zinc.

When ascorbic acid was added to the ferrous iron, no further decrease in the absorption of plasma zinc was seen. The researchers cautioned, however, that because the test dose was administered in an acidic soft drink the maximum effect of ferrous iron may have already occurred. When the ascorbic acid (1 g) was added to the ferric iron dose, the decrease in zinc absorption was significant at one hour. For this test plasma iron levels were available from studies in which the test was conducted both with and without the addition of ascorbic acid; in addition to decreasing zinc absorption, the ascorbic acid significantly increased the ferric iron absorption. Iron status as determined by plasma iron also affected zinc absorption in the 2:1 iron:zinc test dose. Those with lower plasma iron levels and, hence, in greater need of iron showed greater inhibition of zinc absorption than those with higher plasma iron levels. These researchers also determined that consuming iron on consecutive days did not decrease the plasma zinc response to test doses more than the depression that occurred with the iron-zinc dose. The researchers stated that these pharmacological doses (25, 50 and 75 mg of iron per test) might not reflect the true picture that occurred with dietary intakes, but these doses were commonly available in nutrient supplements.

Effect of Protein

Greger and Snedeker (57) investigated the effect of dietary protein and phosphorus on the utilization of zinc in eight young adult male subjects. The balance study consisted of a three day adjustment period followed by four 12 day balance trials. Diets supplied either 8.1 g or 24.1 g of nitrogen and either 1010 mg or 2525 mg phosphorus daily. The subjects consumed test diets in which the protein level was modified by the use of either a "high" or "low" protein bread. The phosphorus was consumed with meals as potassium monobasic phosphate in solution. Zinc was held constant throughout the test periods. The basic diet was supplemented with fats and sugars to maintain caloric balance in each subject. When subjects were fed high protein moderate phosphorus diets, less zinc was lost in feces than when fed the other three test diets, but urinary zinc increased when high protein diets were fed. Serum zinc levels were higher when fed the high protein diets with no effect from phosphorus level or interaction of phosphorus and protein. Serum zinc was significantly correlated with urinary zinc but not with fecal zinc. Thus, the authors concluded that both level of dietary protein and phosphorus affect zinc absorption.

Colin et al. (58) used college age women to study the effect of two levels of protein and of zinc on zinc absorption and retention. Twenty-four subjects were randomly

assigned to one of four dietary treatments. Results showed that urinary excretion of zinc was significantly greater on the high protein diet regardless of zinc intake. Although the retention of zinc did not differ significantly between the four treatments, only in the high protein high zinc diet was the net retention of zinc negative. Thus, at the levels of protein and zinc supplied in these test diets, increasing the protein did not affect zinc retention.

Another balance study conducted at the Grand Forks Human Nutrition Research Center studied moderate protein intakes and the effects of these on zinc balance (59). The protein intakes of both diets were within the amounts commonly consumed by men and could not be considered high; they did exceed the Recommended Dietary Allowances, however. The diets were analyzed at 65 and 94 g protein. Again greater urinary excretion of zinc was found with the higher protein intake; while many minerals were held constant between the two diets, the high protein diet contained more phosphorus than the low protein diet.

The zinc content of the two diets was held constant. Yet, urinary zinc increased when dietary protein increased. Thus, protein level is important in assessing zinc status.

Regulation of Zinc Balance

Evans et al. (60) postulated that the absorption of zinc may be regulated at both the apical and basolateral plasma membrane in rat intestine. If this applies to humans

as well, cellular structure in addition to intestinal contents might play a role in the absorption rate of zinc.

In a study of healthy young adult males, Istfan et al. (61) reported that the zinc absorption rate was dependent on the zinc load. They found, using a liquid purified diet, that absorption of the ^{70}Zn tracer (either 1.19 or 4.52 mg dose) was approximately 81% when the zinc dose of 15 mg/day was divided equally into three meals. The rate of absorption decreased to 61% when the total zinc dose was 24.52 mg/day. Istfan et al. suggested that the zinc nutritional status of the subject affected the rate of absorption. They also found a statistically significant increase in the absorption of zinc after only six days of maintenance on a diet which was low (1.5 mg/day) in zinc. When the dietary zinc dropped from adequate (15 mg/day) to low (1.5 mg/day), there was an immediate reduction in urinary and fecal zinc loss. If these results were found in mixed diets as well as in purified diets, zinc absorption and excretion rates would appear to be sensitive to dietary load.

Possible roles for influence of carbonic anhydrase and albumin on the absorption of zinc during pregnancy and lactation were hypothesized by Moser and Reynolds (62). They found low intakes of dietary zinc both in ante and postpartum women whether or not they lactated. While the plasma zinc levels declined significantly during pregnancy, at delivery the cord plasma zinc was high. They inferred

the maternal decline in plasma zinc was a physiological response to the increase in blood volume, decrease in albumin, and increase in circulating estrogens of pregnancy. Erythrocyte zinc levels fell significantly one month postpartum to three months postpartum and again at six months postpartum. The researchers hypothesized that declining erythrocyte zinc was due to a decrease in carbonic anhydrase no longer needed at high levels due to decreasing the carbon dioxide load with delivery. This study suggested that an internal homeostatic mechanism for regulation of zinc absorption partly based on need may exist because no changes were found in plasma zinc during lactation and because erythrocyte zinc decreased during the six months postpartum with and without lactation. Further investigations are needed on the mechanisms regulating zinc absorption, metabolism and homeostasis.

Baer and King (63) reported that the kidney may be a regulatory site for zinc homeostasis. This role was suggested based on experimental work with young adult men who showed a rapid increase in urinary zinc excretion at repletion following a low zinc diet. Thus, they believed that when intake of zinc was high, renal mechanisms functioned to excrete excess zinc and preserve tissue homeostasis.

Evidence exists for association of several proteins with zinc in blood. Parisi and Vallee (64) found human plasma zinc associated with two protein fractions--albumin

and a zinc macroglobulin--by separation of serum or plasma chromatographically and with gel filtration. Zinc peaks paralleled those for alpha macroglobulin and albumin.

Hahn and Evans (65) found in rats that following oral administration of ^{65}Zn the radioactive isotope was found in four peaks with 40% in a low molecular weight ligand. Evans (66) has since reported evidence for several proteins associated with zinc in plasma. He suggested that albumin may be the transport protein to body cells and that transferrin in the portal blood transported zinc from the basolateral plasma membrane to the liver. Evans (66) reported that isolated rat intestinal cells when treated with a radioactive zinc dose did bind zinc to albumin and that albumin added to a solution of zinc bound cells draws the zinc to the albumin from the cells. Thus, they believed that albumin may have promoted the release of zinc from intestinal cells. It appeared that the association of zinc with different proteins may depend on the stage of absorption or transport to cells in which the zinc is found.

Measures of Zinc Status

Many studies have been conducted in attempts to determine appropriate measures for determining zinc status. None of these measures have provided conclusive evidence for a reliable measure using relatively easy non-invasive techniques.

Plasma Zinc

Plasma has been recognized as an easily obtained tissue for evaluating components of body fluids. The validity of plasma as a measure of nutritional status depends on the existence (or lack of existence) of equilibrium between plasma and other body tissues. Plasma may be the means of transport from the gastrointestinal tract to functioning tissues rather than being in equilibrium with other body tissues. Nevertheless, zinc in plasma has been frequently evaluated as one measure of zinc nutritional status.

Because of differences in reported zinc values from plasma and serum, researchers have expressed concern regarding the use of serum for zinc analyses. Serum may be contaminated with zinc from prolonged exposure to erythrocytes during coagulation or from rupture of platelets (67). Smith et al. (67) reported differences ranging from 8 to 21% between serum and plasma zinc in a series of samples from ten subjects when 30% sodium citrate was used as an anticoagulant. Makino (68) stated a shift of water from erythrocytes to serum occurred accounting for lower zinc values in plasma than in serum. Zinc may be carried to serum in the water shift.

The choice of anticoagulant may also affect comparability of results. Smith et al. (67) reported work of others which revealed that oxalated or citrated plasma had significantly ($p<.1$) lower zinc concentrations than did

plasma or serum in which heparin was the anticoagulant. The lower values in citrated and oxalated plasma were due to chelation of the ion. Thus, comparison of results between studies may be difficult. Heparin is frequently contaminated with zinc; Whitehouse et al. (69) used an ion exchange column (Chelex 100) to remove zinc from heparin and reported that three drops of 10,000 USP units zinc-free heparin was sufficient to prevent coagulation of three to seven milliliters of whole blood.

Other sources of contamination also exist. Air, reagents, skin and saliva from technicians as well as blood collection apparatus must either be controlled and/or tested for contamination before use. Laminar flow clean air hoods with a rating of 100 are desirable in all sample preparations. Ultrapure acids and tested deionized water also must be used. Plastic labware must be tested because plastics suitable for one trace metal analysis may be contaminated for another (67). Sample storage was another possible source of error. Samples stored frozen may dehydrate especially when frost free freezers are used. Storage with ice cubes in sealed bags may prevent dehydration and thus concentration of the analyte (67).

Smith et al. (67) stated that an analytical method must not only be "preferred, excellent and needed" but that it must also be "precise, relatively simple, inexpensive, and rapid." Most commonly zinc in plasma or serum has been measured using diluted samples with flame atomic absorption

spectrophotometry (AAS). The dilution level and the viscosity of the resulting solution must also be taken into consideration.

In 1979 Smith and Butrimovitz (70) reported on a flame AAS method which used dilutions of one part plasma to four parts deionized water and standards of 50mL/L glycerol and water. The use of glycerol and water standards simulated the viscosity of plasma and thus controlled for differences in aspiration rate which would otherwise have interfered with the validity of the results.

Rodriguez et al. (71) modified the procedure of Smith and Butrimovitz (70) by decreasing the concentration of glycerol to 30 mL/L in the standards. They reported that this glycerol concentration closely matched the aspiration rate found with a 1:4 plasma deionized water solution in a Perkin Elmer 5000 AAS.

Solomons (72) has synthesized the work of many researchers to report factors which may affect plasma zinc levels. Zinc is known to be bound to albumin; if hypoalbuminemia existed in the subject then plasma zinc would be low. Hormones, both endogenous and exogenous, affect circulating levels as well.

Plasma zinc levels vary in healthy subjects with time of day, fasting state, gender, medication usage, and age. Pilch and Senti (73) reported the serum zinc data from the second Health and Nutrition Examination Survey (NHANES-II)

which showed that fasting and time of day of sample collection had great effects on the resulting values.

In the NHANES-II study males had significantly higher values than females for ages 9-74, but, in childhood, males had values lower than females of similar ages. Serum zinc rose during adolescence and peaked during young adulthood. Caution must be exercised in using these values as references, however, because in children the samples were drawn non-fasting. Age categories also present some difficulties because the ages preceding the expected growth spurt through maturation are grouped together. Mean serum zinc for white boys age 3-8 was 80.5±13.71 (mean±SD) and for 8-19 years 86.0±15.27 ug/dL. The data were adjusted for age and morning or afternoon collection of all samples, and for fasting using the fasting data for the males 20-74 years of age (73). Wagner et al. (48) reported serum zinc levels ranging from 101±12 to 119±18 ug/dL (mean ± SD) for males 11 to 19 years of age according to stage of maturation. Buzina et al. (46) reported plasma zinc in children with impaired growth ranging from 50 to >90 ug/dL with the mean of 74.7 ug/dL. However, this group used tricholoracetic acid to precipitate the protein in the plasma; TCA has been shown to cause a decrease in plasma zinc levels because some zinc is also precipitated (70). Hambidge et al. (45) reported a mean ± SEM value for plasma zinc in boys age 33 to 90 months of 81.9±2.2 ug/dL. In a group of healthy preschool children who served as a control group, Hambidge found mean plasma

zinc of 84.2±2.9 ug/dL. Many researchers have reported values of <70 ug/dL to be associated with deficiency; however, due to changes in technology, age, maturation and gender of the subjects, setting a value for deficiency remains difficult.

Plasma zinc was found to measure depletion only after variable periods of zinc deprivation. Apparently the length of time before plasma zinc levels fell was dependent on previous nutritional status of the subjects (63). Plasma zinc decreased only after urinary zinc fell to below 150 ug/day. The level of plasma zinc continued to fall while the urinary zinc did not.

Wada et al. (74) conducted a 75 day metabolic study in which a conditioning diet containing 15.5 mg Zn/d was fed for 12 days to six young adult men. A low zinc diet (5.5 mg/d) was fed for 54 days, and then the conditioning diet was refed for the remainder of the study. Using stable isotopes they found that the mean decrease in serum zinc was insignificant when the diet changed from adequate to low. Even after seven weeks on the low zinc diet the lowest serum zinc concentration was 100 ug/dL. This concentration was not low enough to be considered abnormal by current definitions.

Rabbani et al. (75) developed a model for the production of experimental zinc deficiency in humans. Their seven day cycle diet was fed for one year to volunteers in phases for stabilization, depletion (mean of 4.8 mg Zn/d for

28 weeks), and followed by a 20 week repletion phase (30 mg Zn/d). Plasma zinc decreased by 25% when the depletion diet had been fed for eight weeks. The differences between plasma zinc during baseline, depletion and repletion were not statistically significant, however. Lymphocytes were found to be more sensitive to both depletion and repletion than was plasma. The differences in lymphocytes between the three phases were significant at $p<0.02$. In this experiment not only was the depletion diet low in zinc, but also the phytate:zinc molar ration was high probably decreasing the absorption of zinc.

While plasma zinc levels have been shown to vary with dietary treatment, Abdulla (76) stated that plasma zinc may not be a good measure of zinc status because about 98% of the total body zinc is intracellular; low plasma zinc could simply represent a shift from the transport media to another body compartment. While variation in plasma zinc levels can be demonstrated, Abdulla also stated that clinical signs of zinc deficiency are not usually seen until plasma zinc falls below 0.6mg/L. Because many factors can affect plasma zinc levels, this measure can be considered only a part of the assessment of zinc status.

Erythrocyte Zinc

Erythrocyte zinc is thought to reflect longer term status due to the slower turnover of zinc in red blood cells than in some other zinc responsive tissues. Depressed

erythrocyte zinc was found in studies of children with protein energy malnutrition (72). However, analysis of erythrocyte zinc and the significance of this measure were limited by research problems in analysis and interpretation. Some researchers reported that erythrocyte zinc remained high when plasma levels were low (72). In a study of preadolescent females, Kenney et al. (77) found that erythrocyte zinc increased with age and maturation stage although zinc intake decreased with increasing age. Thus, erythrocyte zinc, while not often measured in females of this age, may not be a good indicator of zinc status.

Baer and King (63) investigated the response of tissues to zinc deficient diets in order to measure variation in tissue responses. Subjects were depleted until plasma zinc fell to <70ug/100 g. Length of depletion varied from four to nine weeks. They found that erythrocyte zinc did not fall during the five week depletion period in young men although plasma, urinary, and fecal zinc levels declined significantly. Their subjects' erythrocyte zinc levels were considered to be in the low normal range at the beginning of the study; therefore, further decreases in red cell zinc may not have occurred in the short period if they were marginal at the outset. It is questionable whether red cell zinc can be a useful measure of changes in zinc status. More information is needed about the mechanisms regulating the deposition of zinc in various body tissues.

Leukocyte Zinc

Leukocytes contain about 25 times more zinc than a similar number of red cells, and total leukocyte zinc amounts to about 3% of all blood zinc (76). Meadows et al. (78) reported that the zinc content of peripheral blood leukocytes and of operative and percutaneous muscle biopsy samples were strongly correlated in healthy individuals. In addition, this group reported that concentration of the nucleated tissue zinc could not be predicted from the zinc levels in plasma, urine or red blood cells. Abdulla (76) suggested that leukocyte zinc might be a good measure of zinc status but that careful separation and quantification of cells were required.

Cholesterol Levels in Adolescents

Serum cholesterol levels in adults have long been associated as a risk factor in atherosclerotic disease. Recently, concern has been expressed about the effect of childhood lipid levels on the development of heart disease later in life. While the current recommendations are that all adults should know their cholesterol levels, routine screening of children and adolescents has not yet been undertaken.

Based on evidence that fatty streaks and raised atherosclerotic lesions have been found in young adults, Chase et al. (79) undertook an investigation of the

cholesterol levels and risk of coronary artery disease in offspring of persons known to have had heart attacks early in life. Medical charts were screened for individuals in the Denver area known to have had a heart attack prior to age fifty. Both parents and all children were invited to participate resulting in a sample (58 heart attack victims, 55 spouses, and 179 offspring) of approximately fifty percent of the eligible subjects. All families were asked if they knew or had measurements of serum lipids prior to this study. Because only 4 of the total 71 families were aware of lipid levels prior to this study, knowledge of lipid levels was not thought to be a biasing factor for participation. Fasting blood samples were drawn from all subjects and from a control group of youth ages 12-20 from families with no history of heart disease. Mean cholesterol levels in the control group were within normal ranges for age. The study results revealed that 39% of the offspring of early heart attack victims had elevated lipid levels and that the pattern of abnormal lipids in the offspring was most commonly the same as the parent who had the heart attack. Fredrickson classifications of Type IIa and IV (elevated cholesterol and low density lipoprotein or elevated triglycerides respectively) were most common in both the offspring and the parent.

On the basis of their results, Chase et al. (79) recommended that all children of early heart attack individuals be screened for cholesterol level. If elevated,

further definitive tests should be conducted. Screening only these children had the benefit of cost effectiveness because the normal controls has mean cholesterol levels within normal limits for their ages.

A study conducted in Beaver County, Pennsylvania (80), followed individuals nine years after original screening at age 12 to determine if early childhood screening could predict adult cholesterol values. Fifty-six percent of those eligible for the follow-up study actually participated. Because laboratory methods for analyzing cholesterol values had changed over the time period considered, results were compared by quintile rankings from initial testing to quintile rankings at retesting nine years later. Sex-specific distributions were used at both testing periods. Only data from the original screening for which follow-up data were also available was used in these analyses. Fifty percent of the men and 46.8% of the women in the top quintile during initial screening were found in the top quintile at follow-up; 66.7% of the men from the original top quintile were in the top two quintiles at follow-up. Those individuals who were incorrectly identified as high risk in youth due to placement in the top quintile were found to have healthier lifestyles than those who were incorrectly identified as low risk at initial screening. These researchers recommended that screening in childhood should be conducted for all adolescents because waiting for a familial coronary event delays screening and

treatment. In addition, a positive family history was not more common in those correctly identified subjects than in those who were incorrectly classified as low risk during the initial testing. Orchard et al. (80) further recommended that the screening should include second screenings for total cholesterol and high density lipoproteins for those in the top quintile in the initial testing.

A study conducted by Vartiainen et al. (81) on 15 year old adolescents in Finland was designed to determine if children at risk for cardiovascular disease can be identified from the parent's history of disease. From a population of 4283 children, a sample of 1280 was selected; 580 males (90.6% of the eligible males) and 610 females (95.3% of the eligible females) participated. Anthropometric measurements, sexual maturation according to Tanner standards, blood pressure and serum levels of total and HDL cholesterol were measured. The mean total cholesterol for males was 4.47 mmol/L (173 mg/dL) with 21% of the males having total cholesterol over 5 mmol/L (193 mg/dL). The data were also examined by sexual maturity stage. Decreases of 14% in HDL-cholesterol were associated with increasing maturation from Stage 1 to Stage 5. No association was found between total cholesterol and sexual maturation in either sex. No significant differences were found between mean values of the assessed risk factors in subjects from families with and without a history of cardiovascular disease. In addition, the proportion of

children with either high total cholesterol or low HDL-cholesterol was the same in the group with a positive family history of heart disease as in those without such a family history. The researchers concluded that in this population children at risk for cardiovascular disease could not be identified solely by parents' history of the disease.

In 1980 Christensen et al. (82) reported the results from the Prevalence Study of the Lipid Research Clinics Program for individuals under 20 years from the seven North American sites of data collection. This report provides data on a large number of children and adolescents (13,665) from industrial populations. While not a national probability sample, the values may be used as references because a variety of socioeconomic groups were included. Racial groups included were white and black. Trends in cholesterol values were detected when data were separated by age, sex, and race. White males showed no consistent pattern of differences up to age 11, but from age 11 until age 17 there was a consistent drop in the mean values (167 to 156 mg/dL). Values for the 5% and 95% for age 12 were 127 and 216 respectively. Standard deviations were similar at each age. For white males a similar inconsistent pattern of triglyceride values was found up to age 11, but, unlike cholesterol values, the triglyceride levels continued to rise to age 19. No data were presented for high density lipoprotein (HDL) values.

The decline in total cholesterol in females began about two years earlier than in the males. This fact as well as the ages at which these values decreased suggested that there might be an interaction between cholesterol levels and factors associated with puberty.

During the 1973-74 school year, lipid data on 3,524 children between the ages of 5 and 14 years were collected from the racially mixed community of Bogalusa, Louisiana (83). This sample represented 93% of all eligible children in that community. Results were presented by age, sex, and race as well as overall values. White males were found to have a similar lipid profile as reported in the Lipid Research Clinics data. Values remained rather consistent until age 11 at which time decreases in total cholesterol were seen as well as increases in serum triglycerides. Black children were found to have higher total serum cholesterol than white children. The authors cautioned that due to analytical error even in carefully controlled labs, which in this study resulted in a variation of 18 mg/dL, care should be observed in constructing a lipid profile from a single observation.

A further report on the lipid values in the Bogalusa study presented data on lipoprotein levels in this group of children (84). High density lipoprotein (HDL) cholesterol concentrations were not found to correlate with age in any of the four race-sex groupings. Mean values for each age and the fifth and the ninty-fifth percentile were given. In

white males (n=134) at age 12, the mean HDL cholesterol was 67 while the fifth and ninty-fifth percentiles were 31 and 101 respectively. A highly significant relationship was found between very low density lipoproteins and total cholesterol ($r=.745$, $p<.0001$) and triglyceride levels ($r=.798$, $p<.0001$) among white males. Highly significant correlations were also found for the relationship between total cholesterol and high density lipoproteins in both races; in addition, when differences between the racial groups were compared, blacks had significantly higher HDL levels than whites.

Lauer et al. (85) investigated youth between the ages of 8 and 18 in Muscatine, Iowa, who were originally examined for lipid levels and were reexamined 12 years later as young adults. Reexamination studies included anthropometric measurements, family history, lifestyle practices as well as health histories. The purpose was to discover relationships between childhood and adult cholesterol levels. About 67% of those eligible actually participated resulting in a sample of 2,446 white subjects. Data similar to that found in other studies were reported. In males mean cholesterol values increased from age 5 to approximately age 10, followed by falling levels until about age 15 at which time levels of total cholesterol began rising and continued to rise throughout the ages studied. Triceps skinfolds in males rose from age 7 until approximately age 12 when falling skinfold thicknesses were recorded. The decrease in

skinfold thickness continued until approximately age 16 at which time the thickness of the fat layer began to increase. This increase with age in fat layer thickness continued throughout the study. Correlations of childhood cholesterol in males with those levels in adulthood ranged from 0.51 to 0.72 and were significant. There were no significant correlations of childhood total cholesterol with adult high density lipoprotein cholesterol, but there were significant correlations between childhood total cholesterol and adult low density lipoprotein cholesterol levels although the correlations were somewhat lower (r=0.47 to 0.60). When factors related to obesity and other health habits were examined for prediction of adult cholesterol values, childhood cholesterol values were the best predictors; however, changes in the W/S^2 added significantly to the prediction equation. Of the children who had cholesterol levels above the 90th percentile in the original testing, 43% were above the 90th percentile at the adult measurement and 62% of these children were above the 75th percentile in adulthood. Based on the results of this study the researchers stated that lifestyle habits should be monitored as well as blood lipids because many of the habits acquired during adolescence persist into adulthood. The predictive value of childhood cholesterol levels was not above 50%, therefore, one should be cautious of single measurements for predicting adult levels or risk of coronary disease.

Fripp et al. (86) reported a study designed to measure the association between atherosclerotic risk factors and physical fitness and obesity. The sample was selected from 289 tenth grade male adolescents who scored in either the low-moderate or moderate fitness level as determined by the Cooper walk-run test. One hundred males met the criteria and 37 volunteered to participate. Body mass index (W/S^2) was used as the measure of obesity. In order to evaluate the effect of aerobic fitness on risk of atherosclerosis, subjects were divided into three groups based on oxygen consumption levels during fitness testing. The differences in risk factors between the three fitness groups were significant for body mass index and triglyceride levels. No differences were found in maximum heart rate between the three fitness groups, suggesting that all three groups were stressed to a similar degree. Body mass index, blood pressure (both systolic and diastolic), and triglycerides were positively related in linear regression analysis. There was a negative relationship between body mass index and triglyceride levels. In this group of male adolescents who were in the low-moderate or moderate fitness category, body mass index was the best predictor of the interindividual variability in the risk factors considered. While this sample was small, the results were similar to those of the larger Muscatine study (85).

In a repeat testing five years after the initial study of the subjects in the Bogalusa Heart Study, researchers

examined the relationship between changes in obesity and in serum lipids (87). Subjects ranged in age from 10 to 17 at retesting. Results indicated that increases in triceps skinfolds were positively related to changes in levels of total cholesterol, triglycerides, and both low density cholesterol (LDL-C) and very low density cholesterol (VLDL-C). There was a weak negative relationship to decreased HDL cholesterol levels. With the exception of follow-up levels of HDL cholesterol, all the associations were stronger in males than in females and were stronger for whites than for blacks. When regression models were constructed to assess the ability to predict follow-up serum lipid and lipoprotein levels from change in triceps measurements and initial lipid concentrations, the predictability increased significantly in white and black males for total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol by adding triceps skinfold thickness changes to the model.

Black et al. (88) studied the effect of zinc supplements on serum lipids in non-smoking non-obese male volunteers between the ages of 19 and 29 from a university community. Subjects were given either supplements containing 50 or 75 mg Zn as zinc gluconate per day or a placebo containing all the ingredients of the supplement except for the zinc. Results from the twelve week study showed that the subjects receiving the zinc supplements had decreases in HDL-cholesterol; there was a significant time

by treatment effect for the group receiving the larger zinc supplement. At week 12 there was a significant decrease in HDL-cholesterol for the 50 mg Zn group. There were no significant differences for any of the groups in total cholesterol, serum LDL- or VLDL-cholesterol. The placebo group had a higher HDL-cholesterol level at week 12 than at baseline. These researchers did not find that zinc supplemented free-living subjects had increases in cholesterol levels as had been found in animal studies. Dietary records kept by subjects did not reveal significant differences in energy, fiber, calcium, or copper between groups although not all days were recorded. The group receiving the 75 mg Zn supplement consumed significantly less zinc, protein, total fat and saturated fatty acids than the other groups. Because the doses in the supplements were amounts commonly available, the researchers stated that long term use of these levels of zinc supplements could have a harmful effect on cardiovascular health.

While many of these studies have shown a tendency for cardiovascular risk factors to be heritable and while many children and youth seem to be "at risk" in terms of serum lipid levels, the Committee on Nutrition for the American Academy of Pediatrics in 1986 (89) restated their earlier opinion on the inadvisability of altering or severely restricting the dietary patterns of children in their growing years. They were particularly cautious about recommendations restricting fat and cholesterol intakes

during the adolescent growth spurt. They supported screening children over two years of age who were at risk for heart disease due to family history for blood lipid levels; screening should consist of at least two measurements and further testing for HDL-cholesterol if the lipid levels were found to be above the 95th percentile for age and sex.

Dietary Assessment Methodology

The purpose of a study has a direct bearing on the method of dietary assessment. If the object is to determine nutrient intakes for groups of people then the methodology should be different from that used to determine relationships between disease and specific nutritional habits of an individual (90). The commonly accepted techniques for dietary assessment each vary in their strengths and limitations. The validity of any method is dependent on the intelligence, motivation, and cooperation of the subjects as well as on the techniques and skills of the interviewer. Rapport needs to be established between the subject and the interviewer if complete information is to be obtained (91).

Most zinc status measures do not respond immediately to low dietary intakes. In fact, it appeared that over time the body adjusted somewhat to lowered intake or less available intakes by increasing the absorption rate (63).

Therefore, dietary assessment measures used to help explain zinc status need to reflect long term or usual intake.

Nutritional History

The diet history developed by Burke (92) in 1947 assessed the usual intake of an individual over a period of time. This method required a highly skilled interviewer and considerable time for data collection. The time period for which data were collected was determined by the purpose of the study, the frequency of collection, and the ability of the subject to remember usual intakes.

In collecting the nutritional histories, the researcher needed to be aware of the subjects' ability to estimate portion sizes and to remember foods consumed. Generally, females under the age of 12 and males less than 13 to 14 were unlikely to give reliable nutritional histories (91).

Training of the subject in observation was essential for accurate results in longitudinal studies. Beal (91) found in the Denver longitudinal studies of children that the first nutritional history from a subject should be used only as an information trial and then discarded due to the multiple inaccuracies. This initial nutritional history served as training of the subject for observation and detail needed in conducting the research.

Twenty-four Hour Intake Records

Research on the validity of the 24-hour dietary intakes revealed that this method, when used as the only basis for data collection, may be acceptable in large group studies in which time, money, and personnel are limited; however, these records were inadequate for determining individual relationships between diet and effects of the diet in longitudinal research (91,93,94). One of the chief advantages of the method was the shorter time required for collection than that required for the nutritional history. In addition, a less-highly trained and skilled interviewer was needed than was required for the history (90).

According to Beaton (93), one of the chief drawbacks to the method is the false negativity in correlations due to the lack of reflection of usual eating patterns. Because of the large intraindividual variation in daily intakes, a single 24-hour recall also biased the slope of the regression analysis toward zero and, thus, did not identify relationships effectively.

Food Records

Food records vary in length of time for which information is recorded. They may be either weighed records or estimates of portion sizes. A seven-day record, thought to give a more accurate estimate of intake than a single day record, demands a high degree of cooperation on the part of

the subjects (90). In addition, an interviewer needs to review the record with the subject to clarify information and to fill in missing information.

Hackett et al. (95) found the diary method satisfactory for a two year study with children who were age eleven at the beginning of the study. They found a high degree of compliance in three day records which were repeated five times during the study. Because they were interested in the relationship between dietary habits and macronutrient intakes, they felt the method was satisfactory. They did not evaluate vitamin or mineral intakes.

In a study of 44 elderly persons Gersovitz et al. (96) found that the first two days of the seven day record significantly correlated with foods actually consumed, but that the accuracy of the record keeping deteriorated during the last three days. Thus, the method may again give good information on group intakes but was more limited for estimates of individual intakes.

Balough et al. (97) compared the results of the average of several 24-hour recalls with a shortened version of a diet history. They found the correlations were high for calories, protein, carbohydrate and fat. The 24-hour recalls were obtained over eight months; thus, this level of precision required subject availability and cooperation. It also increased the length of the study and interviewer time. Due to the high intraindividual variability they found that

nine 24-hour recalls would be required to accurately reflect the usual intake of 90% of their population.

CHAPTER III

RESEARCH METHODS

Methods of data collection and analysis are described in this chapter. Selection of subjects, anthropometric measurements, biochemical measures, and method of dietary intake assessment are included.

Research Design

The present study was designed as a correlational descriptive study. Controls established were age, gender and apparent good health. Because the purpose was to describe differences in adolescents related to maturation, it was necessary to limit the age range of subjects. Controlling for age and gender provided group sizes sufficient for analysis without necessitating a very large sample. Controlling for health reduces the potential for unexplainable intervening variables. This control for health can also be considered valuable in reducing the total sample size needed.

Data were collected between February and June 1988. Subjects were scheduled for laboratory visits in the order in which interviews were conducted, as much as possible. Care was taken to prevent a child who was interviewed early

in the study from being scheduled for laboratory measures late in the study. Interviews were conducted in settings in which the adolescent was comfortable; most were conducted in the adolescent's home.

Selection of Subjects

The target population was adolescent males in different stages of maturation. The subjects were volunteers from the ages of 10.6 to 14.3 years who were invited by letter to participate.

A preliminary announcement (Appendix B) of the project was sent to parents of eligible subjects. The announcement, on bright yellow paper, was sent to elicit interest before the longer more detailed letter was sent. The announcement was timed to arrive one week before the letter.

The adolescents attended the Stillwater Middle School, Stillwater, OK. From a total male student population of 333, letters of invitation were sent to 241 for whom addresses could be located using a cross-listed telephone directory. Of the 241 letters which were sent, nine were returned as unable to be located. Cards, enclosed in the letters, expressing interest in the study were returned or telephoned inquiries were received from parents of 66 adolescents. Three parents also volunteered younger sons who met the age criteria but who were not enrolled in Middle School. Three volunteers were lost either by not being able to contact a parent for an appointment (2) or by family

relocation before the study began. The final sample included 66 adolescent males. All subjects completed all parts of the study with the exception of one for whom a complete blood sample could not be obtained.

Prior to the selection of subjects, the study received approval from the Internal Review Board at Oklahoma State University for studies involving human subjects. Informed consent was obtained from at least one parent and from the subject (Appendix C).

Rewards for participation were given. All subjects received a coupon for a fast food item[1], tokens for two games at a video game entertainment center, and two dollars. A drawing for six $25.00 gift certificates was held. All participants' names were entered into the drawing on the day of blood collection. Each child had a one in eleven chance of winning a gift certificate.

Anthropometric Measures

All anthropometric measurements were made on the day that blood samples were collected. Mid-arm circumference and three triceps skinfold measures using Lange calipers (Cambridge Scientific Industries, Cambridge, MD) were taken on the right arm by one trained nutritionist. Height was measured without shoes using a steel tape affixed to a

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1 Donated by McDonald's, Inc., Stillwater, OK

perpendicular surface with a right angle head board. Subjects were weighed in street clothes without shoes on a beam balance calibrated before the study began. Arm muscle area and body mass index (W/S^2) were calculated from these measurements.

Height percentiles were determined using maturation based growth charts (12) obtained from Castlemead Publications (Hertsfordshire, England) and are included in Appendix D. Skinfolds were evaluated using the reference values from Frisancho (18). All anthropometric procedures are included in Appendix D.

Blood Collection and Analyses

Fasting blood samples were drawn by a registered medical technologist using plastic syringes with plastic pistons (Sarstedt, Numbrecht, W. Germany) and 21 gauge stainless steel butterfly needles (Deseret Medical, Inc., Sandy, Utah). Three drops of heparin (10,000 USP units/mL) which had been passed through a prepacked ion exchange column (Chelex 100, Bio-Rad Industries, Richmond, CA) to remove contaminating zinc were used as an anticoagulant in the tubes for collecting plasma. The whole blood was kept in an ice bath until analysis of hemoglobin and separation of plasma.

Blood was separated in a Beckman TJ-6 refrigerated centrifuge at 1520 x g for 22 minutes at 4°C. Plasma was transferred to acid washed plastic tubes, capped and stored

frozen until plasma zinc and DHEAS were analyzed. Plasma for cholesterol determinations was stored refrigerated overnight.

Hemoglobin was measured by the cyanmethemoglobin method using prepared reagents (Sigma procedure 525, Sigma Chemical Co., St. Louis, MO). Whole blood measurements were made within three hours of blood collection.

Total and high density lipoprotein cholesterol in plasma were measured by an enzymatic colormetric method using a kit (Sigma procedures 351 and 352-3). Absorbance was read at 500 nm on a Gilford Response Spectrophotometer (Oberlin, Ohio). Values were compared to those for adolescents from the Lipid Research Clinics trials (82).

Maturation status was assessed using dehydroepiandrosterone sulfate as a marker. This adrenal androgen has been shown to have little diurnal variation and to increase progressively with age and maturation status (31). A double antibody radioimmunoassay kit (Cambridge Medical Diagnostics, Billerica, MA) was used to determine the plasma level of the hormone.

Plasma zinc was determined using an air-acetylene flame with a Perkin-Elmer 5100 PC atomic absorption spectrophotmeter (Norwalk, CT). Wavelength was set to 213.7 nm with a slit of 0.7 nm. Acetylene flow rate was 1.9 L/min and air was 10.5 L/min. The integration time was 3 seconds. Calculations were based on mean peak area for two replicates.

A modification of the procedure of Rodriguez et al. (71) was used for plasma zinc analyses. Samples were prepared by dilution of 400 uL plasma with 1.6 mL 0.5% nitric acid (GFS Chemicals, Columbus, Ohio). Purified water tested to contain less than 0.005 ug/mL zinc was used in preparation of the nitric acid. The same bottle of water was used for all steps of the analysis. Standards of 0 and 0.4 mg/L zinc were prepared in 30 g/L certified glycerol/0.5% nitric acid. Glycerol, used in the standards, alters the viscosity of the solution resulting in an aspiration rate very similar to the diluted plasma samples. Aspiration rates are important because the calculations are based on mean peak area within a specified time. Nitric acid in the standards and samples enhances the absorption signal to the photomultiplier tube. After each sample reading the tubing and nebulizer were rinsed with 0.5% detergent (ERA, Proctor and Gamble, Cincinnati, OH) solution to prevent clogging of the tubing, nebulizer or burner head. Water was then aspirated until a normal flame returned. Standards and a control sample of plasma were periodically reread. Corrections were made to readings based on slight baseline drift. Specific procedures for each biochemical determination are included in Appendix E.

Dietary Intake Records

Estimations of usual eating habits were determined using a specially constructed food frequency questionnaire

(Appendix C). The questionnaire was developed using foods commonly eaten in Oklahoma with the additions of foods known to be especially rich sources of zinc. The questionnaire was pretested in volunteers (n=137) at the Stillwater Junior High School in the Fall of 1986. Foods not commonly consumed by youth of this age were eliminated unless they were outstanding sources of zinc. This group was selected for pretesting the questionnaire because of the similarity of age without biasing the actual sample.

Frequency of consumption and estimations of usual portion sizes were determined. To assist the subjects in estimating portion sizes specially constructed food models were used which have been shown to be non-biasing (98). Food model sizes ranged from one teaspoon to 2 cups. Models for estimating meat and cheeses were made from various thicknesses of cardboard using the directions from OSU Cooperative Extension Weigh-Off program. Glasses with marks for 4, 6, 8 and 12 ounces were used for beverages. When brand name items were reported by subjects, portions sizes were based on published values.

From the frequency and portion estimates amounts consumed per day were calculated using the figures of seven days per week and 30 days per month. For example, the portion size (quantity) was multiplied by the frequency of consumption and then divided by the appropriate time period. If a food were eaten three times per week, the quantity was multiplied by three and divided by seven. Subjects were

asked to recall foods consumed for approximately 4 months. The Food Processor II (ESHA Research, Salem, OR) was used for dietary analyses. The data base included fast food items commonly consumed. For foods not listed in this software product, values were calculated from product labels and added to the data base.

Health Background

A questionnaire for collecting health habits and family and subject background information was completed by interview at the time of the dietary intake collection (Appendix C). This questionnaire was developed to identify unmeasured variables which might explain unexpected or "abnormal" results.

Statistical Analyses

All data were analyzed by the Statistical Analysis System (SAS) version 5.18 (SAS Institute, Cary, NC) on a mainframe IBM model 3081 computer.

Correlation coefficients were calculated in preliminary analyses to identify relationships between key variables identified or hypothesized from published research. Means and standard errors of means were calculated by age and DHEAS groups in growth and maturation characterization of the subjects.

In analyzing plasma zinc, data were examined for differences using the GLM procedure by grouping data within

variable categories. Plasma zinc groups were divided into tertiles. Differences in least squares means of categorical groups were analyzed by the general linear models (GLM).

Regression models were constructed by stepwise regression calculations entering variables which were related from previous analyses or which had been identified in published research as associated with the dependent variables. Overall statistical significance was set at p=0.05 for all analyses in this study.

CHAPTER IV

DEHYDRODEPIANDROSTERONE SULFATE, CHOLESTEROL, HEMOGLOBIN AND ANTHROPOMETRIC MEASURES RELATED TO GROWTH IN ADOLESCENT MALES

Introduction

Adolescence is a time of rapid growth and sexual maturation and of self-consciousness. Physiological changes during this period necessitate adequate nutrient intake. In order to assess adequacy of nutrient intake a measure of maturation status is desirable. Plasma dehydroepiandro-sterone sulfate (DHEAS) rose with increasing maturation evidenced by Tanner staging (1) and was related to changes in bone age (2,3), another measure of increasing maturation. Maturation may be a determinant of increasing hemoglobin levels found with increases in age in males during adolescence (4-7). During adolescence cholesterol levels in males decreased with age up to usual maturation age and then again rose (8,9). The purpose of this study was to determine relationships between measures of maturation and DHEAS and to evaluate the usefulness of DHEAS for surveys of adolescent males.

Methods

Sample

The sample consisted of 66 apparently healthy white male adolescent volunteers between the ages of 10.6 and 14.3 years. Subjects were generally from an upper middle income background, primarily children of university faculty members. The study was approved by the Internal Review Board at Oklahoma State University for studies involving human subjects. After explaining all aspects of the study to the subjects and parents, informed consent was obtained from at least one parent and from each subject. All subjects, except for one from whom a complete blood sample could not be obtained, completed all aspects of the study.

Anthropometric Measures

Subjects were weighed and measured in light street clothing without shoes on the morning of blood collection. A calibrated beam balance scale and steel tape with right angle head board were used for these measures. The same person measured and weighed all subjects. Triplicate triceps skinfolds using Lange calipers (Cambridge Scientific Instruments, Cambridge, MD) and midarm circumference were measured on the right arm by the same individual. These measurements were compared with national norms from NHANES-I

data (10). Height percentiles were determined using maturation based growth charts (11).

Biochemical Measures

Blood Collection

Fasting venous blood was drawn using 21 gauge stainless steel butterfly needles (Deseret Medical, Inc., Sandy, Utah) and plastic syringes (Sarstedt, Numbrecht, W. Ger.) with heparin, which had been passed through a chelating resin column (Chelex 100, Bio-Rad Industries, Richmond, CA), as an anticoagulant. Blood samples were kept on ice until processed. Plasma was separated by centrifugation at 1520 x g for 22 min at 4° C. Plasma was immediately transferred with polyethylene pipets to acid washed polyethylene storage tubes. Plasma for total and high density lipoprotein (HDL) cholesterol was refrigerated for no more than 24 h until analysis. Plasma for DHEAS determinations was stored frozen.

Hemoglobin and Cholesterol Measures

Hemoglobin was determined on whole blood within three hours of drawing by the cyanmethemoglobin method (Sigma Procedure 525, Sigma Chemical Co., St. Louis, MO). Results were compared to norms for age. Total and HDL cholesterol were determined enzymatically (Sigma Procedures 351 and

352-3). Results were compared to those in similar aged males in the Lipid Research Clinics trials (9).

Maturation Status

Dehydroepiandrosterone sulfate (DHEAS) was used to assess maturation status. A double antibody radioimmunoassay (Cambridge Medical Diagnostics, Billerica, MA) requiring 10 uL plasma was used to assay this adrenal hormone. DHEAS concentrations were compared to reference values established for a pediatric population (1) with categories set at <3 umol/L for Group I, 3-6 umol/L for Group II and >6 umol/L for Group III.

Age

Age was determined from birth to day of anthropometric measurements and blood collection and was expressed in years. If a subject were less than 12.00 years on the day of data collection, he was classified in "Age Group 11." Those more than 12.00 and less than 13.00 were classified as "Age Group 12", and those more than 13.00 were classified as "Age Group 13." Only one subject was actually less than 11.00 and two more than 13.00 years.

Nutrient Intake

Nutrient intakes were determined using a pretested quantitative food frequency questionnaire. Portions were estimated using nonbiasing food models (12) in amounts

ranging from 1 tsp to 2 c. Subjects were asked to recall food consumed for approximately four months. Data were converted to amounts consumed per day and analyzed using the Food Processor II (ESHA Research, Salem, OR). Nutrient supplement data were obtained from label values and were included in total daily intakes. Values consumed were compared to appropriate standards for age in the Recommended Dietary Allowances (13).

Statistical Analyses

Results are presented as means $\pm$ SEM by age, DHEAS, and iron supplementation groups. Two subjects whose triceps skinfolds exceeded 2 SD of the mean were eliminated from all calculations. Correlation coefficients were calculated for variables related to growth and maturation. When significant relationships were found, analysis of variance for predetermined groups was calculated using the General Linear Model (GLM) for unbalanced groups. Regression equations were constructed to determine the variables which best predicted maturation. The Statistical Analysis System (SAS) version 5.18 (SAS Institute, Inc., Cary, N.C.) was used for all calculations.

Results

Age

Adolescence is a period of rapid growth and development. However, maturation is highly individualized

by development and not by chronological age. Means of anthropometric data for the sample were within 5% of national norms (10) in all age groups.

Age categories revealed significant differences in height at each age category but significant weight differences were reflected only between the youngest and oldest groups (Table 1). Significant differences existed for midarm muscle area (MAMA) between Age Group 11 and Age Groups 12 and 13 and not between Age Groups 12 and 13. There were no significant differences in body mass index (W/S^2) nor were there significant differences in DHEAS, hemoglobin or total and HDL cholesterol concentrations among any of the age groups (Table 1).

Maturation

DHEAS groupings documented differences in subjects' development according to maturation. Age of the subjects in the three groups was not significantly different (Table 2). Significant differences between Group I and those in Groups II and III were seen in height, weight, muscle development and hemoglobin (Table 2). Differences in body mass index (W/S^2) were found between Group I and Group II. In contrast, by age groups there was no pattern to body mass index (W/S^2). Significant positive correlations ($p<0.01$) were found between DHEAS concentration and height ($r=0.34$), weight ($r=0.35$), and MAMA ($r=0.33$), as well as significant

correlations ($p<0.05$) between DHEAS and W/S^2 ($r=0.25$) and hemoglobin ($r=0.28$).

Cholesterol

Cholesterol concentrations were similar to those reported by Christiansen (9). The tendency for cholesterol to decline during adolescence was seen in this sample by age groupings although the differences were not significant (Table 1). These trends, again not significant, were also seen between DHEAS groups I and II with groups II and III lower than those in group I. Significant negative correlations were found between cholesterol and height ($r=-0.36$, $p<0.004$) and measures of muscle development MAMA ($r=-0.35$, $p<0.005$). Differences in HDL cholesterol values between maturation groups II and III were significant, but no significant differences were found between group I and groups II or III. No differences were seen in HDL levels with age changes.

Hemoglobin

The differences in mean hemoglobin concentration among the DHEAS groups were between Group I and Groups II and III (Table 2). The same pattern of significance among the DHEAS groups existed for hemoglobin as for other measures which were associated with greater maturation: height, weight, W/S^2, and MAMA. Mean hemoglobin levels were the same for

all age groups. Hemoglobin levels were significantly correlated with DHEAS ($r=0.28$, $p<0.03$) but not with age.

When subjects were grouped by those receiving supplements containing iron and those not receiving supplemental iron, no differences were seen in age, DHEAS, hemoglobin, height,or weight (Table 3). While there were differences in estimated mean intakes of nutrients between these two groups, there were no differences in the nutrient ratios of iron:vitamin C, zinc:copper or iron:zinc. Hemoglobin was not significantly related to total intakes of any of these nutrients nor was it related to the ratios of nutrients in the diet. Nutrient intake as estimated in this study did not affect hemoglobin level.

Prediction of Dependent Variables

Height, weight, W/S^2, MAMA and hemoglobin were all related either to age or DHEAS or both. Regression coefficients were calculated with age and DHEAS as independent variables and these characteristics of maturation as dependent variables to determine the better predictor. DHEAS was a better predictor for W/S^2, MAMA, and hemoglobin and was similar to age in predicting weight. Age was a stronger predictor than DHEAS only for height.

To illustrate the interplay of these variables in relation to a maturation characteristic a regression equation was generated for MAMA using DHEAS, weight,

hemoglobin, age, W/S^2, and height as variables. The R^2 was 0.55 and overall significance was $p<0.0001$.

Nutrient Intake

This sample was well nourished as evidenced by adequacy of mean nutrient intakes. As a group, nutrient intakes exceeded the RDA for protein, iron, zinc, calcium, folacin and vitamin C. No differences were found in nutrient intakes either between age groups or between maturation groups except for protein. Overall, subjects in DHEAS group III reported consumption of significantly less protein than the subjects in DHEAS group II; however, the calculated intake for all groups was well above the Recommended Dietary Allowance (13) for adolescents. Mean copper intake was slightly below the lower limit of the estimated safe and adequate level for adolescents. While intakes of vitamins and minerals were higher in supplement users, most users consumed multivitamin or multivitamin-mineral preparations resulting in no change in mean ratios of nutrients.

Discussion

Adolescence is a period of rapid growth, maturation and self-consciousness. The age of onset of puberty is highly variable, generally occurring later in males than in females. Because nutrient needs and nutritional status may vary with maturational state, assessment of nutritional status in this age group should consider the pubertal

changes in the individual. Due to the self-consciousness in this age, maturation assessment without the embarrassment of physical examinations should increase willingness to participate in research. Reiter et al. (14) have reported continuous increases in DHEAS with increases in bone age up to bone age of 16 y. Katz et al. (2) also related DHEAS levels to bone age. Those with low levels of DHEAS had delayed bone age, and increasing levels of DHEAS were associated with increases in bone age. Lee and Migeon (3) found that elevations of DHEAS occurred after Tanner pubertal Stage I and occurred with the appearance of pubic and axillary hair. In a large pediatric population Babalola and Ellis (1) related plasma DHEAS to Tanner's stages of sexual maturation. Plasma DHEAS <3 umol/L was not found in those males in Stage V pubertal development. Those between 3 and 6 umol/L were primarily in Tanner's stages II and III. Considerable overlap in DHEAS levels were found in Tanner' Stages IV and V. Katz et al. (2) also found significant relationships between DHEAS and increased body fat. In this study evidence for DHEAS as a marker for maturation was given support by increases in body mass index, arm muscle area as well as increased height and weight that occurred with increases in plasma DHEAS concentration.

Dallman and Siimes (4) developed percentile curves for hemoglobin levels throughout infancy and childhood showing an increase in the slope of the curve at age 12 y; the increased slope was particularly noticeable for those above

the 90th centile. Others have also related increases in hemoglobin to increased age (5-7). Several groups (4-6) have expressed the need for determination of sexual maturation in interpretation of hemoglogin values because the onset of puberty contributes to the spread in values seen within age groups. Variability in iron status measures was commonly found in males between the ages of 11-14 when NHANES-II data were analyzed (5). The data did not show significant differences between hemoglobin by age groups but a noticeable change in hemoglobin values occurred as the subjects changed from the DHEAS group I to DHEAS group II ($p<0.03$). The fact that the hemoglobin level did not appear to be related to dietary factors or to the use of nutrient supplements in adolescent male populations has also been documented by others (6,15).

Screening children for cholesterol levels as a means of predicting coronary artery disease risk in adulthood has been discussed for several years. Based on present knowledge the American Academy of Pediatrics does not recommend routine screening at this time nor do they recommend dietary changes for children and adolescents (16). Cholesterol values have been shown to decrease with increasing age during adolescence (9). Other studies have related childhood risk factors to adult coronary artery disease (17-19). In this study plasma cholesterol concentration was negatively correlated with age, DHEAS, and hemoglobin, but these relationships were not significant.

Nevertheless, cholesterol was negatively correlated with height ($p<0.004$), weight ($p<0.01$), and MAMA ($p<0.005$), measures which were positively associated with DHEAS levels ($p<0.008$). The decrease in cholesterol concentration was greater between DHEAS groups I and II than were the decreases between any of the age groups. Perhaps there were no significant differences in cholesterol levels either by age or by maturation groups in our sample because our subject selection was from a deliberately narrow age range in the attempt to identify maturation changes.

It is well known that during puberty males increase rapidly in height and undergo great changes in body composition. The data support the relationship between DHEAS and maturation when evidence of maturation includes the characteristics of increased height, weight, muscle development (MAMA), W/S^2, and increased hemoglobin. These differences were seen between DHEAS group I (DHEAS <3 umol/L) and group II (DHEAS 3-6 umol/L). While chronological age also separates some of the maturation characteristics of these subjects, fewer were significant by age groups than by DHEAS groups. Significant differences in variables were not found between the same age groups for all characteristics. Because nutritional needs of adolescents are associated with growth and development rate, determination of maturational status in survey work is important. We suggest the use of DHEAS as a relatively simple indicator of change in maturation status which

eliminates the embarrassment of physical examinations in nutritional surveys of adolescent males.

TABLE 1

Indices of growth and maturation by age groups[1]

Variable	Age 11 n=8[2]	Age 12 n=24	Age 13 n=32
Age, y[3]	11.5±0.2[a]	12.5±0.1[b]	13.5±0.1[c]
Height,cm [4]	148.0±2.1[a]	156.7±1.6[b]	161.5±1.6[c]
Weight, kg [4]	41.2±3.3[a]	46.0±1.6[ab]	49.4±1.8[b]
W/S^2	18.8±1.3	18.6±0.5	18.8±0.5
MAMA, cm^2 [4]	28.1±1.5[a]	34.1±1.3[b]	35.7±1.4[b]
Hemoglobin, g/L	134.2±3.5	137.7±3.5	144.3±2.1
Cholesterol, mmol/L	3.97±0.2	3.74±0.11	3.72±0.09
HDL, mmol/L	1.32±0.08	1.19±0.04	1.27±0.03
DHEAS, umol/L	4.1±1.0	4.00±0.4	4.9±0.3

1 Row means with different superscripts are significantly different

2 Data based on 7 subjects for DHEAS and total and HDL cholesterol

3 $p<0.0001$

4 $p<0.05$

TABLE 2

Indices of growth and maturation by DHEAS groups[1]

Variable	Group I <3 umol/L n=13	Group II 3-6 umol/L n=40	Group III >6 umol/L n=10
Age, y	12.7±0.2	12.9±0.1	13.2±0.2
Height, cm [2]	152.8±2.1[a]	159.0±1.5[b]	162.3±2.6[b]
Weight, kg [3]	39.1±1.7[a]	49.3±1.4[b]	49.1±3.6[b]
W/S^2 [3]	16.6±0.3[a]	19.4±0.4[b]	18.5±1.1[a,b]
MAMA, cm^2 [3]	28.4±1.2[a]	35.6±1.1[b]	36.0±2.6[b]
Hemoglobin, g/L[2]	131.9±3.1[a]	142.1±2.3[b]	145.5±4.1[b]
Cholesterol, mmol/L	3.94±0.14	3.70±0.10	3.76±0.08
HDL, mmol/L[2]	1.23±0.05[ab]	1.22±0.03[a]	1.36±0.06[b]
DHEAS, umol/L[4]	1.9±0.2[a]	4.5±0.1[b]	7.4±0.3[c]

1 Row means with different superscripts are significantly different

2 $p<0.03$

3 $p<0.01$

4 $p=0.0001$

TABLE 3

Characteristics of users and non-users of supplements containing iron[1]

	Non-users n=47	Users n=17[2]
Physiological characteristics		
Age, y	12.9±0.1	12.8±0.2
Height, cm	158.0±1.3	157.9±2.5
Weight, kg	47.4±1.5	46.2±2.0
DHEAS, umol/L	4.4±0.3	4.7±0.5
Hemoglobin, g/L	140.1±2.1	141.8±3.3
Cholesterol, mmol/L	3.76±0.08	3.74±0.14
HDL, mmol/L	1.24±0.03	1.27±0.04

(continued)

TABLE 3 (continued)

	Non-users n=47	Users n=17[2]
Nutrient intake characteristics		
Protein, g	110±6	124±10
Iron, mg [3]	18±1[a]	33±2[b]
Zinc, mg [3]	16±1[a]	27±3[b]
Copper, mg [3]	1.6±1.0[a]	2.7±0.3[b]
Folate, ug [3]	427±31[a]	674±53[b]
Vitamin C, mg [4]	164±18[a]	277±34[b]
Iron:zinc, mg:mg	1.18:1	1.36:1
Iron:Vit. C, mg:mg	0.16:1	0.14:1
Zinc:copper, mg:mg	10.1:1	10.6:1

1 Row means with different superscripts are significantly different

2 DHEAS and total and HDL cholesterol values based on 16 subjects

3 $p<0.0001$

4 $p<0.003$

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CHAPTER V

PLASMA ZINC IN RELATION TO GROWTH AND MATURATION IN A SAMPLE OF ADOLESCENT MALES

Introduction

Zinc is required for growth and sexual maturation. In the 1960's clear documentation of the importance of zinc in maturation was shown by investigators in the Middle East (1-4). Others have reported suspected zinc deficiency in children with growth retardation (5-10). IIn some studies zinc supplementation has resulted in significant changes in growth achievement (7,10). These studies have frequently reported greater incidence of suspected zinc deficiency in males than in females (5,7,11-13).

Because adolescence is the period of sexual maturation and rapid growth, the need for zinc would be expected to be high. Many biochemical measures have been suggested for assessing zinc status (14), yet plasma zinc is commonly reported as the only biochemical determination in studies involving zinc status (6,15-17). The present study was undertaken to examine the relationship between plasma zinc and dietary intakes, growth and maturation in adolescent males.

Methods

Sample

Sixty-six apparently healthy male adolescent volunteers between the ages of 10.6 and 14.3 years from an upper middle income background participated in a nutritional assessment study in the Spring of 1988. The study was approved by the Internal Review Board at Oklahoma State University for studies involving human subjects. After explaining all aspects of the study to the subjects and parents, informed consent was obtained from both parents and subjects.

Anthropometric Measures

One person using a calibrated beam balance scale and steel tape with right angle head board weighed and measured all subjects in light street clothing without shoes on the morning of blood collection. Triplicate triceps skinfolds using Lange calipers (Cambridge Scientific Instruments, Cambridge, MD) and midarm circumference were measured on the right arm by another individual. These measurements were compared with national norms from NHANES-I data (18). Height percentiles were determined using maturation based growth charts (19).

Biochemical Measures

Blood Collection

Fasting venous blood was drawn using 21 gauge stainless steel butterfly needles (Deseret Medical, Inc., Sandy, Utah) and plastic syringes (Sarstedt, Numbrecht, W. Ger.). Three drops of 10,000 USP units/mL heparin, which was passed through a prepackaged chelating resin which scavenges for divalent ions including zinc (Chelex 100, Bio-Rad Industries, Richmond, CA), was the anticoagulant. Blood samples were kept on ice until separated no more than three hours after collection. Plasma was separated by centrifugation at 1520 x g for 22 min at ^{4}o C and immediately transferred with polyethylene transfer pipets to acid washed polyethylene storage tubes. Plasma for zinc and dehydroepiandrosterone sulfate (DHEAS) analyses was stored frozen. Plasma for total and high density lipoprotein (HDL) cholesterol was stored refrigerated until analysis within 24 h.

Plasma Zinc

Plasma zinc was determined by flame atomic absorption spectrophotometry (FAA) using a Perkin Elmer 5100 PC Atomic Absorption spectrophotometer (Norwalk, CN) and a modification of the procedure of Rodriguez et al (20). Wavelength of 213.7 nm and slit of 0.7 nm were used with the air and acetylene flow rates set to 10.5 L/min and 1.9 L/min

respectively. Duplicate readings integrated over 3 s were used for calculations based on mean peak area.

Samples were prepared by dilution of 400 uL plasma with 1.6 mL of 0.5 % double distilled nitric acid (GFS Chemicals, Columbus, Ohio) in a laminar flow clean air hood. Water tested to contain <0.005 ug Zn/L was used in all steps of the analysis. Standards of 0 and 0.4 mg/L zinc were prepared in 30 g/L certified gylcerol in 0.5 % nitric acid. Glycerol, tested to contain undetectable amounts of zinc, was used to increase the viscosity of the standards to that of diluted plasma thereby eliminating a frequent problem in plasma analyses. Maintaining similar aspiration rates for both plasma and the glycerol solution provided more accurate results than when standards were prepared only in diluted acid because calculations were based on the peak area in a preset time period. A dilute acid solution enhanced the absorption signal to the photomultiplier tube. After each sample 0.5 % detergent (ERA, Proctor and Gamble, Cincinnati, OH) solution was aspirated to prevent clogging of the tubing, nebulizer or burner head. Water was then aspirated until the flame returned to normal color and appearance. Standards and a control sample of plasma were periodically reread. Corrections were made to readings to adjust for minor baseline drift. Care was taken throughout sample collection, storage and dilution to prevent environmental contamination.

Hemoglobin and Cholesterol

Hemoglobin was determined on whole blood within three hours of drawing by the cyanmethemoglobin method (Sigma Procedure 525, Sigma Chemical Co., St. Louis, MO) using 10 uL of whole blood. Total and HDL cholesterol were determined enzymatically (Sigma Procedures 351 and 352-3).

DHEAS

Dehydroepiandrosterone sulfate (DHEAS) was used to assess maturation status. Duplicates of 10 uL of plasma were used to assay this adrenal androgen with a double antibody radioimmunoassay kit (Cambridge Medical Diagnostics, Billerica, MA). DHEAS concentrations were compared to reference values established for a pediatric population (21).

Nutrient Intake

Nutrient intakes were estimated from a pretested quantitative food frequency questionnaire. Portions were estimated using nonbiasing food models (22) in amounts ranging from 1 tsp to 2 c. Subjects were asked to recall food consumed for approximately four months. Data were converted to amounts consumed per day and were analyzed using the Food Processor II (ESHA Research, Salem, OR). Nutrient supplement data were obtained from label values and were included in total daily intakes.

Statistical Analyses

The results are presented as means_+ SEM. Two subjects whose triceps skinfolds were more than 2 SD above the mean and one with an unusually high plasma zinc of 20.1 umol/L were eliminated from these analyses. Statistical significance was defined as $p<0.05$. After preliminary calculations of correlation coefficients, analysis of variance was calculated for predetermined groups with the General Linear Models for unbalanced groups using version 5.18 of the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

Results

Mean plasma zinc was 13.8 umol/L (900 ug/L) and ranged from 9.7 to 17.1 umol/L (633 to 1119 ug/L). Plasma zinc was not correlated with any of the anthropometric or biochemical measures in this study.

There was no relationship between plasma zinc and total intake of zinc. When subjects were grouped by zinc intake no differences were found in any of the anthropometric or biochemical measures associated with growth and maturation (Table 1). This sample was generally well-nourished; only eight of the subjects reported food intakes resulting in calculated zinc intakes of <10 mg Zn/d. Even for those eight subjects, neither growth, maturation, nor plasma zinc appeared to be affected. Calculated protein intakes were significantly lower in the low intake group than in those

consuming more zinc, but the calculated protein intake still exceeded the Recommended Dietary Allowances (RDA) for adolescent males (23). Mean reported nutrient intakes for certain nutrients which have been shown to affect zinc absorption or utilization are shown in Table 2. Mean nutrient intakes for all subjects met or exceeded the RDA (23).

Because severe zinc deprivation affects growth resulting in nutritional dwarfism, the subjects were categorized by growth achievement (Table 3). Subjects who had achieved the 25th height centile or less were grouped as short and those with greater height achievement were grouped as average. Highly significant differences were seen between these groups for height, weight and MAMA. Significant differences also existed between the groups for body mass index (W/S^2) and DHEAS. However, there were no differences in plasma zinc, hemoglobin or cholesterol. Neither dietary intakes of protein nor zinc were significantly different.

Subjects were then grouped by tertiles of plasma zinc (Table 4). None of the anthropometric or maturation-based biochemical variables were significantly different based on plasma zinc concentration. Significant differences were found in total plasma cholesterol among the lowest and highest tertiles while no differences were seen between the middle and either the lowest or highest tertile groups. These same groups had significant differences in dietary

zinc intakes, but there was no correlation between total zinc intake and cholesterol concentration.

There were significant differences in calculated protein and zinc intakes between the lowest and highest plasma zinc groups. No significant dietary differences existed between the lower two tertiles. Even though the lowest tertile of plasma zinc reported consumption of significantly less dietary zinc than the highest tertile, the mean amount was above the RDA for adolescent males.

Discussion

Zinc is known to be necessary for growth and maturation. Plasma zinc concentrations were not related to calculated nutrient intakes, to maturation or to growth variables in this study of 10.6 to 14.3 year old adolescent males. There was no significant relationship between total zinc intake and plasma zinc levels. In addition, height, weight, MAMA, and hemoglobin known from previous analyses to be related to growth and maturation were not significantly different based on total zinc intake. The mean intake of zinc in this sample was above the RDA for adolescents; these subjects appeared to be adequately nourished based on growth achievement.

Supplements of iron and folacin have both been related to inhibition of utilization of zinc (24-28). Those subjects taking nutrient supplements did not take large doses of single nutrients; nutrient ratios did not approach

those suggested to affect zinc status (25,26,28). No relationships were found between these dietary factors and plasma zinc, growth or maturation.

While Butrimovitz and Purdy (16) found a second degree polynomial relationship in plasma zinc in males their age span was wide - infants to age 18. They also reported decreasing plasma zinc from a peak of about 12.9 y until approximately 16 when concentrations again rose. Their subjects were non-fasting, and time of blood drawing was not controlled; thus, comparisons are difficult, because plasma zinc decreases during the day and after food consumption (30).

Pilch and Senti (30) also reported lower serum zinc during adolescence than occurred in older subjects from analyses of NHANES-II data. Their data grouped subjects from 9-19 y in one adolescent group; none were fasting. At all percentile rankings adolescent values for plasma zinc were higher than those in the younger males aged 3 - 8 years. In the studies of Butrimovitz and Purdy (16) and Pilch and Senti (30) data were analyzed comparing plasma (serum) zinc levels to height. One found a second degree polynomial relationship between plasma zinc and growth index plotted against age (16) while no relationship was found between height and plasma zinc in the NHANES-II data (30). In both of these studies subjects ages were in wider groups than were in our sample. In our sample, as in the NHANES-II

sample, there was no relationship between plasma zinc concentration and any of the measures of growth.

Severe zinc deficiency in the Middle East resulted in marked growth and maturation retardation; those diets were known to be low in animal protein and high in dietary fiber (2-4). Our subjects consumed adequate amounts of protein. Meat consumption was reported daily. Even in our group with low growth achievement mean dietary protein and zinc intakes were adequate (Table 3).

In adolescent males Wagner et al. (15) reported increases in serum zinc only for those with the adult sexual maturity rating; the plasma zinc concentrations in Tanner stages 1 - 4 were similar but were significantly different from mean concentration in Tanner stage 5. Variables identified as related to sexual maturation in our study included increased height, increased muscle mass, and increased hemoglobin concentration. DHEAS was used as a marker of maturation. No relationship was found between plasma zinc and DHEAS. It was unlikely that we had subjects in adult sexual maturation stages, however, as the oldest subject was 14.3 y; the age range in our study was deliberately narrow to detect small differences during growth years. Because Babalola and Ellis (21) found widely overlapping values between Tanner stages particularly in stages four and five and wide ranges of normal adult male concentrations, it was difficult to determine if any of our subjects had achieved adult sexual maturation.

Experimental studies have investigated plasma zinc levels in different stages of restriction. Baer and King (31) found that plasma zinc fell less rapidly than did urinary zinc and declined to <10.7 umol/100 g (70 ug/100 g) only after urinary zinc declined to <2.4 umol/d (150 ug/d). After seven weeks on a diet restricted to 5.5 mg Zn/d Wada et al (32) found mean serum zinc declined only 0.9 umol/L which was insignificant. Rabbani et al. (33) reported that plasma zinc fell more slowly than did lymphocyte zinc during depletion; lymphocyte zinc declined significantly after eight weeks of depletion. Plasma zinc declined and increased with depletion and repletion, but the differences between baseline, depletion, and repletion were not significant. The mean zinc content of the depletion diets was 4.8 mg/d. Thus, in our subjects with relatively high reported mean zinc intakes, the lack of low plasma zinc concentrations seems reasonable.

Blood is an easily accessible body tissue. Ease of access does not suggest that all components will be equally useful in monitoring nutritional status. The present study found relationships between easily determined anthropometric variables and maturation, but these were not related to the plasma zinc level. Zinc nutrition is important in the maturation process and should be assessed in populations during growth years. From NHANES-II data Pilch and Senti (30) estimated that the occurrence of low plasma zinc levels was about 1% in the 9-19 y male age group. Low plasma zinc

was defined as less than 70 ug/dL (10.71 umol/L). They expressed concern that the concentration defined as low has been arbitrarily used in other published reports. If <70 ug/dL as the definition of low plasma zinc is accepted, only two of our subjects met the criteria. These occurrences of low plasma zinc are actually more than would be expected if the incidence were 1% of the adolescent population. Neither of these adolescents was short nor had low plasma DHEAS concentrations.

Further, Abdulla (34) stated that 98 % of the body zinc was intracellular and that plasma zinc may only be a transport medium. If plasma contains only 2 % of the body zinc, the lack of association between plasma zinc and growth and maturation seemed rational. Abdulla also stated that clinical signs of zinc deficiency were generally not seen until plasma zinc was <0.6 mg/L (9.1 umol/L). None of our subjects had plasma concentrations <9.7 umol/L.

It is possible that during maturation zinc deficiency must be severe and coupled with other physiological stresses before plasma zinc is affected. Those severely affected in the Middle East consumed diets low in animal protein, practiced geophagia, and had high fiber intakes with few fresh fruits and vegetables. None of our subjects reported such restricted food intakes, and none acknowledged geophagia.

Based on national estimates of potential low plasma zinc concentrations and the lack of relationships to

anthropometric and maturation measures, plasma zinc does not appear to be a useful measure of zinc status for nutritional surveys of healthy adolescent males. There may not be a "single" indicator for zinc status; concentrations of zinc in other relatively accessible tissues such as erythrocytes and various white blood cells as well as zinc dependent enzymes should be investigated for relationships to growth and maturation. Perhaps the survey assessment tool for zinc will involve multiple measures as has been found necessary for iron.

TABLE 1

Plasma zinc, growth and maturation measures by zinc consumption[1]

Variables	<10 mg Zn/d n=8	>10 mg Zn/d n=5^{5}2	Mean n=63
Age, y	13.1±0.2	12.8±0.1	12.8±0.1
Height, cm	157.9±2.0	158.0±1.3	158.0±1.2
Weight, kg	48.3±3.8	46.9±1.3	47.0±1.2
W/S^2	19.3±1.2	18.6±0.3	18.7±0.3
MAMA, cm^2	33.4±2.0	34.3±1.0	34.2±0.9
Hemoglobin, g/L	140.4±3.5	140.2±2.0	140.3±1.9
Cholesterol, mmol/L	3.89±0.15	3.73±0.08	3.75±0.07
HDL, mmol/L	1.20±0.04	1.25±0.03	1.24±0.02
DHEAS, umol/L	5.6±0.8	4.2±0.2	4.4±0.2
Plasma zinc, umol/L	13.4±0.7	13.8±0.2	13.8±0.2
Dietary protein, g [3]	57±13[a]	123±5[b]	114±5
Dietary zinc, mg [3]	8+3[a]	21+1[b]	19±1

1 Row means with different superscripts are significantly different

2 DHEAS, total and HDL cholesterol and plasma zinc based on 54 subjects

3 $p<0.0001$

TABLE 2

Mean daily intakes of selected nutrients

Variable	Mean n=63	Range
Protein, g	114±5	34-239
Dietary fiber, g	22±1	6-63
Folates, ug	495±30	124-1249
Vitamin C, mg	196±17	19-668
Iron, mg	22±1	7-48
Zinc, mg	19±1	4-52
Copper, mg	2±0.1	0.5-5
Iron:zinc mg:mg	1.2:1	0.5-3.4:1

TABLE 3

Plasma zinc, dietary protein and zinc, growth and maturation by height groups[1]

Variable	Short <25th centile n=9	Average ≥25th centile n=5[4]2
Age, y	13.2±0.2	12.8±0.1
Height, cm [3]	148.8±1.6[a]	159.6±1.2[b]
Weight, kg [4]	37.9±1.2[a]	48.6±1.3[b]
W/S^2 [5]	17.1±0.3[a]	19.0±0.4[b]
MAMA, cm^2 [6]	28.7±1.7[a]	35.1±1.0[b]
DHEAS, umol/L	3.3±0.6	4.6±0.3
Hemoglobin, g/L	134.1±4.0	141.3±2.0
Cholesterol, mmol/L	4.02±0.18	3.71±0.07
HDL, mmol/L	1.28±0.03	1.24±0.03
Plasma zinc, umol/L	13.8±0.4	13.8±0.2
Dietary protein, g	96±12	117±6
Dietary zinc, mg	20±5	19±1

1 Row means with different superscripts are significantly different

2 DHEAS, total and HDL cholesterol and plasma zinc based on 53 subjects

3 $p<0.001$

4 $p<0.002$

5 $p<0.05$

6 $p<0.02$

TABLE 4

Growth, maturation and related variables by plasma zinc tertiles[1]

Variable	Lowest Tertile	Middle Tertile	Highest Tertile
		umol/L	
	<13.1	13.1-14.5	>14.5
	n=21	n=20	n=21
Age, y	12.7±0.1	13.1±0.1	12.9±0.2
Height, cm	158.1±2.0	158.2±2.0	158.5±2.0
Weight, kg	47.6±2.1	47.9±2.5	46.0±1.8
W/S[2]	18.9±0.6	18.9±0.7	18.3±0.6
MAMA, cm^2	33.3±1.3	35.6±0.7	33.6±1.4
DHEAS, umol/L	4.8±0.5	4.2±0.4	4.3±0.3
Hemoglobin, g/L	138.0±3.6	138.4±3.5	144.1±2.2
Cholesterol, mmol/L	23.61±0.13[a]	3.70±0.10[ab]	3.94±0.12[b]
HDL, mmol/L	1.21±0.03	1.24±0.03	1.27±0.05
Plasma zinc, umol/L	312.1±0.[2]a	13.7±0.1[b]	15.5±0.2[c]
Dietary protein, g[4]	100±9[a]	106±9[a]	137±9[b]
Dietary zinc, mg[4]	16±2[a]	19±2[ab]	21±2[b]
Dietary iron, mg	20±2	23±2	23±2

1 Row means with different superscripts are significantly different

2 $p<0.05$ 3 $p<0.0001$ 4 $p<0.02$

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CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

Sixty-six white adolescent males between 10.6 and 14.3 years were assessed for growth, dehydroepiandrosterone sulfate (DHEAS), and plasma zinc in the Spring of 1988. The study was approved by the Internal Review Board at Oklahoma State University, and informed consent was obtained from each subject and at least one parent. Subjects were interviewed for health background, use of medications and nutrient supplements and frequency of food consumption. Each subject was weighed and measured for height, arm circumference and triceps skinfold on the day that fasting blood samples were drawn.

Significant differences among groups were found for height, weight, and mid-arm muscle area (MAMA) when subjects were grouped by age. The differences were not found between the same age groups for all measurements.

When subjects were grouped by DHEAS categories, significant differences existed between the lowest group and the two higher groups for height, weight, body mass index

(W/S^2), MAMA and hemoglobin concentration. All of the increased anthropometric measurements and the increased hemoglobin concentration are characteristics of physiological maturation and support the use of DHEAS as a marker for maturation in adolescent males.

Plasma zinc concentration was not significantly related to age, DHEAS concentration, or to dietary intakes of protein or zinc. Except for two subjects all had plasma zinc concentrations within the commonly accepted normal range. The lack of association of plasma zinc with any of the maturation or growth measures or with dietary zinc suggests that in apparently healthy adolescent males, plasma zinc may not be a good indicator of zinc status.

Conclusions

The objectives of the study included assessing adolescent males for growth, maturation and plasma zinc as parts of nutritional status evaluation. Based on the results of these analyses the following conclusions were reached.

Hypothesis One

There will be no statistically significant differences by ages in nutritional status measures among healthy male adolescents.

Hypothesis one was rejected. These subjects as evidenced by growth and estimated nutrient intake were

healthy. Differences in anthropometric measurements existed between age groups for height, weight and midarm muscle area. These differences in anthropometric measurements were not correlated to nutrient intakes and did not occur between the same age groups for all measurements. There were no differences in estimated nutrient intakes when the adolescents were grouped by age.

Hypothesis Two

There will be no statistically significant differences in growth measures relative to maturation status among healthy male adolescents.

Hypothesis two was rejected. Significant differences were found among the maturation categories for height, weight, body mass index (W/S^2) and midarm muscle area (MAMA). The differences were between the group with the lowest DHEAS concentration and the two higher groups. There were no significant differences in growth measures between the two higher DHEAS concentration groups.

Hypothesis Three

There will be no statistically significant difference in plasma zinc concentration based on maturation.

Hypothesis three was not rejected. No significant differences were found among DHEAS groups or by plasma zinc concentration. There was no significant relationship between plasma zinc and any of the measures associated with

maturation including increased height and weight, MAMA or hemoglobin.

Hypotheses Four

There will be no statistically significant difference in plasma lipids based on maturation.

Hypothesis four was not rejected for total cholesterol. Differences were found between DHEAS group I and groups II and III but these differences did not reach the significance level of 0.05. HDL cholesterol differences existed between DHEAS group II and III. The highest DHEAS concentration group had a significantly greater concentration of HDL cholesterol than did the middle group. Therefore, H_4 was rejected for HDL cholesterol.

Hypothesis Five

There will be no statistically significant relationship between dietary zinc and measures of plasma zinc, growth, maturation and plasma lipids.

Hypothesis five was not rejected. Dietary zinc was not correlated to height, weight, W/S^2, MAMA, hemoglobin, DHEAS, plasma zinc or total and HDL cholesterol. While zinc is known to be essential for growth and maturation, all except eight subjects reported an estimated food consumption providing more than two-thirds of the RDA for zinc. They reported consumption of greater quantities of protein than estimated to be needed for growth and much of this protein

was of high quality. Therefore, these subjects consumed zinc with probable high bioavailability. There were insufficient numbers of subjects with reported low zinc intakes or zinc intakes low enough to apparently affect growth and maturation. In addition, nutrients reported to decrease zinc absorption, namely high intakes of fiber, folacin and iron, were not consumed in unbalanced ratios. Therefore, it was concluded that these subjects consumed adequate levels of zinc and other nutrients for adequate rates of growth and maturation.

Adolescent males grow and develop at different ages and at different rates. The fact that only some differences were seen by ages for anthropometric measures is a clear illustration of this commonly acknowledged information. When subjects were grouped by concentrations of an adrenal androgenic hormone (DHEAS), the differences in growth rates were apparent. By DHEAS groups no significant differences existed for age, but the measures of growth (height, weight, W/S^2, and MAMA) were significantly different. Thus, DHEAS was useful in distinguishing those adolescents either who had not entered puberty or were in very early stages (Group !) from those who were more mature (Groups II and III). The subjects were intentionally limited in age range in order to provide groups for studying maturation, and the changes associated with it. However, if these adolescents were observed over time, significant differences might appear in other measures as maturity approaches.

The sample for this study was primarily from upper middle income families. Outwardly, they all appeared healthy. The mean nutrient intakes met or exceeded the RDA for all nutrients investigated. The fact that plasma zinc did not correlate with any of the anthropometric or biochemical measures suggests several possibilities. These boys may have all been well enough nourished to have plamsa zinc concentrations within normal limits. There was no justification for concluding that zinc malnutrition existed in this sample; however, due to the wide ranges of normal plasma zinc concentration, one cannot state that some of these males were not in marginal zinc status. If any were marginally deficient in zinc, apparently this deficiency was not great enough to affect growth in ways identified by usual anthropometric measurements. Thus, the question of zinc status still exists for these adolescents. Longitudinal data from these subjects might provide some of the answers. Zinc supplementation studies in similar males might also provide needed information on the usefulness of plasma zinc as a measure of zinc status during adolescence.

Other physiological changes that occur during maturation include changes in cholesterol and hemoglobin concentrations. Had other measures of iron status been included, one could better relate low hemoglobin to immaturity and not to iron deficiency. Nevertheless, the increases in hemoglobin concentration paralleled the changes in DHEAS concentration. Estimated dietary intake data

supported adequacy of iron consumption which also lends support to the maturation-hemoglobin relationship. Nevertheless, additional measures of iron status would clarify the maturation-hemoglobin relationship.

While statistically significant differences were not found in plasma cholesterol by either age or maturation groups, trends for decreasing concentrations for total cholesterol were apparent by age group. The food frequency questionnaire was not designed to identify sources of fat in the diet other than that contained in dairy products and meats. Therefore, no analyses could be done on the dietary fat-cholesterol relationships in these adolescents. None of these subjects had extremely elevated cholesterol concentrations, and family history of cardiovascular disease was not assessed. Based on the data collected one can only conclude that the means were within normal ranges and that decreases in cholesterol concentration with age were not clearly identified within the maturation limits of this study. The important relationship between changes in cholesterol concentration over time awaits further studies.

The lack of significant relationships between total zinc intake and growth, maturation, plasma zinc and plasma lipids was not a surprising finding. If dietary intakes were sufficient to allow adequate growth, then the differences between adequate growth and optimal cellular function would be difficult to measure. Only eight of the subjects reported estimated consumption of less than two-

thirds of the RDA for zinc. Two-thirds of the RDA is an arbitrary, but commonly used, limit. Based on the RDA the need for zinc is estimated to be 6 mg/d in preadolescent children and also 6 mg/d in adults. No estimates of need have been published for adolescents. If adolescents needed the same amount as adults or children, practically all of these subjects met or exceeded their requirement. Therefore, one would not expect to find clinical zinc deficiency.

This study introduces questions which require further study. It does provide guidelines and comparison points for those studies, however. DHEAS is relatively easy to measure; it did provide information on which to base maturation categories. Further studies could evaluate DHEAS concentration changes and its subsequent relationship to completion of maturation.

Currently information documenting growth, maturation, and plasma lipid and mineral concentrations in the same individuals does not exist in the literature. Following the changes longitudinally in this group of apparently healthy adolescent males might provide additional insight into the processes surrounding growth and maturation.

Recommendations

The following recommendations for future research were developed from this study. While support for use of DHEAS as a marker of maturation existed in this study, further

research using this hormone is needed. Adolescent males should be followed longitudinally to determine the relationship between DHEAS and other measures of growth and maturation in individuals over time.

Adolescent males should be evaluated during maturation for zinc status to determine the effect of maturation on plasma zinc and other measures of zinc status in individuals. Several possible zinc status measures including blood cells and zinc dependent enzymes need to be evaluated in adolescent males to determine those which are responsive to growth and to maturation changes for use in nutritional assessment studies of adolescents.

Adolescent males need to be studied longitudinally for the relationship between plasma cholesterol, other blood lipids and growth and maturation. Repeated measures over time related to changes in maturation may be useful in identifying characteristics of cardiovascular risk present in youth. Those characteristics so identified could lead to early intervention and treatment.

Investigations on methods of assessing dietary intakes of adolescent males are needed. Information on the reliability of food frequency records for estimating mineral intakes needs to be collected. Comparison studies of nutrients estimated from food records of various lengths in different seasons and from food frequency records need to be conducted.

An improved nutrient data base should be sought to reduce the number of food items for which estimates of composition must be made. Data bases should be identified which are frequently updated with not only complete but also reliable information regarding nutrient composition of food.

Males from low socioeconomic background need also to be studied to provide a more complete overall description of the nutritional status of adolescent males during rapid growth and maturation.

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APPENDIXES

APPENDIX A

TANNER MATURATION STANDARDS

Genital Development Stages for Males

Stage 1 - Preadolescent. Testes, scrotum and penis are similar in size and proportion to early childhood.

Stage 2 - Scrotum and testes enlarge. The skin color and texture of the scrotum change. There is little to no enlargement of the penis a this stage.

Stage 3 - Enlargement of the penis primarily in length. Additional growth of testes and scrotum.

Stage 4 - Increased size of penis primarily in breadth. Development of the glands. Continued enlargement of the scrotum and testes. Continued darkening of the skin of the scrotum.

Stage 5 - Adult genitalia in size and shape.

Pubic Hair Stages

Stage 1 - Preadolescent. No pubic hair.

Stage 2 - Slight growth of long lightly pigmented fine hair appearing primarily at the base of the penis or along the labia. Not photographable by usual techniques.

Stage 3 - Hair is much darker, coarser and more curled spreading over the junction of the pubes.

Stage 4 - Hair resembles adult in type but area covered is less than in the adult. There is no spread to medial surface of the thighs.

Stage 5 - Adult distribution and quantity of hair. Hair is spread to medial surface of the thighs but above the base of the inverse triangle.

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APPENDIX B

SUBJECT RECRUITMENT

HELP WANTED

WHO: Adolescent males ages 11 through 13

WHAT: Human nutrition research investigating zinc status in males*

WHY: Zinc is a mineral required for growth and development, for ability to taste, and for healing of wounds. There are indications that males have a greater need for zinc than females and that the need for zinc is great prior to the growth spurt of adolescence. This project will evaluate various measures of zinc status related to maturity and to growth.

HOW: Measurements include those for growth--height, weight, body fat stores; for usual dietary intake of zinc and other nutrients; and for zinc in the blood.

WHEN: January and February 1988

WHERE: Department of Food, Nutrition, and Institution Admin.
College of Home Economics
Oklahoma State University

BENEFITS: Experience in and exposure to research currently being conducted. Reports of growth achievement, hemoglobin and cholesterol levels will be provided to parents of all participants.

REWARDS: All participants will receive a movie pass, coupons for various restaurants and recreational activities, and breakfast on the day of data collection. IN ADDITION, all participants will be entered into a special drawing for one of four $25.00 gift certificates at a local sporting goods store. This drawing is for participants in this study only!!

FOR FURTHER INFORMATION CONTACT:
Andrea Arquitt, M.S., RD/LD
Instructor, Dept of Food, Nutrition, and
Institution Administration
Home: 372-8048
Office: 624-5039
watch for a letter providing more information about this project coming soon.

* This project has been reviewed and approved by the Internal Review Board at Oklahoma State University for research involving humans. It has also been reviewed and approved by a local pediatrician.

APPENDIX C

FORMS

INFORMED CONSENT FORM
for
Participants in the Research Study
Zinc Status of Preadolescent Males: An Evaluation of Measures

I give permission for my son/ward ______________________ to participate in the above named research. I understand that he will participate in the following procedures:

1. completion of a health background questionnaire;
2. completion of a food frequency questionnaire;
3. anthropometric measurements of height, weight, mid-arm circumference, and triceps fatfold thickness; and
4. twenty milliliters of blood will be drawn using sterile techniques by a certified Medical Technologist.

Two questionnaires will be completed in an interview of one hour or less. The anthropometric measurements and blood collection will be obtained in the Department of Food, Nutrition, and Institutional Administration at OSU in one time period to be arranged. The participant should not eat after midnight the night preceding blood collection.

In addition, I agree to supply background health information regarding birth weight and length, history of illnesses, and parental height, if known.

I realize that this study has been reviewed and approved by the Internal Review Board for research involving human subjects at Oklahoma State University and by a local medical doctor.

I realize that I may withdraw my child/ward from this study at any time without penalty by contacting the principal investigator at the telephone numbers listed below. I also understand that all test results will remain confidential ant that my son's/ward's name will not be associated with the results. I understand that the Department of Health and Human Services and the Federal Drug Administration may inspect the records of this investigation.

I have been informed of all procedures and I understand them.

Principal investigator:___________________________
office telephone: 624-5039
home telephone: 372-8048

Parent/guardian: ___________________________

Participant's name: ___________________________

Person obtaining consent:_________________________

Date: _________________________

Subject No.____________

Food Frequency Questionnaire
Adolescent Nutrition Study
FNIA Department
College of Home Economics
Oklahoma State University
Stillwater, Oklahoma 74078

You are participating in a project designed to identify the foods which are sources of zinc and fiber commonly eaten by teenagers In order to learn which foods are eaten I will read a list of foods to you one at a time. I will also read some frequency of consumption categories to you: "Never, more than once a day, once a day, more than once a week, number of times last week, once a week, less than once a week, but more than once a month, and less than once a month."

You will also identify from food models the usual size of the portion that you eat.

Do you have any questions?

Thank you for your help.

Before we start the actual questionnaire let us go over some examples. Below you will find listed foods which you may or may not commonly eat. I want you to think about whether or not you ever eat that food item. Then if you do eat it, please think carefully and try to estimate how often you eat it. Tell me the correct category as I state it; then you will select from the food models for the portion size.

EXAMPLES: If you eat beef 5 times a week mark as follows

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Beef					5					

If you never eat an item for any reason, mark the "never" column beside the food item and proceed to the next food item.

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	
???	X									

If you eat an item infrequently, but do eat it several times a year, please mark the "less than 1x/month" column.

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	
???								X		

If you eat some of these items in food combinations, please mark as a single item. For example, if you eat tuna-rice casserole once a week, mark both tuna and rice in addition to the tuna and rice you may eat separately.

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Apple w/ peel: 2 3/4" 154 3 1/4" 155										
Applesauce Swtn. 169 Unswtn. 168										
Banana 190										
Blueberry Fresh 197 Frozen 199										
Cantaloupe Cubes 253 Melon 254										
Fruit Cocktail Heavy 220 Juice 221 Light 222										
Grapes Thomp 233 Tokay 235										
Orange Navel 265 Valencia 267 Average 263										
Orange Juice 271										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	
Peach Fresh 280 Heavy 283 Juice 285 Light 287 (cups: Heavy, Juice, Light)										
Pear Bartlett 292 Bosc 294 D'Anjou 295 Heavy 297 Light 301 Juice 299 (halves: Heavy, Light, Juice)										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Pineapple CHUNK RINGS Fresh 304 Heavy 305 306 Juice 307 308 Light 309 310										
Plums Fresh 317 Heavy 321 Juice 323 Light 325										
Raisins 330										
Strawberries Fresh 336 Frozen 337										
Watermelon 344										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Any other fruit not mentioned?										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Gr. Beans Fresh 377 Froz. 378 Cnd 379										
Broccoli, spears Fresh 421 Froz. 422										
Cabbage raw 426 cooked 427										
Carrots raw 436 cooked 438										
Celery 447										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Corn Fresh 456 Froz. 458 Cnd. 460										
Lettuce 490										
Mixed Veg. Froz. 652 Cnd. 653										
Mushrooms Raw 496 Sauteed 497 Cnd 498										
Okra Fresh 502 Froz. 503										
Peas, Gr. Fresh 527 Froz. 525 Cnd 527										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	
Potatoes, Baked Flesh & skin 563 Flesh only 564 Skin only 565										
Potatoes Boiled 570										
Potatoes French Fries Home 575 Fast Food 1917										
Potatoes, Mashed Fresh 581 Dried 582										
Potato chips 593										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Spinach Raw 604 Fresh ckd 605 Froz 606 Cnd 607										
Squash Kind?										
Tomato Fresh 640 Cnd 642										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Any other vegetable not mentioned?										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Cheeses, yellow American 001 Cheddar 010 Brick 006										
Cheeses, White Monterey Jack 030 Mozzarella 032 Swiss 048										
Cottage 014 Cheese										
Cocoa 086										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Milk whole 67 2% 70 1% 72 Skim 74										
Yogurt Plain 139 Frt Flavored 140										
Eggs Boiled 148 Fried 147 Poached 151 Scrambled 148										
Ice cream 130										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Almonds 1510										
Cashews 1527										
Peanuts 1559										
Peanut Butter 1560										
Pecans 1564										
Sunflower Seeds 1585										
Tofu 638										
Baked Beans 395										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Beef, ground 799										
Roast Chuck 782 Rump 790 Rib 786										
Steak Sirloin 794 T-bone 796										

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Hot Dogs 938										
Pork Chops 833 Roast 839										
Ham 844										
Sausage 932										
Luncheon meats Bologna 923 pepperoni 961 liver loaf 953 other										

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Chicken Wing 879 Breast 859 Thigh 872 Drumstick 866										
Turkey Light 905 Dark 904										
Fish Fresh Water										
Fish Sticks 711										
Tuna 779										
Shrimp 771										
other fish										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Bagel 975										
Corn Bread 1095										
Dinner Roll 1031										
French Bread 988										
Hamburger 1033 Hot dog 1034 Bun										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
White, Sliced 1002										
WW Sliced 1010 (Blend)										
Graham Crackers 1067										
Saltines 1072										
Bran Muffins 1092										
Corn Muffins 1095										
English Muffins 1085										
Other Breads										

Food Item	Never	more 1x/da	1x/ da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/ mo	Fd Mod Code	Portion
Macaroni 1280										
Noodles 1283										

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Spaghetti 1293										
Pancakes 1140										
Popcorn 1289										
Rice White Regular 1267 Instant 1271										
Rice Brown 1265 Wild 1272										

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Taco Shell 1141										
Tortilla Corn 1144 Flour 1146										
Waffles 1150										
Brewers yeast 1718										
Other Breads										

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Beef & bean Burrito 1353										
Chili/Beans 1335										
Hamburger Reg/bun 1375										
Hamburger 1/4 w/bun 1376										
Cheeseburger /bun-reg 1368										

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Cheeseburger 1/4 w/bun 1369										
Macaroni/ cheese homemade 1343										
Macaroni/ cheese package										

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk 1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Meat Loaf 1350										
Pizza 1365-reg 2076 thick										
Toppings										
Refried Beans 398										
Spag. Sce without meat 1791										
with meat 1792										
Taco Beef 1360 Chix 1361										

Breakfast cereals contain a variety of nutrients and may vary considerably due to different fortification practices. In the next section please name ALL of the breakfast cereals you eat and give the frequency of consumption.

Food Item	Never	more 1x/da	1x/da	more 1x/wk	# times week	1x/wk	<1x/wk >1x/mo ?x 1st month	<1x/mo	Fd Mod Code	Portion
Dry Cereals										
Cooked Cereals										

Subject No.__________

HEALTH INFORMATION QUESTIONNAIRE
Adolescent Nutrition Survey
FNIA Department
Oklahoma State University

Health History: Subject
Birth date:__________
Birth length:__________
Birth weight:__________
Race: W___ B___ Other__________

Do you have or have you ever had any of the following:

Inherited disease	No	Yes	Specify__________
Uremia	No	Yes	Specify__________
Sickle cell anemia (or sickle cell trait)	No	Yes	
Neoplastic disease	No	Yes	
Diabetes	No	Yes	
Liver diseases	No	Yes	Specify__________

Are you on any type of special diet:

Food allergies?	No	Yes	Specify__________
Weight loss?	No	Yes	Specify__________
Weight gain?	No	Yes	Specify__________

Other? (Specify)__________

Have you recently had any of the following:

Serious illness	No	Yes	Specify__________
Surgery	No	Yes	
Fractured bones	No	Yes	Specify__________
Dermatitis or skin rash	No	Yes	Diagnosed?__________
Weight gain	No	Yes	How much?__________
Weight loss	No	Yes	How much?__________

Subject No. ____________

Do you currently use any drugs or medications? ______________________

____

Do you take any nutrient supplements? No Yes Specify: ____________
Brand ____________
Number per day ____________

Have you ever been treated for anemia? No Yes When? ____________
How long were you treated? ____________
Was your blood retested after treatment? ____________
Was the treatment considered successful? ____________

How would you describe your appetite?

Excellent
Good
Fair
Poor
I'm really not interested in eating

Do you ever eat or crave substances not usually considered as food?

Dirt	No	Yes
Clay	No	Yes
Starch	No	Yes
Bones	No	Yes
Ice	No	Yes
Other (specify)	No	Yes

What is your impression of your overall health?
Excellent
Good
Fair
Poor
Very poor

Family Background

Mother's barefoot height ____________
Father's barefood height ____________
Mother's occupation ____________
Father's occupation ____________

APPENDIX D

PROCEDURES FOR ANTHROPOMETRIC MEASUREMENTS AND GROWTH CHART

Weight Measurement

Subjects were weighed to the nearest quarter pound on a previously calibrated double beam balance scale in light street clothing.

1. To calibrate the scale six labeled concrete blocks were weighed individually and in planned combinations.
2. Total weights were compared with the sum of the weights of the individual blocks.

Height Measurement

Subjects were measured to the nearest sixteenth of an inch standing in street clothing without shoes.

1. Subjects stood with calves, hips, shoulders, and the back of the head against a surface perpendicular to the floor to which a steel tape was affixed.
2. With the subjects looking straight ahead, a right angle head board was brought down against the perpendicular surface to the top of the subjects head.
3. Height was read at the point at which the head board met the top of the subjects head.

Mid Arm Circumference

1. Stand subject erect with the right arm bent at the elbow.
2. Using a non-woven tape the midpoint of the upper arm was marked by measuring the length between the tip of the acromial process of the scapula and the oleocranon process of the ulna.
3. The midpoint was marked with a small dot using a felt tipped pen.
4. Using an "Insert-a-Tape" (Ross Labs) and without compressing the soft tissue, the mid arm circumference is measured at the midpoint dot to the nearest 0.1 cm.

Triceps Skinfold Thickness

1. With the subjects right arm hanging at the side, a lengthwise fold of both skin and fat was grasped approximately one inch above the mid arm dot.
2. Using Lange calipers which were held parallel to the floor, the skinfold was measured at the midpoint mark by releasing the caliper onto the skinfold.
3. Three measurements were averaged completely releasing the skin between measurements.

Mid Arm Muscle Area Calculation

$$MAMA_{cm2} = (MAC_{cm} - (0.314)(Triceps_{mm}))^2 / (4)(3.14)$$

HEIGHT

BOYS

DATE	HEIGHT

Centiles for boys maturing at average time — 95, 50, 5

Early (+2SD) maturers — 95, 50

Late (-2SD) maturers — 5

Penis stage 2+ 3+ 4+ 5+ — 97 90 75 50 25 10 3

Pubic hair stage 2+ 3+ 4+ 5+ — 97 90 75 50 25 10 3

Testes vol. 4 ml 12 ml — 97 90 75 50 25 10 3

cm / in.

YEARS AGE

APPENDIX E

PROCEDURES FOR BIOCHEMICAL DETERMINATIONS

Cyanmethemoglobin Method for Hemoglobin Concentration
(Sigma Procedure 525, revised 1984)

1. Reconstitute Drabkin's reagent with 1000 mL deionized water; store in amber glass in the dark at room temperature.
2. Prepare Cyanmethemoglobin standard solution by reconstituting Hemoglobin standard with 50 mL Drabkin's reagent. Mix well and let stand at least 30 min. Store refrigerated protected from light.
3. Prepare standards to provide concentrations of 0, 6, 12, and 18 g/dL as follows by mixing appropriate quantities of Drabkin's reagent and the cyanmethemoglobin standard. Store tightly capped refrigerated protected from the light.
4. Label duplicate tubes for each subject.
5. Pipet 2.5 mL Drabkin's reagent into each tube.
6. Carefully pipet 10 uL whole blood into each appropriate tube. Rinse the pipet tip in the tube to remove all blood.
7. Mix well and let stand at least 15 minutes at room temperature. Color is stable for several hours.
8. Read absorbance at 540 nm.

Total Cholesterol

(Sigma Procedure 351, 1085)

1. Prepare cholesterol reagent according to label directions. Mix by gentle inversion. Store refrigerated.
2. Date all reagents as each is reconstituted.
3. Prepare fresh or pool prepared cholesterol assay solution in sufficient amounts to complete all tests.
4. Duplicate blanks and standards must be read with all samples.
5. Prepare tubes for duplicate determinations for all samples.
6. Pipet 0.01 mL deionized water into each tube labeled "Blank," and 0.01 mL cholesterol aqueous standard (200 mg/dL) into each tube labeled "standard."
7. Pipet 0.01 plasma into each appropriate sample tube.
8. Pipet 1.0 mL Cholesterol Assay Solution into all tubes.
9. Incubate 10 min in a 37° C water bath.
10. Read and record absorbance at 500 nm. Complete all readings within 30 minutes.

HDL Cholesterol

(Sigma Procedure 352-3, 1987)

1. Prepare cholesterol assay solution as for total cholesterol.
2. Prepare HDL cholesterol reagent according to label directions.
3. Label tubes for controls and samples.
4. Separate HDL fraction by:
   a. pipet 500 uL plasma into small tube
   b. add 50 uL HDL cholesterol reagent
   c. vortex
   d. allow to stand at room temperature for 5 min
   e. mix briefly by vortexing
   f. centrifuge all tubes for 10 min at 2200 x g.
5. Pipet 1 mL cholesterol assay solution into fresh subject tubes.
6. Pipet 50 uL water (bland), calibrator (50 mg/dL), or supernatant from step 4 into each tube.
7. Mix by inversion. Incubate tubes for 10 min at 37° C.
8. Read absorbance at 500 nm using the blank and calibrator for the standard curve.
9. Complete all readings within 30 min.

Dehydroepiandrosterone Sulfate Radioimmunoassay

(Cambridge Medical Diagnostics, Feb., 1987)

1. Prepare work surface in a manner suitable for radioactive isotopes.
2. Use appropriate precautions in handling all materials and waste.
3. Reconstitute reagents according to directions and store as instructed.
4. Careful pipeting is essential for optimal results.
5. Run samples and standards in duplicate; a standard curve must be run with each set of samples.

Assay Procedures

1. Label glass or polystyrene tubes as follows:

   1 - 2 Total Counts (TC)

   3 - 4 Non-specific binding (NSB)

   5 - 6 Maximum binding (B_o)

   7 - 20 Standards B - H

   21 on Samples
2. Pipet 10 uL DHEAS zero to tubes 3 - 6,
3. Pipet 1 mL assay buffer to tubes 3 - 4.
4. Pipet 10 uL of standards or sample plasma to the appropriate tubes.
5. Pipet 100 uL ^{125}I to all tubes
6. Shake the rack of tubes gently.
7. Pipet 1mL DHEAS antiserum to all tubes except 1 - 4.

8. Vortex tubes and incubate for 30 min at 37°C.
9. Pipet 200 uL goat-anti-rabbit gamma globulin to all tube except 1 & 2.
10. Vortex and incubate for 30 min at 37°C.
11. Centrifuge at 1500 - 1600 x g for 15 min at 2 - 8 °C.
12. Immediately decant the supernatant except for tube 1 & 2 (Total Counts) into a suitable radioactive waste jar. Pour off smoothly to avoid disturbing the pellet.
13. Blot the rim of each tub on absorbant paper as the decanting is completed without returning the tube first to an upright position.
14. Cap tubes. Count radioactivity in all tubes for two minutes.
15. Calculation of results may be done by computer programs designed for semi-log data.

VITA

Andrea Bender Arquitt

Candidate for the Degree of

Doctor of Philosophy

Thesis: DEHYDROEPIANDROSTERONE SULFATE, GROWTH AND PLASMA ZINC IN ADOLESCENT MALES

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