INDICES OF ZINC STATUS IN ADOLESCENT FEMALES

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CHAPTER I

INTRODUCTION

The essentiality of zinc for humans had been suggested by Todd in 1934 (1). The intake of zinc was not considered a problem, however, until clinical manifestations of zinc deficiency were identified in the early 1960's. The severe growth and sexual retardation found in adolescent males in Egypt (2) and Iran (3) was thought to be a result of zinc deficiency. Supplementation of the diet with oral zinc sulfate resulted in rapid growth and sexual maturation of these Egyptian adolescents (4). These findings were confirmed when Iranian dwarfs, fed a well-balanced, zinc-supplemented diet, had improved growth and reached sexual maturation sooner than Iranian dwarfs fed the same diet without supplementation, thus identifying zinc deficiency as the major cause of the endocrinopathies (3).

The extreme dwarfism and lack of sexual maturation represented one end of the spectrum of types of effects of deprivation of zinc. The effects of mild zinc deficiency on outwardly healthy adolescents remains to be investigated, as zinc may be a limiting factor during periods of growth. Recent evidence suggests that marginal zinc deficiency may be a problem in many nations, including the United States.

The assessment of zinc status and intake of various populations in nutrition surveys has been limited. There is currently no standardized procedure for assessing zinc status in humans (5, 6). Analyses

of zinc concentration in commonly used materials such as blood, urine, and hair are currently being questioned as indices of zinc status. The identification of zinc as a component of more than 20 enzymes stimulated interest in the use of enzyme assays as a measure of zinc status. Because of the inadequacy of any one measure, the use of several indices to measure zinc status is considered imperative at this point in time (7). In addition, relatively easy-to-conduct procedures are essential for use in nutrition status surveys.

Few data are available on the zinc intake of groups in the United States. Recent research provides evidence of marginal intake, relative to current recommendations, in segments of our population particularly in relation to low income (8) and low meat consumption (8, 9). Future surveys of populations considered to be at nutritional risk need to assess the prevalence of marginal zinc intake. One of the groups of people identified by nutrition surveys to be at nutritional risk generally is teenagers. Dietary studies for that group have shown low intakes of calcium, iron, vitamins A and C, and riboflavin (10, 11). Little information on dietary zinc was collected because standard food composition references did not include zinc content of foods. Although analyses of dietary intake indicated teenagers had lower intakes of various nutrients than were recommended, the recommendations for nutrient needs of adolescents are based on limited data, especially for protein, calcium, iron, zinc, magnesium, fluoride, iodine, and for vitamins D and C, riboflavin, and thiamin (12). As adolescence is a period of increased nutrient needs due to the demands for growth, zinc intake should be further investigated in relation to present recommendations.

Significance

Little information is available on zinc intake or status of adolescent females. In a recent study on trace mineral status, Greger et al. (13) reported that one-third of the adolescent girls consumed less than two-thirds of the Recommended Dietary Allowance (RDA) for zinc. However, only 3% of the girls were considered to be in marginal zinc status based on hair and plasma zinc levels. Indices of zinc status for adolescents, such as erythrocyte zinc, plasma ribonuclease, and alkaline phosphatase, have received little examination. Reliability of present and proposed indices needs to be determined before dietary records can be reevaluated. Consideration of the measurements best suited for nutrition status surveys is important, as information on relatively large samples needs to be obtained. Therefore, relating dietary zinc intake to indices of zinc status is an extremely important area for research.

Purpose

The purpose of this research was to determine the means and ranges of values for zinc intake, erythrocyte zinc concentration, plasma ribonuclease activity, and serum alkaline phosphatase activity among adolescent females according to age, race, and menarcheal status. In addition, the relationships among erythrocyte zinc concentration, plasma ribonuclease activity, serum alkaline phosphatase activity, and zinc intake were investigated. The practicality of these proposed measures of zinc status for use in nutrition surveys was assessed.

CHAPTER II

REVIEW OF LITERATURE

The reports relating zinc intake to impaired growth and sexual maturation, and depressed levels of various parameters of zinc status stimulated considerable research (2, 3). The World Health Organization (WHO) challenged nutrition scientists to intensify their research on trace element status of populations in 1973 (14). Specific recommendations included developing indices or parameters for measuring zinc status and assessment of the occurrence of zinc deficiency in all populations. Since then, the importance of zinc in nucleic acid metabolism (15), taste perception (8), wound healing (16), and the treatment of acrodermatitis enteropathica (17) has been identified. However, estimations of zinc status of many healthy populations, including adolescent females, have been limited.

The following presents information on the absorption and metabolism of zinc. Data on the requirement for zinc and zinc intake of adolescent females were reviewed. In addition, various methods used in assessing zinc status and their applicability to nutrition status surveys were evaluated. These include measures of serum and plasma zinc, hair zinc, urinary zinc, erythrocyte zinc, and zinc-related enzyme activities.

Zinc Absorption and Metabolism

The factors affecting zinc metabolism are not well understood.

The process and control of absorption of zinc and the factors that may enhance or hinder these processes are being studied. Research indicates that the bioavailability of zinc is a major factor in the maintenance of zinc status.

The primary site of zinc absorption is still under investigation, as few studies have been conducted with animals or humans. Pearson et al. (18) used everted gut sacs from rats and found absorption occurred primarily in the distal portion of the small intestine, by active transport. However, zinc absorption was greater from the duodenum than the distal portion of the small intestine in two studies using ligated rat intestinal sacs (19, 20).

The absorption of zinc in humans was measured using the intestinal perfusion technique (21). Four volunteers, ages 21 to 32, consumed four different meals on four consecutive days. Duodenal and jejunal samples were collected at three sites every 10 minutes from each subject for one hour prior to and five hours after each treatment. The blended meals had different compositions but were isocaloric (464 kcalories) and of similar fat content (21.0 g), volume, osmolality, and pH. Meal A contained 24.0 g protein, primarily from beef, and provided 7.5 mg iron and 4.4 mg zinc. Meals B, C, and D contained 14.8 g protein, mostly from a specially prepared breakfast cereal and milk. Mineral contents were 1.4 mg iron and 2.4 mg zinc (B); 5.3 mg iron and 5.5 mg zinc (C); and 4.6 mg metallic iron and 5.5 mg zinc (D). Iron disappeared from the intestinal juices at sites in the duodenum and proximal jejunum, with little change in iron content of juices in the distal jejunum. The bioavailabilities of the different forms of iron and with different meals were similar.

Sites of zinc absorption were difficult to measure as more zinc was found in the intestinal contents recovered from the duodenal juices than was present in the meal, indicating a mixture of endogenous and exogenous zinc. Recovery peaked at all three sites during the first two hours and was similar for all meals. The sites of zinc absorption were not identified. Further studies need to be conducted to determine the site(s) of zinc absorption.

Studies on zinc absorption reported wide ranges of estimations of efficiency. Most studies estimated retention by measuring the difference between intake and fecal and urinary output. Few studies measured zinc loss in sweat or in sloughed cells. Differences in efficiency of absorption may be due to type of zinc used, methods of administration, techniques for measuring absorption, and individual variation. Mean absorption of 38 percent of a single dose of ^{70}Zn (3.6 mg ^{70}Zn of 11 mg in the diet) was measured in 22 women consuming a semi-purified formula diet (22). The ^{70}Zn was given on day four of the 12 day metabolic study. Average absorption of 36 percent was found in five healthy male subjects given an oral dose of 15 to 24 μCi ^{65}Zn consuming an analyzed diet for 24 days (23). A range of absorption of 40 to 86 percent of an oral dose of 10 μCi carrier free ^{65}Zn was measured two hours after administration to 75 subjects in a fasting state, using the total body counting technique (24). Information on zinc retention in subjects consuming mixed diets is lacking. An estimated efficiency of absorption of dietary zinc between 20 to 40 percent has been estimated by the WHO (14) and 40 percent by the National Research Council (25).

A number of theories exist regarding the homeostatic control of zinc absorption. The presence of a low-molecular-weight zinc binding ligand (LMW-ZnBL) in intestinal secretions is under considerable debate. A number of investigators have identified various LMW-ZnBL involved with zinc absorption, including prostaglandins (26), citric acid (27, 28), and picolinic acid (29). In addition, several investigators provided evidence of LMW-ZnBL in human milk which were indistinguishable from LMW-ZnBL isolated from intestinal secretions (28, 30, 31). Additional support for LMW-ZnBL was provided when a qualitative defect in the binding affinity of LMW-ZnBL containing fraction of human pancreatic secretions in patients with acrodermatitis enteropathica (AEP) was reported (32). Cousins et al. (33), however, have questioned the presence of a LMW-ZnBL. They found no evidence of any LMW-ZnBL in freshly prepared samples of rat intestinal cytosol after oral administration of ^{65}Zn. Considerable amounts of LMW-ZnBL were found when samples were kept at 37°C for 30 minutes or 5°C for one hour. These data suggested that intestinal LMW-ZnBL were products of the isolation procedure rather than products of intestinal secretions (33, 34). Thus, the site of absorption and the mechanism of action of zinc uptake are not entirely understood.

Zinc transport across the mucosal membrane is probably by active transport (18, 35). The proposed homeostatic regulation of zinc includes a mucosal block or feedback regulation of absorption (34). The zinc may become part of cellular high molecular weight zinc metalloproteins, transferred to the plasma, or bound to metallothionein (34). Metallothionein is assumed to be an intracellular

binding protein, rich in sulfur amino acids, that binds excess zinc and traps it within the cell. When the mucosal cells are sloughed, the zinc returns to the lumen. In an experimental study using laboratory animals, metallothionein increased when additional zinc was administered by diet or parenterally (36).

Carrier proteins of zinc in the plasma from the intestinal tract to the liver were different in several studies. Cousins et al. (33) and Suso et al. (37) identified albumin as the main carrier protein in portal blood, while Evans et al. (38) identified transferrin as the major portal transport protein.

Limited data exist on the metabolism of zinc in humans. The major route of excretion is the feces, with urinary excretion relatively independent of zinc intake. In a series of studies on zinc metabolism, ^{65}Zn was administered, intravenously or orally, to patients suffering from a variety of chronic diseases (24, 39, 40). A single dose of labeled zinc chloride ($ZnCl_2$) was given intravenously to eight patients and the ^{65}Zn content of whole blood, plasma, urine, and feces was measured for 45 days (39). Thirteen minutes after injection, the blood contained 42 percent of the dose, with the plasma showing 20 percent activity. After one hour, the whole blood contained only five percent of the dose. The plasma level declined and stabilized by the tenth day with 0.7 percent of the dose, while the whole blood concentration declined to three percent and remained stable for the remainder of the period. The excretion via the feces initially was five percent and increased to 20 percent. The urinary excretion was independent of the blood concentration. Similar findings were reported by Graig et al. (41).

Blood zinc peaked between two and four hours following an oral dose of 15 to 25 uCi ^{65}Zn (23). Initially, plasma ^{65}Zn was higher than that in whole blood. The level in whole blood increased for five days and then declined, while the plasma level consistently decreased after four hours. Sixty-six percent of the tracer dose was excreted in the feces in the first three days. After 21 days, 70 percent of the dose was accounted for in the feces and two percent in the urine. Increased amounts of urinary zinc were found when chelating agents were given to patients who had previously received ^{65}Zn (40).

The distribution of zinc in the tissues was measured during autopsy in 11 patients with metastatic neoplastic disease, following an intravenous dose of ^{65}Zn (39). Zinc uptake was highest in the liver, kidney, and spleen. The uptake by the pancreas, adrenals, and thyroid was high, but slower than the previously mentioned organs. The concentration in the large and small intestine was high, while concentration in muscle tissue was low.

The mixture of endogenous and exogenous zinc must be considered in attempts to estimate true absorption as well as in assessing factors that may influence absorption, such as level of zinc in the diet, and dietary factors. Additional research is needed to determine whether changes in fecal zinc reflect differences in efficiency of absorption or changes in endogenous zinc status. Information on the absorption, transport, and metabolism of zinc is needed for improved evaluation of the zinc status of populations.

Factors Influencing Zinc Absorption

A number of factors are known to influence zinc absorption.

These include phytate, calcium, and other dietary components. In several animal studies, poor growth and other evidence of zinc deficiency developed in animals fed diets composed largely of plant proteins. O'Dell and Savage (42) fed young chickens casein- or soy-based diets and feeds treated with phytic acid. The effects of zinc supplementation of 9 ppm and 15 ppm to both conventional feeds and phytic acid-treated feeds were measured. The growth of the casein-fed chickens was used as the standard. Supplementation of the casein diet did not enhance growth, indicating zinc intake was adequate. Birds fed soy protein grew slower than the casein-fed birds, but supplementation of the soy feeds with 15 ppm zinc produced growth equal to those fed casein. Phytate reduced growth in birds fed either treated soy or casein; however, addition of 15 ppm zinc along with the phytate resulted in growth equal to the standard. Isolated soy protein contained less available zinc than the casein protein and zinc availability was further reduced by phytic acid in both. Pigs fed soy versus casein diets (43, 44) and rats fed soy versus casein or egg white diets (45) showed similar responses to supplemental zinc as the chickens above.

A negative effect of phytate on zinc status had also been noted in humans consuming moderate zinc intakes. The phytate content of the diet consumed by Iranian dwarfs was identified as a major cause of zinc deficiency (46) and zinc supplementation of the diet resulted in improved growth (3). The incidence of dwarfism was higher in Iranian villagers than Iranian urban dwellers, even though the estimated zinc content of both diets was 12 to 15 mg/day (47). Reinhold et al. (47) speculated that the difference was due to the phytate

content of the bread consumed by the villagers. The phytate content of Tanok, the unleavened wholemeal bread staple of the villagers, was greater than the phytate content of the leavened bread of the urban dwellers. Tanok had a greater negative effect on zinc, calcium, and nitrogen balance than purified bread with added sodium phytate (48).

Additional factors thought to interfere with zinc absorption include calcium, phosphate, and mixtures of food. The responses of serum zinc to the oral administration of zinc sulfate ($ZnSO_4$) with various substances were compared to determine the effect on zinc absorption. Healthy volunteers were given various levels of $ZnSO_4$ with either water, coffee, sodium phytate (102 mg phytate), sodium phosphate (48 or 480 mg phosphorus), and combinations of sodium phytate with 500 mg calcium and sodium phosphate with 500 mg calcium (49). In addition, some of the subjects were divided into two groups. They were fasted overnight before receiving each of three different test meals, five days apart. The control meals contained coffee, jam, butter, and either brown bread (102 mg phytate), or white bread (4 mg phytate). The other meals contained milk and cheese or water and dry meat, plus the food in the controls. Blood was drawn before the test feeding and at two hours after the purified substances and at one, two, three, four, six, and nine hours after all other treatments. A peak lower than that found with 50 mg $ZnSO_4$ and distilled water indicated a negative effect of the substance(s) on zinc absorption. Due to the variations within the study, direct comparisons were not made with all treatments; however, some general statements were made. Consumption of the zinc along with coffee, all meals,

and sodium phytate or phosphate gave lower peaks in serum zinc concentration compared with zinc administered with distilled water. The addition of calcium to the purified substances showed inconsistent results. Meals containing the milk and cheese showed lower peak in serum zinc compared to the other meals, regardless of the phytate content. All meals containing 102 mg phytate have lower peaks than meals with 4 mg phytate. Similar peaks were found for meals with without meat. Consumption of zinc with dairy products, brown bread, purified phytate, or phosphate showed lower peaks in serum zinc, with dairy products having the most negative effect.

The greater response of serum zinc to an oral dose of $ZnSO_4$ with water than with various substances supported the theory that dietary components interfered with zinc absorption. Studies measuring true absorption are needed to demonstrate the extent of the inhibitory effect of common foods and substances in a "typical" U.S. diet on uptake of dietary zinc.

Additional research on plant-based diets and zinc status involved investigation of the effects of a lacto-ovo vegetarian diet on plasma zinc and zinc content of whole mixed saliva or saliva sediment in 12 non-vegetarian females (50). Fasting plasma zinc was measured in all 12, and whole mixed saliva was analyzed for seven subjects, and saliva sediment was analyzed for five subjects. The plasma response to an oral dose of 50 mg zinc, given as 220 mg $ZnSO_4$, was determined for seven subjects. All measurements were taken before and after three weeks on a vegetarian diet. The meals contained an average of 15.3 $\pm$ 3.5 mg zinc; 7.8 $\pm$ 1.7 g crude fiber; 1756 $\pm$ 534 mg calcium; and 4638 $\pm$ 1322 mg phytate; and met or exceeded the 1974 RDA for all

other nutrients. Zinc content of the saliva sediment was lower after the diet than before. No change was found in the zinc content of the whole mixed saliva; however, the plasma response to the oral dose of zinc was greater after subjects consumed the vegetarian diet than before. This change was assumed to reflect increased intestinal absorption of zinc. These results supported the existing data on the negative effect of plant-based, high-calcium, high-phytate diets on zinc status.

The effects of calcium on zinc metabolism, with and without added phytate, were investigated in animals and humans. Weanling pigs fed twice their calcium requirement (1.2% diet) and zinc at 30 ppm had decreased feed consumption, with low feed efficiency, poor growth and dermatosis, compared to weanling pigs fed 1.2% calcium and 94 ppm zinc (51). The additional zinc overcame most of the negative effects of this high calcium feed. Weanling rats fed combinations of 0.8% or 1.2% calcium, 12 ppm zinc, and 0.4% or 2.0% phytic acid had slower growth than rats fed the same combinations except with 65 ppm zinc (52). Different levels of calcium (0.8 or 1.2%) without phytate had no negative effects on growth at either zinc levels.

The effects of low and high dietary calcium, with and without phytate, was measured in five male subjects living in a metabolic ward (23). Zinc concentrations in plasma, whole blood, feces, and urine were monitored after an oral dose of ^{65}Zn. The diet contained an average of 2223 kcal, 258 mg calcium, 771 mg phosphorus, and 15 mg zinc. During the second trial, calcium gluconate was added to increase the calcium to 1981 mg/day. Both diets were fed for 28

days. Similar absorption of the ^{65}Zn was found in both trials. The possible inhibition of zinc absorption with high calcium intake, by stimulating more zinc-phytate complexing, as proposed by several researchers, needs additional investigation as results are now confusing.

Zinc Requirements of Adolescent Females

Although data on human zinc requirements have been collected almost 40 years, there have been few observations of adolescent females. The requirement for a nutrient is the "minimum intake that will maintain normal function and health" (25, p. 3). The balance method was the principal technique employed in estimating zinc requirements and was based on the difference between intake and fecal and urinary excretion.

Zinc balance or net retention of zinc was reported for subjects on wide ranges of zinc intakes. Zinc retention in three male subjects fed specified levels of zinc, from 4.9 to 22.9 mg/day, for four-day periods, varied from -0.1 to +2.7 mg (53). Positive balance occurred with higher zinc intakes. During a 30-day balance study, nine women, ages 19 and 20, consumed 11.2 $\pm$ 2.6 mg zinc/day from mixed foods (54). The diets were based on five-day cycle menus. Four subjects were in slightly negative balance; one was in equilibrium; and four were in positive balance. Positive balance was measured in four women during a 27-day metabolic study consuming meat loaf, ice cream, tea, coffee, and orange juice (55). The zinc intake ranged from 16.1 to 20.9 mg, depending on food intake. Only one study reported a large apparent retention of zinc. Thirteen females, ages 17 to 27, retained an

average of 6.6 mg zinc on self-selected diets containing 9.8 to 14.4 mg zinc/day (56). The large retention was unexplained. Based on these studies, zinc intake of 12 mg/day was suggested for adults to maintain equilibrium (23, 24, 54).

Engel et al. (57) estimated preadolescent females needed to consume 6 mg zinc/day to maintain equilibrium or positive balance. Thirty-six females, ages 7 to 10, were involved in a 30-day metabolic study. Various diets were consumed for six day periods with the protein ratio varied from 74% animal : 26% plant to 100% plant protein. The dietary zinc varied from 4.6 to 9.3 mg/day, with the higher levels associated with higher animal protein. Zinc retention of 14% was found, with little differences in types of protein or zinc levels.

The effects of different levels of calcium and protein on zinc balance were measured in 15 preadolescents (58). Combinations were 25 or 45 g protein; 260 or 620 mg calcium; and 4.8 or 6.9 mg zinc. Each diet was fed for six days and apparent absorption was measured. More zinc was retained on 46 g protein (27%) versus 25 g protein (14%) diet, which is in contrast to the balance study mentioned earlier (56). Few differences were found in zinc retention between the low and high calcium levels at either protein level.

Only one study measured zinc retention in adolescent females between 12.5 and 14.5 years old (59). Fourteen subjects consumed diets with two levels of zinc (7.4 and 13.4 mg/day) and defatted soy substituted for 15% and 30% of the meat at lunch. Urinary zinc excretion was similar to those for preadolescents (57, 58) and adults (60), regardless of soy or zinc level. Fecal zinc was greater when subjects consumed 13.4 mg zinc than with 7.4 mg zinc, regardless of soy level.

Sixty-four percent of the subjects were in negative zinc balance on 7.4 mg zinc, compared to 40% on 13.4 mg zinc, indicating more girls achieved equilibrium or positive balance on higher zinc intake than on the lower zinc intake. Additional data on zinc balance of adolescent females is needed. Growth velocity peaks just prior to menarche (12) and increased zinc may be needed to maintain positive balance during this time. Investigation of the difference between pre- and post-menarcheal girls regarding zinc balance is needed.

True absorption of zinc of 2.5 to 4.0 mg/day has been estimated to support growth and maintain zinc equilibrium for children and adults (14). The balance studies with preadolescents and adults indicated dietary intakes of 6 mg and 12 mg/day, respectively, were needed to maintain equilibrium or positive zinc balance. As these studies did not account for the zinc lost in sweat and in sloughed cells, the RDA was set at 10 mg/day for children 1 to 10; 15 mg/day for all other males and females; at 20 mg/day for pregnant females; and 25 mg/day for lactating females in 1974 (25). No changes were made in the 1980 RDA for zinc (61). Additional evaluation of the zinc requirements of populations is necessary. Evaluation of the bioavailability of zinc from different foods needs to be investigated. For adolescent females, the relationships among dietary zinc, parameters of zinc status, and growth need to be studied in order to expand the information to assess zinc status.

Dietary Zinc in Adolescent Females

Studies on the zinc intake of populations were limited in part because of the lack of data on zinc content of foods. Expanded

information became available in recent years and stimulated evaluation of diets for zinc content.

The zinc content of self-selected diets of females reported in published research ranged from 1.0 to 21.7 mg for one day. The zinc content of one-day diet records of 13 females, ages 17 to 27, ranged from 11.8 to 14.4 mg (56). The zinc content of 48 one-day diet composites collected from 15 adolescents (ages 14 to 16), living at home, was 1.0 to 19.0 mg and for 21 women (ages 18 to 30), living at home or in university dormitories, was 4.8 to 21.7 mg (62).

The zinc to energy ratio of the diets, reported by White (62) for adolescents, was 8.6 $\pm$ 4.5 mg/1000 kcal; for adult women, 8.8 $\pm$ 2.6 mg/1000 kcal. The zinc content of metabolic diets was 11.2 $\pm$ 2.6 mg (54), and the zinc to energy ratio was 6.6 mg/1000 kcal (63). Zinc levels in the metabolic diets were higher in diets containing beef or liverwurst than in diets containing turkey or ham (63). No explanation for the higher zinc to energy ratio of the self-selected diets was given.

Comparison of one-day diet records collected in the fall and spring from 184 adolescent females, age 12.8 $\pm$ 0.8, showed no seasonal variation in zinc intake, with 11.3 $\pm$ 14.1 and 11.3 $\pm$ 4.7 mg, respectively (13). Although 39% of the girls in the fall and 36% in the spring consumed less than two-thirds of the 1974 RDA for zinc, all subjects had hair zinc concentrations within the normal range ($\geq$ 100 µg/g). The serum zinc concentration, measured in a sub-group of 94 girls, was also at the normal level. Only 3% of the sub-group had low serum zinc levels. These girls were similar to the total sample in all other measurements except for serum zinc. There was no

difference in either indicator of zinc status between pre- and post-menarcheal girls. The question of whether hair and serum zinc concentrations reflected zinc status was unanswered.

The mean zinc content of 132 self-selected diet composites from 22 subjects, analyzed by atomic absorption spectrophotometry (AAS), was 8.6 $\pm$ 0.5 (SE) mg (64). Based on the analyzed zinc values, 68% of the subjects consumed less than two-thirds of the RDA for zinc. The calculated zinc to energy ratio was 4/1000. Zinc status was not assessed. Fourteen-day food consumption records kept by these subjects showed low to borderline protein and energy intakes related to low zinc intakes. The diets described simply as having "large" amounts of beef and eggs contained more zinc than diets with other types of protein.

Data obtained from diet composites and calculated from composition tables indicated a wide range in zinc intakes. Several studies reported more than one-third of the subjects consumed diets with zinc content less than two-thirds of the RDA for zinc. From the study on zinc balance in adolescents, this level may be marginal in meeting the requirements for some adolescents. Interpretation of the dietary data alone cannot be used to assess zinc status of populations; however, the consistently low values indicate a need for further research on the relationship between dietary intake and zinc status, especially for adolescent females.

Indices of Zinc Status

Major technological advances in trace mineral analysis have been made in recent years. However, no single measurement is accepted as

an indicator of zinc status. The indices most often used to evaluate zinc status included plasma and serum, hair, and urinary zinc levels. In addition, erythrocyte zinc and assay for zinc-metalloenzymes have been suggested.

Plasma and Serum Zinc

The most widely used measure of zinc status is plasma or serum zinc level. However, concentrations of zinc in the blood are influenced by many factors, including serum albumin, steroids, and conditions under which the blood was drawn.

Plasma zinc is totally bound to protein, with 30 to 40% bound to an α-2 macroglobulin and 60 to 70% loosely bound to albumin (65). Some investigators demonstrated a linear relationship between serum or plasma albumin and zinc concentration in various disease states, including cirrhosis (66, 67), renal allotransplantation (68), and cystic fibrosis (69). However, Solomons et al. (70) found plasma zinc decreased while plasma albumin increased in a prospective study on the effects of unsupplemented total parenteral alimentation on plasma minerals.

The role of zinc in human protein metabolism during total parenteral nutrition (TPN) was recently assessed (71). The concentrations of albumin, transferrin, prealbumin, and serum zinc were measured before and after zinc repletion of seven severely deficient patients receiving TPN. Serum zinc concentration of $\leq$ 50 μg/dl and presence of acrodermatitis were used as evidence of deficiency. Supplementation of TPN with zinc (220 mg zinc sulfate for adults, one to three times per day and 500 μg zinc/kg/day for pediatric

patients) was the sole nutritional therapy during the 22 day study period. Serum zinc concentration increased from 21.6 ± 2.4 to 63.0 ± 8.0 µg/dl. The initially low levels of albumin, transferrin, and prealbumin returned to near normal during the treatment with the degree of improvement correlated with the half-lives of the proteins. The prealbumin levels responded the most rapidly, indicating this measure best reflected short-term changes in protein metabolism.

The influence of steroids on circulating plasma or serum zinc levels was investigated. Oral contraceptive agents (OCA) affected circulating zinc inconsistently. Differences may be due to types of OCA used, length of time on OCA, and variety of methods used to analyze circulating zinc. A higher, but not significantly different, mean plasma zinc level was found for 16 women on OCA for 19 days (189 µg/dl) than for non-OCA users (134 µg/dl) or pregnant women (117 µg/dl) (72). However, other investigators reported significantly decreased plasma zinc during OCA use (73-76). Differences in results were also reported by two investigators using hair and plasma/or serum zinc measurements. Deeming et al. (77) reported higher mean serum zinc (186 µg/dl) and lower mean hair zinc (199 ppm) in seven non-OCA users, compared to seven OCA users (149 µg/dl and 233 ppm, respectively). However, in a larger study with 69 subjects, no differences in serum zinc or hair zinc were found between OCA users and non-users after three or six months of use (78).

In addition to the factors mentioned above, the manner in which blood was drawn influenced plasma/serum zinc levels. Hemolysis of the sample during extraction or processing caused an apparent increase in serum or plasma zinc values (79). In addition, zinc

concentration of the serum was approximately 16% greater than the plasma, probably due to hemolysis, platelet disintegration, and slightly greater dilution in plasma (79). Hemolysis increased zinc because 75 to 80% of the zinc in human blood is found in erythrocytes (80). Contamination of the sample from glassware, collection tubes, and rubber stoppers represented additional factors influencing plasma and serum zinc levels (81).

Although several factors influence circulating zinc concentrations, studies have shown serum and plasma zinc reflected zinc status. Solomons et al. (70) measured the plasma zinc concentration in 13 patients with gastrointestinal disease receiving TPN. The TPN contained only 0.3 - 0.4 mg zinc/liter, given at the rate of three liters/day. Plasma zinc concentration declined during the treatment by an average of 4.9 µg/dl week, while plasma albumin increased.

The effects of a mild zinc-deficiency state in humans were investigated (82). Four male volunteers received a semi-purified diet based on texturized soy protein. The daily zinc intake during zinc restriction for two patients was 2.7 mg/day (for 24 weeks) and for two other patients, 3.5 mg/day (for 40 weeks). During zinc repletion, 30 mg zinc acetate was given for 12 or 8 weeks, along with the experimental diet, followed by an eight week period with the supplement and a standard hospital diet. The zinc concentration in the plasma, erythrocytes, leukocytes, and urine decreased during both zinc restriction regimens and returned towards normal during zinc repletion.

The simple determination of circulating zinc alone is insufficient for assessing zinc status of individuals or groups. The large

bulk of research on serum and plasma zinc have identified factors that influence these measurements but additional indices of zinc status must be measured for a more complete picture.

Hair Zinc

The zinc content of hair had been used by several investigators in assessing zinc status. Values $\leq$ 70 ppm were found in zinc-deficient individuals (83). The zinc concentration of hair in 338 healthy subjects ranging from birth to 40 years of age was determined by AAS (8). Mean values were: neonates, 174 $\pm$ 8 ppm; 3 months to 4 years, 88 $\pm$ 5; 4 to 17 years, 153 $\pm$ 5; and 17 to 40 years, 180 $\pm$ 4. In this investigation, a number of young children with hair zinc values of $\leq$ 70 ppm, who had a history of poor growth, appetite, and taste acuity were identified. In assessing the zinc status of low income preschool children, hair and plasma zinc, and alkaline phosphatase were measured for 148 children (84). The 74 low income children had less plasma and hair zinc than 74 middle income children; however, plasma and hair zinc concentrations were not correlated.

In contrast to Hambidge's data (84), a negative correlation between hair zinc and serum zinc was found by Greger et al. (13). The serum and hair zinc concentrations and dietary zinc intake of 184 adolescent females, mean age 12.8 $\pm$ 0.8, were measured in the spring and fall. Mean concentration of zinc in hair sampled during the spring was 216 $\pm$ 64 and 191 $\pm$ 36 ppm for hair resampled in the fall. No values below 100 ppm were found. The mean serum zinc value of 94 $\pm$ 15 µg/dl was reported for 102 subjects.

Additional considerations necessary in evaluating hair zinc concentrations include possible contamination of the sample from the environment, shampoo, and bleach. The content of hair zinc depends on the delivery of zinc to the root as well as the rate of hair growth. As zinc deficiency may also impair hair growth, the use of this measure requires careful interpretation (84).

Urinary Zinc

A number of problems limit the usefulness of urinary zinc as an index of zinc status. The standard procedure involves a 24-hour urine collection, which would impose significant problems in survey studies. Possibility of contamination is high and the biochemical analysis is complex and time consuming (6). In addition, both hypozincemia and hyperzincuria were reported in various conditions associated with zinc deficiency such as cirrhosis (67, 85) and TPN (86). The data on urinary zinc of populations have indicated that excretion was independent of intake.

Erythrocyte Zinc Concentration

The zinc content of erythrocytes was first investigated by Vallee and Gibson (80). Using the dithizone method, a mean value of 14.4 $\pm$ 2.7 µg/ml packed red blood cells was determined by 31 healthy adults. The zinc content of erythrocytes of premature infants, full-term infants, children up to 11 years of age, and adults was determined by Berferstram between 1948 and 1952 (87). A progressive rise from 3.8 $\pm$ 1.1 µg in full-term infants to 11.53 $\pm$ 1.8 µg/ml at age 11 was found. The value determined for 11 years of age was not

significantly different than a value of 12.4 ± 1.8 µg/ml obtained for adults.

The zinc content of erythrocytes ranged from 10 - 14 µg/ml in various studies. Comparing the dithizone method and atomic absorption spectrophotometry in 14 normal adult male volunteers, no differences were found (14.0 ± 1.5 and 13.7 ± 1.2 µg/ml, respectively) (88). Neither were differences found in fasting and postprandial erythrocyte zinc (10.1 ± 1.2 and 10.1 ± 1.0 µg/ml) or plasma zinc (103 ± 13.4 and 104 ± 13.1 µg/dl) in 10 adult subjects (89). Mansouri et al. (90) found the zinc content of erythrocytes in 51 fasting adults to be 11.8 ± 1.7 µg/ml.

Erythrocyte zinc content in various pathological conditions was reported. Measures of zinc status, including plasma, erythrocyte, hair, and urine zinc and activity of plasma ribonuclease were obtained in adult, sickle cell anemia patients (SCA), and in healthy, non-anemic adults (91). Mean values for plasma, erythrocyte, and hair zinc were lower in SCA than non-anemic adults, while mean urinary zinc excretion and plasma ribonuclease activity were greater for SCA patients. After treatment with 660 mg zinc sulfate in nine patients (for varying periods of time), plasma zinc increased, while a trend toward increased erythrocyte zinc and decreased plasma ribonuclease activity was measured. In a three-year follow-up study on SCA, plasma, erythrocyte, and hair zinc remained lower than in healthy adults (92).

Reduced erythrocyte zinc concentrations have been reported in primary zinc deficiency. Prasad et al. (2) reported reduced erythrocyte, plasma, and hair zinc in zinc-deficient dwarfs compared to normal Egyptian subjects. In addition, the removal of ^{65}Zn from plasma

was more rapid in dwarfs, while urinary and fecal zinc were lower than in normal subjects.

Ronaghy and Halsted (93) presented a detailed report on the first cases of female dwarfs identified in Iran. The women, ages 19 and 20, presented with the syndrome of dwarfism, hypogonadism, iron deficiency anemia, and geophagia similar to the male zinc-deficient dwarfs (2, 3). Initial plasma zinc levels (40 and 29 µg/dl) in both patients were low and the erythrocyte zinc level measured in one patient was 5.5 µg/ml. Treatment consisted of a 2500 kcal diet based on the RDA, 100 mg ferrous fumarate, and 120 mg zinc sulfate. In one patient, all three components were given simultaneously. The plasma zinc rose to 111 µg/dl and erythrocyte zinc rose to 10.5 µg/ml. In the second case, diet and iron supplement were given for eight months. Plasma zinc rose to 78 µg/dl and erythrocyte zinc was 11.1 µg/ml. The addition of zinc sulfate for three months resulted in a plasma zinc of 180 µg/ml and a greatly enhanced sexual maturity compared to diet and iron supplementation alone.

During a mild, dietarily induced zinc deficiency, erythrocyte zinc was measured during stabilization, depletion, and repletion (82). Erythrocyte zinc levels decreased in two patients after 12 weeks on a restricted diet containing 2.7 mg zinc/day and increased during the repletion stage, with 30 mg zinc, to slightly above the initial value. Two other subjects fed 3.5 mg zinc/day showed similar decrease and increase in erythrocyte zinc; however, only one subject's values were significantly greater after repletion. As previously mentioned, plasma zinc decreased in all four subjects during zinc restriction and increased following supplementation. Additional parameters of

zinc status were measured and were consistent with the diagnosis of mild zinc deficiency.

The use of erythrocyte zinc as a diagnostic tool in identifying zinc deficiency in humans has been suggested. Low erythrocyte zinc values may be indicators of long term rather than acute deficiency. The mechanism of zinc uptake by erythrocytes is not clearly understood. Kruckeberg et al. (94) investigated the uptake of zinc by red cells in four different media: Krebs bicarbonate buffer (KBB), phosphate buffered saline (PBS), homologous plasma, and human serum albumin (HSA) in KBB. The zinc uptake by cells suspended in the KBB was 20 to 24 times more than by cells suspended in the other media. This indicated a major control of erythrocyte zinc was the environment or the medium. The KBB medium contained no zinc-binding constituent, whereas the plasma and PBS contained protein and phosphate, respectively, as zinc-binding constituents. Zinc efflux from erythrocytes preloaded with high or low levels of zinc was small and independent of the pre-efflux zinc concentration. The zinc influx was much faster than the efflux, indicating that once zinc was associated with the cell it tended to stay. Other researchers found similar results (95, 96).

No data are currently available on the zinc content of erythrocytes in adolescent females. Knowledge of the range of zinc content in erythrocytes for this age group is essential for this index to have general application in nutrition status surveys.

Plasma Ribonuclease

The severe growth retardation found in zinc-deficient animals

and humans stimulated research in identifying the biochemical changes associated with an inadequate supply of zinc. The effects of zinc deficiency on zinc content of tissues and enzyme activities of zinc-deficient tissues in rats (97, 98) and in young pigs (99, 100) were investigated. The zinc content of various tissues was significantly lower in zinc-depleted animals versus pair-fed controls or ad libitum-fed controls. The ribonucleic acid (RNA) content of zinc-deficient tissues of the young pigs was decreased, while the deoxyribonucleic acid (DNA) content was similar to that of pair-fed or ad libitum-fed controls (100). In addition, growth retardation was noticed before the food intake was decreased in zinc-deficient animals. This early effect on growth led to the investigation of activity of zinc metalloenzymes associated with protein metabolism.

Somers and Underwood (101) measured the zinc, RNA, DNA, and protein content and activity of ribonuclease (RNase) in the testes of zinc-deficient rats. An elevated RNase activity and lowered zinc, RNA, DNA, and protein content were found in zinc-deficient animals, compared to controls. The activities of RNase and deoxyribonuclease (DNase) in testes, thymus, kidneys, and bones of zinc-deficient rats were examined (103). The DNase activity showed no differences between pair-fed controls and zinc-deficient rats, whereas the activity of RNase was increased in zinc-deficient rats. Similar findings were reported by Fernandez-Madrid et al. (15). RNase degrades RNA to 3'-mononucleotides. The action of RNase was completely inhibited by zinc ions at concentrations of 10^{-4} M (103). Thus, the zinc content of the cell regulated the activity of RNase, which, in turn, regulated the catabolism of RNA. An increased RNase activity led to decreased

protein synthesis or increased protein catabolism and was partly responsible for the growth retardation observed in zinc-deficient animals (15, 101, 102).

Despite the increase in RNase cited in the above studies, Chesters and Will (104) found the activity of RNase unaltered in the liver, kidney, and testes; increased in thymus; and decreased in esophagus of the zinc-deficient animals compared to pair-fed controls. Also, the total RNase concentration and free RNase inhibitor concentration were unaltered in the testes, kidney, and liver of both groups. The question of whether zinc deficiency alters RNase activity due to a direct effect of zinc on the enzyme system or as a generalized response to altered growth remains unanswered.

The findings of altered RNase activity in zinc-deficient animals prompted investigation of this enzyme in humans. Comparison among studies was difficult because of differences in procedures used and expression of enzyme activity. Activity, in units/minute/milliliter, indicated the difference in absorbance of the sample plus substrate minus absorbance of pre- or post-incubation controls.

Increased RNase activity had been found along with other indices of zinc deficiency in humans in a number of investigations. In patients with sickle cell anemia, the plasma RNase activity (4.4 $\pm$ 0.02 units) was greater than for the control subjects (3.1 $\pm$ 0.01 units) (91). Significantly lower plasma and erythrocyte zinc was found in the sickle cell anemia patients. After treatment with 660 mg $ZnSO_4$ between 7 and 49 weeks in nine patients, a rise in plasma zinc and a decrease in plasma RNase were found in five of the seven patients for whom complete data were available.

The activity of plasma RNase was higher during zinc depletion than during zinc repletion in all four subjects with mild primary zinc deficiency (32). Values for the two groups were 5.1 $\pm$ 0.1 and 7.3 $\pm$ 0.1 units during depletion compared to 3.7 $\pm$ 0.1 and 4.1 $\pm$ 0.1 units during repletion. These changes corresponded with the patterns in plasma, erythrocyte, and urinary zinc concentrations.

These results suggest that determination of plasma RNase activity is a useful parameter in the diagnosis and assessment of zinc status in humans. However, as a sole measure of zinc status, it is impractical, as altered levels have been found in other conditions. Higher serum RNase activity was found in weanling rats fed wheat gluten compared to rats fed animal protein (105). RNase activity decreased with increased nitrogen intake, indicating RNase activity may reflect protein status.

In studies involving humans, elevated serum RNase activity was found in 10 of 14 patients with cirrhosis and in five of nine patients with leukemia (16). Elevated plasma RNase levels were measured in young children suffering from kwashiorkor compared to healthy, age-matched, well nourished children. After treatment, the levels for both groups were similar (107). Scott et al. (108) found elevated levels of plasma RNase in 14 low birth weight infants.

Data on plasma RNase activity of normal, healthy subjects are limited. The plasma RNase activity, measured between birth and 10 days of age in 10 healthy infants (12.1 $\pm$ 2.0 units), was higher than at 20 days of age (5.8 $\pm$ 1.1 units) (107). The activity at 20 days of age was not different than the mean value obtained on

10 subjects, ages 19 - 37 (5.7 $\pm$ 0.7 units). A positive association between age and RNase activity was found when the mean value for 73 subjects (mean age of 33) was compared to the mean value for 124 subjects (mean age 78) (109). Lower serum RNase activity was reported in individuals after a 20 minute exercise period than before exercise (110).

No data exist on the plasma or serum RNase activity of adolescent females. Assessment of the range of values for healthy females is essential in order to evaluate the implications for using this measure in nutrition status surveys.

Serum Alkaline Phosphatase

The presence of zinc in alkaline phosphatase (AP) was first demonstrated by Mathies (111). AP is classed as a zinc metalloenzyme with two to three g-atoms zinc per mole of enzyme required for catalytic activity (112-114). The physiological functions of AP are not well understood. The suggested roles for this enzyme, including hydrolysis of phosphate esters, synthesis of phosphate esters, calcification, transport of substances across cell membranes, and regulation of cellular processes, are reviewed by McCombs, Bowers, and Posen (115).

Interpretation of reported AP activity is hampered because of the wide variety of assay methods resulting in different units in which activity is expressed. Expressing activity in terms of International Units (IU), defined as the amount of enzyme that forms 1 μmol product/minute is recommended (116). However, the IU does not take into account differences in substrate concentration or pH;

thus, results from various studies expressed in IU have different numerical values. Conversion factors have been employed to convert activity expressed in one method to a different method; however, this must be done with caution, as results from experiments run under different conditions are not directly comparable. For purposes of this review, reported values were converted to Sigma (Σ) units for the convenience of the reader (115).

A number of factors are known to influence serum alkaline phosphatase levels. Levels rose rapidly during the first few months of life and gradually declined throughout the first year, but remained higher than adult values (117, 118). The mean serum AP activities remained relatively unchanged from one year of age until puberty, with values consistently higher than adults (119-122). No significant differences were found by sex between 6 and 10 years of age (119, 121-124). Most investigators noted a peak in serum AP values in females earlier than in males. Peak values were reported between 11 and 12 years of age in preadolescent females (120); between 8 and 12 years of age (3.23 to 3.73 Σ units/ml) in female British school children (121); in preadolescent females between 9 and 12 years of age (6.6 to 7.3 Σ units/ml); and at 11 years of age (6.2 $\pm$ 0.1 Σ units/ml) in low income preadolescent females (125). In an early report, no peak in serum AP was found in females ages 8 to 12 (124). A fall in serum AP was noted after the peak in the above mentioned studies, with a gradual lowering to adult values within six to eight years. For adolescent males, peak activity occurred approximately two years later than for females and gradually declined, but remained higher than adult values at 18 years of age (120-126).

A number of dietary components are known to influence AP levels. The consumption of a diet high in sucrose (40% of kcal) increased serum AP values in males and females, compared with a diet containing 40% of the energy from wheat starch (127). Protein malnutrition was associated with decreased AP values (128-131), as well as depressed plasma zinc levels (131). Serum AP varied little in volunteers ingesting 300 to 2000 mg calcium per day (132). Acute magnesium deficiency in humans presented conflicting results (133-136). Patients suffering from hypophosphatemic osteomalacia had normal (135) or elevated (136) serum AP activities. In vitamin D deficiency, resulting in rickets or osteomalacia, levels of AP were greatly elevated (137-139). Serum AP was measured in surveys of the vitamin D status of populations (121, 140, 141) and in response to supplemental dietary treatment (142).

In animal studies, AP was sensitive to zinc deficiency. Induced zinc deficiency resulted in a fall in tissue AP activities in chicks (143), turkeys (144), and rats (145). Serum AP decreased in rats (146, 147), pigs (148), and Holstein cattle (149) fed zinc deficient rations.

Depressed serum AP levels were reported in humans in both severe and mild zinc deficiency. Low serum AP and low plasma zinc were reported in zinc-deficient Egyptian dwarfs (2). Zinc supplementation of the diets of zinc-deficient Iranian subjects resulted in increased AP activity (3). In contrast, Carter et al. (150) found low plasma zinc levels, but normal serum AP in a group of adolescent Egyptian boys with evidence of mild growth retardation.

Low plasma zinc, serum AP, and erythrocyte zinc were found in a young female suffering from acrodermatitis enteropathica (AE) (151). The administration of zinc sulfate improved all three indices. Similar findings were noted by Bohane et al. (152) in the treatment of an infant female suffering from AE. A positive correlation between serum zinc and serum AP in four patients with AE was reported (153).

Kasarskios and Schuna (154) retrospectively evaluated the response of serum AP in three patients who developed zinc deficiency while receiving total parenteral nutrition (TPN). Serum AP activities were within the normal range prior to zinc supplementation; however, activities rose above normal for 14 days. The values returned to normal for the remainder of the 28-day treatment period. A parallel decrease in serum AP and plasma zinc developed in children with clinical symptoms of zinc deficiency, while receiving unsupplemented TPN (155). Increased plasma zinc and serum AP followed supplementation with zinc. Similar findings were reported by Principi et al. (156).

Plasma AP was monitored carefully by Prasad et al. (82) in two males in whom a mild primary zinc deficiency was induced. The activity of the enzyme slowly declined when dietary zinc intake was 3.5 mg zinc/day. The activity nearly doubled during the eight week repletion, with zinc supplemented at 30 mg/day.

The wide area of involvement of alkaline phosphatase in various disease conditions as well as its response to factors ranging from growth rate and age to dietary components other than zinc requires care in its use and interpretation. Consideration of these factors known to influence alkaline phosphatase is essential. Serum AP

levels should be investigated in apparently healthy adolescent females in relation to the nutrient content of their diets and to other measures of zinc status for the best use of this measurement in clinical and nutritional assessment.

Summary

The best clinical assessment of trace mineral deficiency is careful observation of the clinical response to supplementation of the nutrient under controlled conditions (5-7). Plasma zinc has been the most widely used measure of zinc status. The problems of obtaining appropriate samples for analysis of zinc has limited its use in surveys. For more widespread assessment of populations, however, less tedious and less expensive diagnostic procedures for zinc nutriture are desirable. Additional parameters suggested include melanosome zinc, carbonic anhydrase activity, retinol-binding protein concentration, taste acuity, and leukocyte zinc. These measures have been reviewed by Solomons (6); however, their applicability for survey work remains to be investigated. As discussed previously, the use of erythrocyte zinc concentration, plasma ribonuclease activity, and serum alkaline phosphatase activity as indices of zinc status have been proposed. Their use in survey work seems feasible. This study investigated these three indices and zinc intake in a group of adolescent females.

CHAPTER III

METHODS AND PROCEDURES

The status of adolescent females in regard to dietary zinc, erythrocyte zinc, plasma ribonuclease, and serum alkaline phosphatase was investigated. Recognition that adolescent females were a high risk group related to nutritional health, coupled with a paucity of data on their zinc status led to this investigation.

Sample Design

One hundred and fifty adolescent females, aged 11.5 to 16.5, participated in the S-150 Southern Regional Project on Nutritional Status of Adolescent Females, served as subjects for this study. The participants were selected from girls attending schools within a 70-mile radius of Stillwater, Oklahoma. Potential participants were contacted through the schools, Girls Scouts, or 4-H Clubs. Volunteers were provided with written and oral information about the study and the procedures. Each subject and a parent or guardian signed a written consent. Girls with known metabolic disorders were not utilized as subjects.

Procedures

Dietary Intake Data

Two 24-hour recalls were obtained through personal interview by

trained survey aides. One record was obtained for the 24-hour period immediately preceding the collection of blood samples. The other record was collected at least two weeks prior to or two weeks after blood was drawn. Food models and various other aids were employed to increase the accuracy of estimations of food intakes. Information on types of foods commonly used at home was obtained during a personal interview with the parent or guardian. Nutrient totals from food and supplements were calculated using the Nutritional Analysis System.[1]

Demographic and Socioeconomic Data

Through a questionnaire, data were obtained for each subject on age, race, education of subject and parents, number of persons in the household, and other background data. In addition, a measure of physical activity indicating type and intensity was obtained using a questionnaire.

Anthropometric Data

Anthropometric measures included height, weight, triceps skinfold, and arm circumference. Measurements were taken by trained survey aides. The height was measured, without shoes, with the subject's heels against a measuring board. The subject was asked to look directly forward, hold her head straight, and stretch to maximum height. The measurement was taken to the nearest quarter inch (157) and converted to metric units. Height was expressed as a percentage relative to U.S. heights for age (158).

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[1]Nutritional Analysis System, Department of Experimental Statistics, Louisiana State University, Baton Rouge, LA.

All subjects were weighed in indoor clothing, without shoes. The weights were recorded to the nearest quarter of a pound. An adjustment was made for weight of clothing worn by each subject, using average weights of common clothing items indicated on a check list. The estimated weight of the clothing at weighing was subtracted from the total weight recorded.

Triceps skinfold and arm circumference were measured at the same site on the right arm. The midpoint of the distance between the lateral margin of the acromial process of the scapula to the tip of the olecranon was marked using a non-stretchable tape. The subject's right arm was relaxed and flexed at 90° at the elbow. The circumference was measured at this point using the tape pulled just to fit. The skinfold was measured approximately 1 cm away from the midpoint by lifting the skin parallel to the long axis of the bone with the arm in a vertical position. Lange calipers were used and the measurement recorded to the nearest 0.1 mm (157).

Clinical Data

A questionnaire, administered by a qualified nurse or physician, was used to obtain data on menstrual cycle, use of oral contraceptives, alcohol use, smoking, and medical history for the subject. Blood pressure was measured.

Blood Collection

A medical technologist drew blood samples from each subject in a fasting state. Two samples of 10 ml venous blood in heparin, one of 5 ml in ethylene-diaminetetracetic acid (EDTA) and one of 10 ml

without anticoagulant were drawn. Blood was collected in evacuated tubes according to standard procedures and kept cold. Serum alkaline phosphatase was determined on the day of collection. EDTA plasma and red cells from heparized blood were kept frozen until assayed for plasma ribonuclease and erythrocyte zinc, respectively.

Biochemical Analysis

Erythrocyte Zinc Concentration

Following separation of cells and plasma, the red cells were frozen in new plastic disposable tubes until assay. Zinc content of the cells was determined using the procedures established for use in the S-150 regional project. All glassware and plasticware used in the ashing procedures and for storage after ashing were soaked in a 50% HCl solution for 24 hours, and rinsed three times with distilled water. The materials were inverted on low ash filter paper and air dried in a closed cabinet. Teflon or teflon-coated instruments were used to handle all materials.

Between 0.47 and 0.51 g of erythrocytes, from each subject, weighed to the nearest .01 g, were placed into 30 ml acid-washed beakers; then 5.0 ml concentrated nitric acid and 2.0 ml concentrated sulphuric acid were added, and the beakers were covered with a watch glass. Blanks were included in each run and treated exactly as the samples. The beakers were allowed to sit overnight under a fume hood.

The next day the beakers were placed on hot plates and heated slowly until gas bubbles evolved. Initially the solutions darkened and dark brown fumes evolved. After 1.5 to 2 hours, the solutions

gradually cleared to a bright yellow after light brown fumes disappeared. As each solution reached this point, the watch glass was removed. The samples and blanks were left to digest for 0.5 to 1 hour, until the nitric acid evaporated, and fumes ceased, leaving only the sulfuric acid solution. If the contents of a beaker started to brown or char, indicating excess carbon, the beaker was removed from the heat and allowed to cool before resuming heating.

After the samples cleared, they were removed from the heat and cooled slightly. Then 0.5 ml 30% hydrogen peroxide was added; the solutions were returned to the hot plate, covered with watch glasses, and allowed to reflux. Additions of hydrogen peroxide were repeated approximately every 30 minutes, or when peroxide and water were driven off, until the solutions were as clear as water. This process took an additional one to two hours. Care was taken in adding peroxide to prevent boiling over. The solutions were removed from the hot plates and allowed to cool for three to five minutes. The sides of the beakers were washed down with 2.0 to 2.5 ml distilled water and solutions were heated gently to dissolve all residual solids. The solutions were cooled and diluted to 10 ml with distilled water and stored in tightly capped, acid-washed plastic bottles until analyzed.

Sample solutions and blanks were compared with zinc standards made up in 8% sulfuric acid. Certified atomic absorption standard zinc reference solution[2] of 1,000 ppm was used to prepare standard solutions ranging from 0.1 µg zinc/ml to 2.5 µg zinc/ml. An atomic

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[2]Fisher Scientific Co., Pittsburg, PA.

absorption spectrophotometer[3] was used for all readings with an air-acetylene mixture, triple slot burner head, and zinc lamp. All the manufacturer's recommendations for zinc analysis were followed for the readings, utilizing the concentration mode. For each set of samples and blanks, two readings were obtained in random order.

To adjust for potential contamination from original vacutainer tubes and plastic storage tubes, a normal saline mixture (0.9%) was prepared. The saline was placed in vacutainer tubes and inverted several times. The solution was held at room temperature for six hours and then transferred to the plastic storage tubes and stored for 48 hours prior to analysis. Zinc was not detectable.

Zinc concentration in erythrocytes was calculated:

$$\frac{\text{Absorbance of cell ash solution}}{\text{Absorbance of 1 ppm standard}} \times \text{dilution (10)} \div \text{weight of sample (g)} = \mu\text{g zinc/gram red blood cell.}$$

Plasma Ribonuclease

Plasma was separated by centrifugation and stored, frozen, in glass until assayed. Plasma ribonuclease (RNase) activity was determined according to the methods described by Roth (159) and Sigulem (107), with some modifications, using organic chemicals from a single source.[4] Additional precipitating reagent was used to obtain a clear supernatant.

The assay mixtures contained 0.35 ml of 0.024 M Tris HCl Buffer (TRIZMA - HCl) pH 7.8 at 37°C; the substrate, 0.2 ml RNA solution

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[3]Perkins Elmer 403.

[4]Sigma Chemical Co., St. Louis, MO.

(1% Type XI yeast ribonucleic acid in distilled water); and 0.05 ml sample. For each sample, post-incubation blanks were prepared with RNA added after incubation.

All samples and blanks were prepared in duplicate and kept in ice water until RNA was added, at which time the tubes were placed in a 37° C water bath for exactly 30 minutes. The reaction was stopped by the addition of 1.8 ml cold acid-alcohol, lanthanum chloride ($LaCl_3$) reagent. This precipitating agent contained 1 N HCl in 76% ethanol with 0.5% $LaCl_3$ (159). The samples and blanks were centrifuged at 2°C for 30 minutes at 2800 rpm.

The supernatant was diluted 1:10 with distilled water, and absorbance was read against distilled water at 260 nm. The units of RNase activity were defined as:

$$\frac{\text{Average absorbance of sample - average absorbance post incubation blanks}}{\text{30 minutes}} \times 1000$$

X 480 (dilution factor) = units/minute/milliliter.

Serum Alkaline Phosphatase

Activity of alkaline phosphatase in serum was determined according to the procedure of Bessey et al. (160) as modified in the Sigma Technical Bulletin (161), using organic chemicals from a single source.[5] Analysis was completed on fresh serum. Repeated analyses were done when indicated in the procedures on rapidly frozen serum, stored for no longer than one week. The substrate

[5]Sigma Chemical Co., St. Louis, MO.

para-nitrophenyl phosphate was mixed with distilled water (40 mg in 13.3 ml) and either used immediately or stored frozen, as recommended. A 0.01 M glycine buffer, pH 10.5, was prepared and stored at 0 - 5°C. Duplicate dilutions of p-nitrophenol standard solution (10 μmoles/ml) were used for the standard curve.

Duplicates for each subject were prepared and the following procedures were used. The assay mixture, 0.2 ml diluted substrate and 0.2 ml glycine buffer, was vortexed and placed in 37°C water bath to warm. Into each tube, 0.04 ml serum was pipetted, vortexed, and the tube was returned to the water bath. After exactly 30 minutes, 4.0 ml of 0.02 N NaOH was added rapidly to each tube and the solutions were gently mixed. The addition of alkali stopped the reaction and developed a color which was stable for several hours, and the intensity of the color formed was proportional to the phosphatase activity (161). Duplicate blanks were prepared each time. Absorbance of test samples and blanks, with distilled water as a reference, was read at 410 nm. To adjust for background absorbance due to the serum, 0.04 ml concentrated HCl was added to each tube to remove the nitrophenol color, and a second reading was obtained. Alkaline phosphatase units corresponding to each reading were calculated based on the calibration curve developed with p-nitrophenol standard after correction for the blanks. The corrected alkaline phosphatase activity for each subject was the average value for the second readings subtracted from the average of the first readings and expressed as Sigma (Σ) units per ml.

Statistical Analyses

The data are presented as least squares means (LSM) with standard

errors (SE). LSM are used with unbalanced designs which are "estimates of the class or subclass arithmetic mean that would be expected had equal numbers been obtained" (162, p. 249). Differences between LSM due to age, race, and menarcheal status were tested using t-tests for the average of two days' intakes of zinc, total protein, animal protein, and energy; and for erythrocyte zinc, plasma ribonuclease activity, and serum alkaline phosphatase activity.

The relationships between zinc intake and each of the other variables were investigated. Subjects were grouped in categories according to percentage of the RDA because relationships to dietary zinc may not be linear. Categories were: $\leq$50% RDA ($\leq$7.5 mg); 50 to 75% RDA (>7.5 to $\leq$11.2); 75 to 100% RDA ($\geq$11.2 to $\leq$ 15.0); and >100% RDA (>15.0). Comparisons of %-RDA-zinc categories were made among LSM for total protein, animal protein, energy, erythrocyte zinc, plasma ribonuclease activity, and serum alkaline phosphatase separately for pre- and post-menarcheal subjects.

Multiple regression analyses were used to examine the relationships of the proposed indices of zinc status being investigated to each other and to other variables that might be involved in the relationships. Detailed information on regression analysis can be found in Applied Regression Analysis by Draper and Smith (163), and the SAS handbook (162). Variables identified in the published research pertinent to each index were used in a stepwise regression procedure, with maximum r^2 improvement, to build the "best fit" model. Computer analysis involved a series of internal comparisons of the appropriate variables and selection of what appeared to be the most useful set of predictors for each index (162). The model chosen as

the "best fit" met the following criteria: an overall F with $p<0.05$; failure of r^2 to increase by 0.025 with the inclusion of the next variable; and each variable included in the model had an F with $p<0.10$. If an entered variable had $p<0.05$ but failed to increase r^2 by 0.025, the smaller, previous model was chosen.

To investigate further the possible relationships between EZN, AP, RNase, and zinc intake, certain variables were "forced" in the model developed for each index. Stepwise regression allows variable(s) to be "forced" into the model, which means the forced variable(s) are included first in the model. They remain in the model as other variables are added, regardless of whether they are significant or not. Zinc intake was "forced" in the model for EZN. For AP analysis, RNase, zinc intake, and EZN were "forced" singly or in combination. For RNase prediction, EZN and zinc intake were "forced" in the model singly or together.

CHAPTER IV

RESULTS AND DISCUSSION

Description of the Sample

The sample consisted of 150 adolescent females who participated in the S-150 regional project, Nutritional Status of Adolescent Females. Obtained data included dietary, biochemical, clinical, and anthropometric measures related to nutritional status. Data thought to be related to zinc status were analyzed. Information was collected on most subjects for each measure investigated. Statistical assessments of intakes of zinc and related dietary components, erythrocyte zinc concentration, plasma ribonuclease, and serum alkaline phosphatase were based on the data from 138 of the adolescents.

The menarcheal status (before or after onset of menstruation) and race of the subjects by age categories are shown in Table I; included were 105 white females and 33 black females. Ages ranged from 11.5 to 16.5 years. Subjects were divided into three age categories: 11.5 to 13.4 years old were in category 12; subjects 13.5 to 15.4 years old were in category 14; and subjects 15.5 to 16.5 years old were in category 16.

Due to the small number or lack of subjects in certain age categories, pre- and post-menarche sub-categories were compared first for all data analyses. Age and race effects were then evaluated within menarche category.

TABLE I

NUMBERS OF SUBJECTS BY AGE, RACE, AND MENARCHEAL STATUS

Age Category	Pre-Menarche white	Pre-Menarche black	Post-Menarche white	Post-Menarche black
12	32	3	5	4
14	6	0	35	20
16	0	0	27	6
Total 138	41		97	

Indices of Zinc Status

Zinc Intake and Related Dietary Components

The average nutrient contents of foods and supplements reported in two 24-hour recalls for each subject were calculated using a computer program. The raw data for each subject, including age in months, race, menarcheal status, average intakes of zinc, total protein, animal protein and energy, and erythrocyte zinc concentration, plasma ribonuclease activity, and serum alkaline phosphatase activity were included in Appendix A. The mean zinc intake of all subjects was 10.4 $\pm$ 0.47 (SE), with a range of 1.6 to 34.7 mg/day. The estimated range of mean zinc intakes for mixed diets consumed by Americans was 10 to 15 mg/day (6, 61). Of the three subjects with zinc intakes of 32 mg/day or more, two consumed zinc supplements averaging 15 mg and 24 mg/day, and one subject reported an average intake of 4131 kcal.

Twenty other subjects reported intakes of 15 to 24 mg zinc, without supplements. Their mean zinc intake was 18.5 $\pm$ 0.4 and an average energy intake of 2420 $\pm$ 45. Thirteen subjects reported intakes of less than 5.0 mg zinc/day. Their mean intakes were 3.7 $\pm$ 0.3 mg zinc, and 1032 $\pm$ 24 kcal/day. While the mean zinc intake was within the usual range for subjects on self-selected diets, the extremes of intake showed wide variation in zinc and energy intakes in this group of adolescents.

The least squares mean (LSM) intakes of zinc, total protein, animal protein, and energy were compared for pre- and post-menarcheal subjects. The intakes of these dietary components were significantly higher in pre-menarcheal subjects than in post-menarcheal subjects (Table II). There was no interaction of age and race within menarche categories for these dietary components. No differences in LSM for these dietary components within menarche categories were found, except that the LSM energy intake of white post-menarcheal subjects was lower than the LSM energy intake of black post-menarcheal subjects.

The RDA for energy for 11-14 year old females is 2200 kcal and for 15-18 year old females is 2100 kcal/day (61). For this study, the average value of 2150 kcal was used to represent the RDA for energy, since subjects were in both age ranges. The RDA for zinc (15 mg/day) and protein (46 g/day) are the same for girls 11 to 18 years old. Based on the RDA, the LSM for pre-menarcheal subjects were 81% for zinc, 166% for protein, and 100% of the average RDA for energy. The LSM for post-menarcheal subjects were 64% for zinc, 128% for protein, and 80% of the average RDA for energy.

TABLE II

LEAST SQUARES MEAN INTAKES OF ZINC, TOTAL PROTEIN, ANIMAL PROTEIN, AND ENERGY BY MENARCHEAL STATUS, AND BY AGE AND RACE, ACCORDING TO MENARCHE

Group	Zinc (mg)	Protein (g)	Animal Protein (g)	Energy (kcal)
Menarcheal Status				
Pre-menarche	12.2 ± 0.8*a**	76 ± 3[a]	54 ± 3[a]	2158 ± 95[a]
Post-menarche	9.6 ± 0.6[b]	59 ± 2[b]	40 ± 2[b]	1728 ± 62[b]
Pre-menarche				
Age				
12	11.4 ± 2.0	78 ± 10	52 ± 7	2312 ± 225
14	9.4 ± 3.3	73 ± 16	51 ± 13	2204 ± 378
Race				
white	11.7 ± 1.4	74 ± 7	54 ± 6	2095 ± 166
black	9.2 ± 4.0	77 ± 20	49 ± 15	2421 ± 460
Post-menarche				
Age				
12	11.5 ± 1.7	60 ± 6	39 ± 5	1724 ± 179
14	9.6 ± 0.7	60 ± 3	41 ± 2	1798 ± 74
16	9.5 ± 0.9	58 ± 3	39 ± 3	1790 ± 101
Race				
white	9.8 ± 0.8	58 ± 3	39 ± 2	1612 ± 82[a]
black	10.5 ± 1.0	62 ± 4	41 ± 3	1929 ± 107[b]

*Least Squares Mean ± Standard Error.

**LSM in a column not sharing a common superscript letter within menarche categories are different $p<0.05$, t-test. If no superscript is given, no differences within menarche categories were found.

The RDA are set as a goal for planning recommended levels of daily nutrient intakes intended to cover the needs of almost all healthy individuals (61). The RDA for adolescents are based on chronological age; however, nutrient requirements are more closely related to physiological age, since they are affected by growth and maturation and the resulting changes in body composition (12). Nutrient needs during adolescence are greatest during pubescence (period of sexual development ending with the capacity for reproduction), usually occurring between 10 and 13 years of age in females. After this period, growth is slower and nutrient needs gradually decrease to adult levels by the end of adolescence. Due to difficulties in assessing physiological age in adolescents and estimating nutrient requirements on this basis, recommendations are based on chronological age (12). Overall, the intakes of selected nutrients of pre-menarcheal subjects in the S-150 study was greater than the intakes of post-menarcheal subjects, which may reflect increased food intake in younger girls to meet the demands for growth. The intakes of the nutrients other than total energy, however, were not affected by age or race, within menarche categories, indicating menarcheal status was the major factor influencing intakes among those investigated.

The relationships between zinc intake and the other dietary components (protein, animal protein, and energy) were investigated by grouping all subjects according to percentage of the RDA for zinc (%-RDA-zinc). The %-RDA-zinc categories were: less than or equal to 50% RDA ($\leq$7.5 mg); 50 to 75% RDA(>7.5 to $\leq$11.2); 75 to 100% RDA (>11.2 to $\leq$15.0); and greater than 100% RDA (>15.0). As there

were differences in intakes of total protein, animal protein, and energy (see Table II) between pre- and post-menarcheal subjects, the LSM for these dietary factors were compared among %-RDA-zinc categories only within each menarche category (Table III).

TABLE III

LEAST SQUARES MEAN INTAKES OF TOTAL PROTEIN, ANIMAL PROTEIN, AND ENERGY BY %-RDA-ZINC, ACCORDING TO MENARCHE

%-RDA-zinc	N	Total Protein (g)	Animal Protein (g)	Energy (kcal)
Pre-menarche				
≤50	10	50 ± 8*a**	35 ± 6[a]	1660 ± 228[a]
50-75	9	67 ± 8[a,b]	47 ± 6[a,b]	1897 ± 212[a,b]
75-100	12	82 ± 7[b]	59 ± 5[b]	2321 ± 204[b]
>100	10	111 ± 9[c]	86 ± 6[c]	2817 ± 244[b,c]
Post-menarche				
≤50	37	43 ± 2[a]	28 ± 2[a]	1321 ± 84[a]
50-75	30	61 ± 3[b]	40 ± 2[b]	1772 ± 92[b]
75-100	19	72 ± 3[c]	48 ± 3[c]	2069 ± 108[c]
>100	11	80 ± 4[c]	57 ± 3[d]	2165 ± 140[c]

*LSM ± SE.
**LSM in a column not sharing a common superscript letter are different within menarche categories, at $p<0.05$, t-test.

As shown in Table III, the intakes of total protein, animal protein, and energy increased with increased zinc intakes. The association between dietary zinc, total protein, and energy had previously been reported (59, 63, 64). Different sources of protein, however, altered zinc content in metabolic diets (63); zinc content was higher in diets including beef and liverwurst than in diets containing turkey or ham. The diets of the pre- and post-menarcheal subjects with $\leq$50% RDA for zinc probably supplied adequate total protein (91 to 98% RDA), but low energy intakes (61 to 77% average RDA). Diets with 75 to 100% of the RDA for zinc contained adequate total protein (158 to 177% RDA) and close to the recommended intake of energy (96 to 108% RDA). The difference in zinc intake probably reflected different choices of animal protein.

Recent reports indicated that one-third or more of the adolescents studied consumed less than two-thirds of the RDA for zinc (10 mg/day). Fifty-five percent of the subjects in the S-150 study consumed less than two-thirds of the RDA for zinc, compared to 37% reported by Greger et al. (13) and 33% reported by White (62). In a balance study with 14 adolescent females (mean age 13.2 $\pm$ 0.6), 40% of the subjects were in negative zinc balance on 13.4 mg zinc/day (89% RDA) and 64% were in negative zinc balance on 7.4 mg/zinc/day (49% RDA), on diets containing mixed foods (59). Although zinc intake cannot be used by itself to indicate deficiency states, these studies reported a large number of subjects below the estimated usual U.S. adult zinc intake of 10-15 mg/day. Understanding the relationship between zinc intake and measures of zinc status is essential to interpret these low intakes.

Table IV showed the mean zinc, protein, and energy intake and zinc-to-energy ratio for the S-150 subjects compared to values reported by others for adolescents consuming self-selected diets. Subjects reported by Greger et al. (13) were 12.8 $\pm$ 0.8 year old females, with menarche experienced by 39%. Subjects in White's study (62) were 14 to 16 years old. No information on menarcheal status was given. In the S-150 project, ages ranged from 11.5 to 16.5 years and 29% were pre-menarcheal. The zinc-to-energy ratio equivalent to the RDA for adolescent females would be 7.0 mg/1000 kcal (61). The adolescent females in the S-150 project consumed 5.5 mg zinc/1000 kcal. The analyzed composites of one day self-selected diets of 15 adolescents (62) contained a higher zinc-to-energy ratio (8.8/1000) than the self-selected diets in Greger's et al. (13) and in the S-150 study. No information was available to explain the higher zinc-to-kcal ratio in White's study (62). Analyzed metabolic diets contained zinc-to-energy ratios of 5.5/1000 (60) and 6.6/100 (63), indicating the zinc content of self-reported diets was similar to the zinc content of analyzed diets.

Several dietary substances reported to interfere with zinc absorption have been fiber, phytic acid, and combinations of calcium and phytic acid. The mean crude fiber content of the diets of the subjects in this study was 2.5 g/day. This level was below the reported crude fiber intake of 7.8 g/day adversely affecting zinc status in 12 non-vegetarian females (50), and below the level causing negative zinc balance reported in two adult subjects on analyzed diets with 30 g dietary fiber/day (mostly in the form of whole meal bread) and 19 mg zinc (164). The mean calcium intake in subjects in

the S-150 project was 822 ± 32 mg/day. Low or high calcium intakes did not affect zinc absorption (23, 58), but combinations of calcium-phytic acid had a negative effect on zinc absorption (59). The phytic acid content of the diets of subjects in the S-150 project was not evaluated.

TABLE IV

COMPARISON OF MEAN DIETARY ZINC, PROTEIN, AND ENERGY FROM SELECTED STUDIES

Group	N	Zinc (mg/day)	Protein (g/day)	Energy (kcal)	Zinc:Energy mg:1000 kcal
S-150	138	10.4 ± 5.4*	64 ± 2	1856 ± 54	5.5
Greger et al. 1979 (13)	183	11.3 ± 4.7	76 ± 2	2030 ± 587	5.6**
White 1969 (62)	15	12.0 ± 5.0**	55 ± 16	1501 ± 425	8.6

*Mean ± SD.
**Calculated from mean data given by authors.

Multiple regression was used to calculate the "best" fitting model to predict (or account for) the variation in dietary zinc. The factors included: animal protein, protein, copper, energy, calcium, magnesium, and fiber intakes. The stepwise regression procedure for all subjects resulted in the following equation:

dietary zinc = -2.289 + 0.163 total protein (g) + 1.570 copper (mg).

The equation accounted for 71% of the variability in dietary zinc. Probabilities for each variable and the model were <0.0001. Descriptions of all other models with up to seven variables were included in Appendix B.

Erythrocyte Zinc Concentration

The mean erythrocyte zinc concentration (EZN) for 138 subjects was 7.6 $\pm$ 0.15 µg/g red cells, with a range from 3.7 to 13.3 µg/g. There was no interaction between race and age, in either menarche category, for EZN. There was a difference in EZN between pre- and post-menarcheal subjects (Table V). Pre-menarcheal subjects had a lower LSM for EZN than post-menarcheal subjects. In addition, there were significant differences due to age among post-menarcheal subjects. The younger post-menarcheal subjects had lower EZN than older post-menarcheal subjects.

The group with the lowest EZN was the post-menarcheal subjects in age category 12. The fact that these adolescents had menstruated indicated at least a minimal degree of adequacy of zinc nutriture prior to menarche. These girls had presumably just experienced the time of most rapid adolescent growth.

The zinc intake of adolescent females in this study decreased with age (see Table III), while the EZN increased with age (Table V). No differences were found in LSM for EZN among %-RDA-zinc categories, either before or after menarche (Table VI).

These results suggested that dietary zinc was not a good predictor of EZN among these adolescent females, except possibly within a

TABLE V

LEAST SQUARES MEAN OF ERYTHROCYTE ZINC CONCENTRATION BY MENARCHEAL STATUS, AND BY AGE AND RACE, ACCORDING TO MENARCHE

Group	Erythrocyte Zinc (µg/g)
Menarcheal Status	
Pre-menarche	6.8 ± 0.3*a**
Post-menarche	7.9 ± 0.2[b]
Pre-menarcheal	
Age	
12	6.9 ± 0.5
14	6.5 ± 0.8
Race	
white	6.6 ± 0.3
black	6.8 ± 0.9
Post-menarcheal	
Age	
12	5.9 ± 0.6[a]
14	7.6 ± 0.2[b]
16	8.7 ± 0.3[c]
Race	
white	7.5 ± 0.3
black	7.3 ± 0.3

*LSM ± SE .

**LSM not sharing a common superscript letter within menarche categories are different $p<0.05$, t-test. If no superscript is given, no differences within menarche categories were found.

narrower age span. Stepwise regression analysis for all subjects included the following variables: serum protein, hemoglobin, height, skinfold, arm muscle mass, race, menarcheal status, and per capita income; and intakes of total protein, zinc, and energy. The following equation accounted for 20% of the variability in EZN in this study:

$$EZN = 0.0541 + 0.0447 \text{ age (months)}.$$

Probabilities for the variable and the model were below 0.0001. Descriptions of other models, including up to 10 variables, were included in Appendix C.

TABLE VI

LEAST SQUARES MEAN ERYTHROCYTE ZINC BY %-RDA-ZINC, ACCORDING TO MENARCHE

%-RDA-Zinc	Erythrocyte Zinc (µg/g)
Pre-Menarche	
≤50	6.3 ± 0.6*
50-75	6.9 ± 0.5
75-100	6.7 ± 0.5
>100	6.6 ± 0.6
Post-Menarche	
≤50	7.8 ± 0.3
50-75	7.1 ± 0.3
75-100	7.4 ± 0.4
>100	7.5 ± 0.5

*LSM ± SE. No differences within menarche categories were found.

No values for EZN in adolescents have been reported in the literature. Values reported for adults were expressed in μg/ml. If one milliliter of packed red cells were equivalent to 1.1 g red cell concentrate (165), the range of values from 10 to 14 μg/ml reported for adults (80, 87-90) would be equivalent to about 9 to 13 μg/g. The range of EZN values for subjects in this study was 3.7 to 13.3 μg/g erythrocytes. The mean EZN (8.7 ± 0.3 μg/g) for older postmenarcheal subjects corresponded to the lower end of the range of adult values; other groups had slightly lower mean values.

The metabolism of EZN has not been fully investigated. Published research (in vitro studies) reported zinc efflux from erythrocytes pre-loaded with high or low levels of zinc was small and independent of the pre-efflux zinc concentration (94); thus, once zinc was associated with erythrocytes it tended to remain. The applicability of in vitro studies on EZN metabolism to in vivo zinc metabolism has not been investigated. In addition, the influences of growth and hormonal changes that occur during adolescence on zinc metabolism in general, and in erythrocytes specifically, have not been studied. The use of EZN in assessing zinc status needs further investigation.

Plasma Ribonuclease

The plasma ribunuclease activity (RNase) has not previously been reported in healthy adolescents. The mean RNase activity for 138 subjects was 4.9 ± 0.06 units/min/ml, with a range of 3.5 to 7.5 units/min/ml. There was no interaction between race and age, in either menarche category for RNase. The LSM for RNase for

pre-menarcheal subjects was greater than the LSM for RNase for post-menarcheal subjects (Table VII). There were no differences in LSM RNase activity among %-RDA-zinc categories, either before or after menarche (Table VIII).

TABLE VII

LEAST SQUARES MEAN PLASMA RIBONUCLEASE BY MENARCHEAL STATUS, AND BY AGE AND RACE, ACCORDING TO MENARCHE

Group	Plasma Ribonuclease (units/min/ml)
Menarcheal Status	
Pre-menarche	5.2 ± 0.2*a**
Post-menarche	4.9 ± 0.1[b]
Pre-menarche	
Age	
12	5.4 ± 0.4
14	5.0 ± 0.4
Race	
white	5.0 ± 0.2
black	5.4 ± 0.4
Post-menarche	
Age	
12	5.1 ± 0.2
14	4.8 ± 0.1
16	4.8 ± 0.1
Race	
white	5.0 ± 0.1
black	4.8 ± 0.1

*LSM ± SE .
**LSM not sharing a common superscript letter within menarche categories are different at $p<0.05$, t-tests. If no superscript letter is given, no differences within menarche categories were found.

TABLE VIII

LEAST SQUARES MEAN PLASMA RIBONUCLEASE BY %-RDA-ZINC, ACCORDING TO MENARCHE

%-RDA-Zinc	Plasma Ribonuclease (units/min/ml)
Pre-menarche	
≤50	5.2 ± 0.3*,**
50-75	4.9 ± 0.3
75-100	5.0 ± 0.3
>100	5.0 ± 0.3
Post-menarche	
≤50	5.0 ± 0.1
50-75	4.9 ± 0.1
75-100	5.0 ± 0.2
>100	4.7 ± 0.2

*LSM ± SE. No differences within menarche categories were found.

Stepwise regression analysis accounted for only a small portion of the variability in RNase activity in this study (r^2=.06). The "best fit" model had the following equation:

RNase = 6.9096 - 0.0052 protein (g) - 0.0097 age (months).

The variables included all at once in the procedure were: serum protein, EZN, hemoglobin, height, skinfold, arm muscle mass; and intakes of protein, zinc, and energy; and age, menarcheal status, race, and per capita income. Probabilities for all variables were below 0.0577, and for the model, 0.0196. Descriptions of other models, with up to 10 variables, were included in Appendix D.

The plasma RNase activity for normal, healthy adults was 3.1 ± 0.01 (SE) units/min/ml (91). The RNase values of four adult males ranged from 3.7 to 4.1 units/min/ml (82). Values reported by authors using a similar procedure to that in this study were: for infants less than 10 days old, 12.1 ± 2.5 (SD) units/min/ml; for 20 day old infants, 5.8 ± 1.1; and for adults, 29 to 27 years old, 5.7 ± 0.7 (107). The mean RNase activity, 4.9 ± 0.06 units/min/ml, for adolescent females in this study was lower than the value for adults in the study using similar procedures (107).

Since menarcheal status influenced RNase values, the range of values for pre- and post-menarcheal subjects needs further investigation. Establishment of normal values is necessary prior to using RNase as an indicator of zinc status.

Serum Alkaline Phosphatase

Values for serum alkaline phosphatase activity (AP) ranged from 1.0 to 9.2 Sigma (Σ) units/ml, with a mean of 3.7 ± 0.16 Σ units/ml. The pre-menarcheal subjects had significantly greater LSM for AP activity than post-menarcheal subjects (Table IX). There was no interaction between age and race, in either menarche category. The younger post-menarcheal subjects (age categories 12 and 14) had greater LSM for AP than the older post-menarcheal subjects (age category 16). There was no difference due to race within menarche categories. Serum AP had been shown to peak between 10 and 13 years of age and then gradually declined to adult values by the eighteenth year in females (121-126). The data for the S-150 adolescents are consistent with these reports.

TABLE IX

LEAST SQUARES MEAN SERUM ALKALINE PHOSPHATASE BY MENARCHEAL STATUS, AND BY AGE AND RACE, ACCORDING TO MENARCHE

Group	Alkaline Phosphatase (Σ units/ml)
Menarcheal Status	
Pre-menarche	5.8 ± 0.2*a**
Post-menarche	2.8 ± 0.1[b]
Pre-menarche	
Age	
12	5.5 ± 0.5
14	5.0 ± 0.9
Race	
white	5.6 ± 0.4
black	4.9 ± 1.0
Post-menarche	
Age	
12	3.7 ± 0.4[a]
14	3.0 ± 0.2[a]
16	2.1 ± 0.2[b]
Race	
white	3.0 ± 0.2
black	2.9 ± 0.2

*LSM ± SE.
**LSM not sharing a common superscript letter within menarche categories are different, $p<0.05$, t-test. If no superscript letter is given, no differences within menarche categories were found.

A scattergram of AP values and months past menarche indicated AP values decreased as months past menarche increased (Figure 1). Regression analysis showed AP decreased by -0.045 units per month

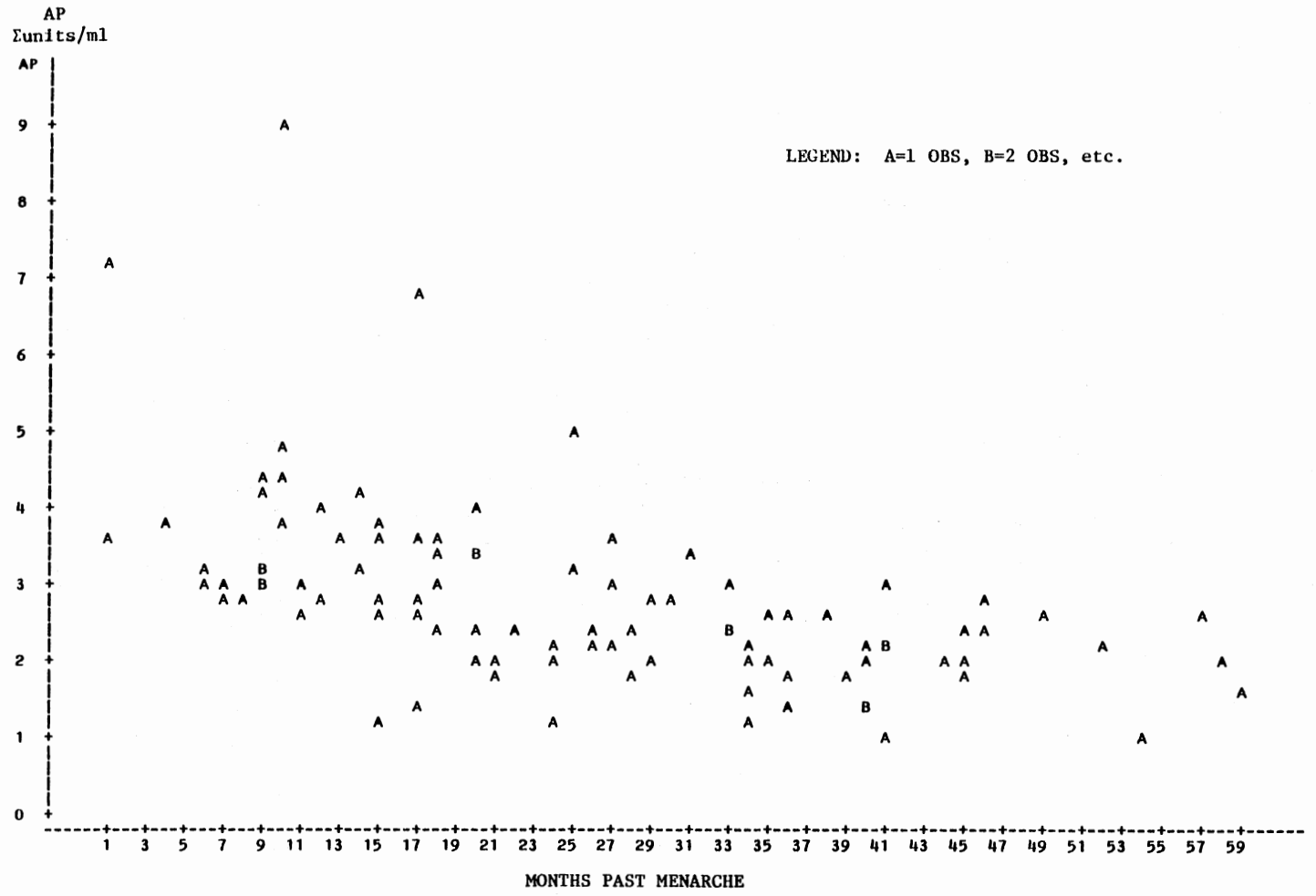

Figure 1. Scattergram of Serum Alkaline Phosphatase and Months Past Menarche

($p<0.001$). This corresponds with the age category 16 having a lower LSM for AP than subjects in age categories 12 and 14.

The LSM for AP among post-menarcheal subjects in <50 and 50 to 75%-RDA zinc categories were lower than the LSM for AP for subjects in >100%-RDA-zinc category (Table X). There were no differences in LSM for AP among %-RDA-zinc categories for pre-menarcheal subjects.

TABLE X

LEAST SQUARES MEAN SERUM ALKALINE PHOSPHATASE BY %-RDA-ZINC, ACCORDING TO MENARCHE

%-RDA-Zinc	Alkaline Phosphatase (Σ unts/ml)
Pre-Menarche	
≤50	5.7 ± 0.6
50-75	5.5 ± 0.6
75-100	5.7 ± 0.6
>100	5.3 ± 0.7
Post-Menarche	
≤50	2.7 ± 0.2[a]
50-75	2.8 ± 0.2[a]
75-100	3.1 ± 0.3[a,b]
>100	3.8 ± 0.3[b]

*LSM ± SE.
**LSM not sharing a common superscript letter within menarche categories are different, $p<0.05$, t-test. If no superscript letter is given, no differences within menarche categories were found.

Table XI shows the comparison of AP values to other studies with subjects of similar ages. The age groups differed among the studies so groups were combined to yield age categories similar to the S-150 categories. Since the studies expressed AP values in different units, the reported units were combined and converted to Sigma units, using standard conversion value (115). Although the results are not directly comparable, the values for the S-150 project seemed consistent with the other studies.

Multiple regression analysis was used to identify those variables that would be the best predictors of AP. The variables, analyzed all together, included: EZN, RNase and serum protein; intakes of energy, vitamin D, calcium, and zinc; height, skinfold, arm muscle mass, age, race, menarcheal status, and per capita income. The model produced this equation:

AP = 9.051 - 0.3999 RNase (units/min/ml) + 0.0018 vitamin D (IU) - 0.0381 age (months) + 1.9551 menarcheal status (1 = post; 2 = pre).

Sixty-one percent of the variability in AP was accounted for by this model, with all variables $p<0.0059$, and the full model $p<0.0001$. Descriptions on all models, up to 10 variables, were in Appendix E.

Relationships Among Zinc Intake, Erythrocyte Zinc Concentration, Plasma Ribonuclease Activity, and Serum Alkaline Phosphatase Activity

The relationships of zinc intake to the biochemical indices of zinc status and to each other were further investigated. There were

no differences in LSM for EZN (see Table VI) or in LSM for RNase (see Table VIII) among %-RDA-zinc categories for either pre- or post-menarcheal groups. The LSM for AP of post-menarcheal subjects showed the LSM for >100 %-RDA-zinc category was greater than for all other groups (see Table X). A scattergram of AP values by zinc intake indicates four subjects had AP values greater than 5.0, but only two of those had zinc intakes over 15 mg/day (Figure 2).

TABLE XI

COMPARISON OF MEAN SERUM ALKALINE PHOSPHATASE VALUES WITH SELECTED STUDIES

Group	Age Categories	Alkaline Phosphatase (Σ units/ml)
S-150*	11.5 - 12.9	5.43
	13.0 - 14.9	3.27
	15.0 - 16.5	2.19
Round, 1973 (121)**	10 - 12	3.62
	13 - 14	2.47
	15 - 16	1.42
Harrison et al. 1948 (123)*	10 - 12	6.97
	13 - 14	5.15
	15 - 16	1.90
Clark et al. 1950 (124)**	10 - 12	5.98
	12 - 14	4.79
	14 - 16	3.38
Bennett et al. 1976 (125)***	10 - 12	5.66
	13 - 14	3.89
	15 - 16	2.07

*Value originally expressed as Sigma units.
**Value converted from King-Armstrong units.
***Value converted from International units.

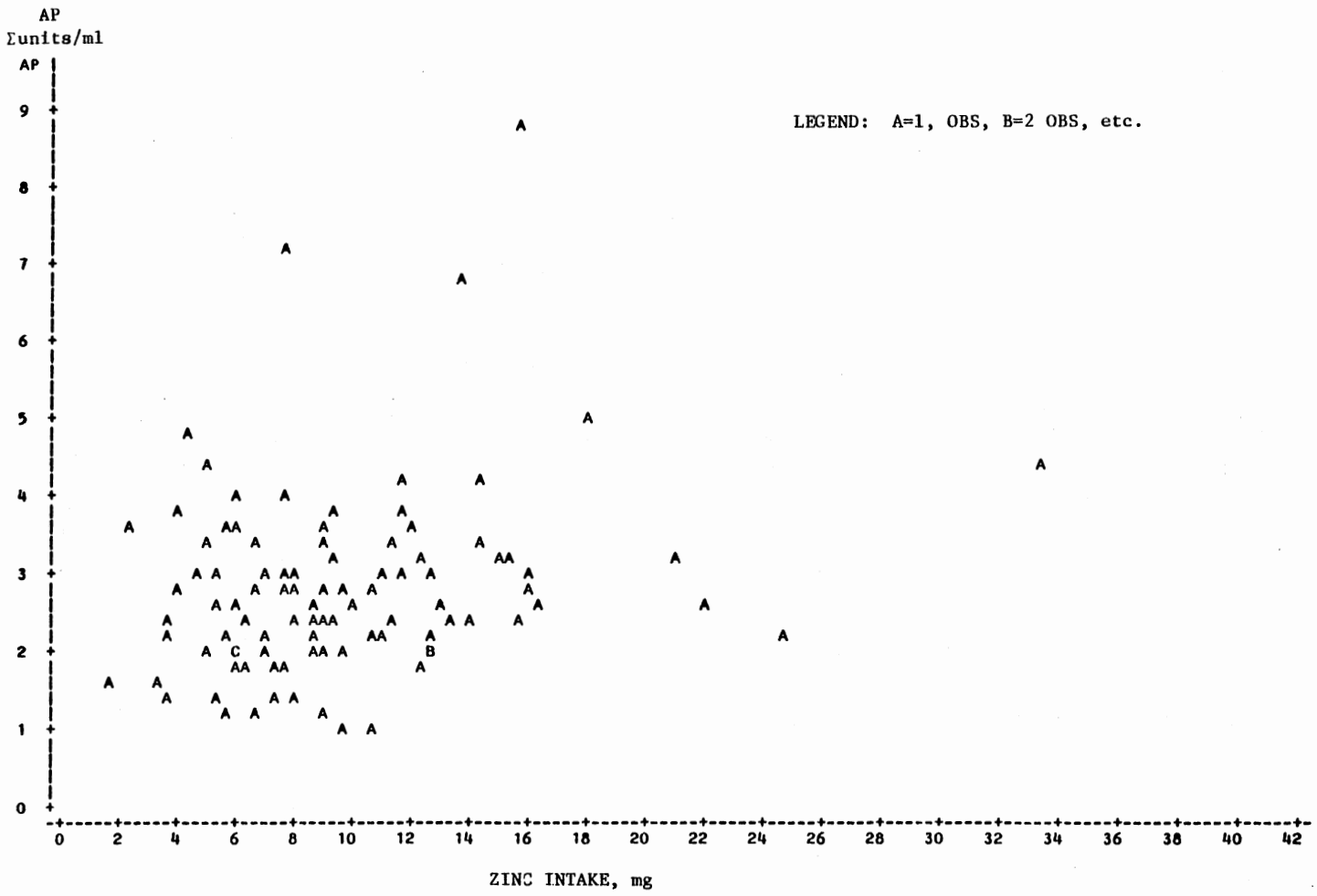

Figure 2. Scattergram of Serum Alkaline Phosphatase and Zinc Intake for Post-Menarche Subjects

Scattergrams for EZN and zinc intake, AP and zinc intake, RNase and zinc intake, AP and RNase, AP and EZN, and RNase and EZN, by menarcheal status, showed no relationships between the variables (Figures 3-13, Appendix F), though RNase did appear in the best equation to predict AP. When subjects were categorized according to EZN groups (<6.5, 6.5 to 8.9 or >8.9 μg/g), no differences in RNase or AP, in either menarche category, were found (Table XII).

TABLE XII

LEAST SQUARES MEAN FOR INDICES OF ZINC STATUS BY ERYTHROCYTE ZINC, ACCORDING TO MENARCHE

Variable	Menarcheal Status	Erythryocyte Zinc (μg/g) <6.5	6.5 - 8.9	>8.9
Plasma Ribonuclease	Pre	5.2 ± 0.2*	5.2 ± 0.2	4.6 ± 0.5
	Post	5.0 ± 0.2	4.9 ± 0.1	4.7 ± 0.1
Serum Alkaline Phosphatase	Pre	5.3 ± 0.4	5.9 ± 0.4	7.0 ± 1.0
	Post	3.1 ± 0.3	2.7 ± 0.2	2.7 ± 0.3

*LSM ± SE. No differences within menarche categories were found.

In marginal zinc deficiency, low AP activity, low EZN, and low zinc intake with high RNase activity were found in a metabolic study with four adult males consuming a semi-purified diet containing 2.7 or 3.5 mg zinc/day (82). Therefore, additional regression analysis

was performed on the S-150 data to determine the effect(s) of "forcing" one or more of these variables into the models already developed. The results of these procedures suggested certain relationships between variables (zinc intake and EZN; and AP and EZN) when only "forced" variables were in the model; however, when the single or best two variable models were presented with a free choice variable, the "forced" variable's probability increased beyond the criteria established in this study (Table XIII).

TABLE XIII

MULTIPLE REGRESSION ANALYSES FOR SELECTED MODELS WITH "FORCED" VARIABLES

Variable for the Model	Included Variable* /2nd Entered**	β-Value	r^2	Prob> F
EZN	Zinc intake*	-0.0478	.02	0.0879
	Zinc intake**	-0.0172	.19	0.5094
	Age (months)	0.0436		0.0001
AP	EZN*	-0.2596	.06	0.0041
	EZN**	-0.0528	.49	0.4417
	Menarchal status	2.8599		0.0001
RNase	EZN*	-0.0485	.01	0.1769
	EZN**	-0.0203	.03	0.6057
	Age	-0.0067		0.1012

*Model with one "forced" variable.
**Best two variable model (with one "forced").

Additional information on the models in Table XIII were included in Appendix G, with up to five variables. Also in Appendix G are the variable models with two or three "forced" variables. These additional regression analyses indicated for this study that the variables menarcheal status and age predicted zinc intake, AP, EZN, and RNase better than these indices predicted each other. Apparent relationships among these indices may have resulted from common associations with other variables.

Summary and Recommendations

Wide ranges in values for zinc intakes, EZN, RNase, and AP were found in the adolescent females studied. Menarcheal status was an important factor in identifying differences in these indices among the subjects. There were no differences due to race. Age, menarcheal status, and RNase explained 61% of the variation in AP. Total protein and copper intakes explained 71% of the variation in zinc intakes. Age alone and age plus total protein intake, respectively, accounted for only a small portion of the variations in EZN (20%) and RNase (6%).

The biochemical determinations of EZN, RNase, and AP were feasible within the limitations of this survey. The amount of sample required for AP (0.04 ml serum) and RNase (0.05 ml EDTA plasma) analyses was small, while the determination of EZN required a greater amount of sample (0.50 g erythrocytes). The need to collect samples in three different media required changing vaccutainer tubes during blood collection. Thus problems of drawing blood from subjects, of

providing adequate refrigeration of all samples, and of separation of the blood are encountered in surveys.

Although the procedures for determination of AP were easily handled, this enzyme is affected by factors other than zinc. These other factors limit the use of AP as a sole indicator of zinc status. The abrupt change in activity after menarche in females, the relationship of AP to bone metabolism, and the relationship of AP to protein status, must be evaluated prior to its use as an index of zinc status. The determination of AP activity prior to and after supplementation with zinc has been proposed as a useful measure of zinc status (6). This procedure, however, would have limited application in a survey.

The determination of plasma RNase activity was more difficult than the determination of AP. In order to obtain a clear supernatant, it was necessary to add additional precipitating reagent, to keep the assay mixture on ice prior to and after incubation, to use covered tubes during centrifugation, and to have exact timing. In addition, special cuvettes were needed as the absorbance was read at 260 nm. Further standardization of the assay method for RNase is necessary to develop routine procedures. As RNase activity was affected by protein status in animals (104) and in humans (106), its use as a sole indicator of zinc status may be limited.

EZN has been proposed as an indicator of long-term zinc status (7). The factors that influence the metabolism of EZN and zinc uptake by erythrocytes are not fully understood. In addition, the impact of the physiological changes associated with growth and maturation during adolescence on erythrocyte zinc has not been

established. The normal range of values for EZN for different stages of maturity should be determined prior to using EZN as an index of zinc status in adolescents.

Even when known influences such as menarche and age are accounted for, as in this study, relationships among zinc intake, EZN, RNase, and AP are not clear. Other proposed indices of zinc status such as plasma zinc concentrations and carbonic anhydrase activity, are not readily applicable for survey work. Preventing contamination of plasma zinc from hemolysis or collection materials in a survey situation are required for plasma zinc concentrations to be a useful index of zinc status. Streamlining the routine analysis of carbonic anhydrase activity with readily available equipment is necessary prior to its use in surveys as an index of zinc status. With advances in research in these areas, the relationships among various currently used or proposed indices of zinc status may help identify the most reliable predictors of zinc status.

Further research is needed in a number of areas. The researcher recommends:

1. Investigation of the effects of physiological changes during growth and maturation on EZN, RNase, and AP to establish normal range of values for different stages of physiological maturity:

2. Improved accuracy and reliability in collecting information on zinc intakes;

3. Investigation of the factors that may influence the metabolism of EZN to determine its sensitivity as an indicator of zinc status;

4. Simplification of methods of determining EZN, carbonic anhydrase activity and plasma zinc concentrations in survey work; and

5. Determination of the relationships among EZN, AP, RNase, and AP and other proposed indices of zinc status, such as plasma zinc and carbonic anhydrase activity, to determine the best combinations of predictors of zinc status in adolescent females.

CHAPTER V

SUMMARY

Limited information on the zinc status of adolescent females was available. The importance of adequate zinc, especially during periods of growth, has been well established. There was, however, no standardized procedure for assessing zinc status in humans. Recent reports have suggested erythrocyte zinc concentration (EZN), serum alkaline phosphatase activity (AP), and plasma ribonuclease activity (RNase) may be useful indicators of zinc status and have applicability to survey work.

In this study, the range of values and the relationships of EZN, RNase, and AP to each other and to zinc intake were investigated in a group of 138 adolescent females. These subjects were part of a larger group who participated in the S-150 regional project, Nutritional Status of Adolescent Females. Dietary, anthropometric, clinical, and biochemical data were collected. Information on intakes of zinc, total protein, animal protein, and energy were obtained from two 24-hour recalls and mean nutrient contents of food and supplements were calculated. Intakes of zinc, total protein, animal protein, energy and EZN, RNase, and AP were compared by age, race, menarcheal status, and %-RDA-zinc categories. Categories used for %-RDA-zinc were: $\leq$50% RDA; 50 to 75% RDA; 75 to 100% RDA; and >100% RDA.

Blood samples were drawn in a fasting state and analyzed for three indices suggested to reflect zinc status. EZN was measured by atomic absorption spectrophotometry following a wet ash procedure. Plasma RNase and serum AP activities were determined by spectrophometric assays.

Effects of menarcheal status, age, and race were evaluated by analysis of variance. Multiple regression analysis was then used to determine the "best fitting" model for each index from a given list of related factors. Additional stepwise regression analysis "forced" into the models a variable that was expected to have an effect, whether or not the criterion for inclusion was met.

The LSM intake of zinc for post-menarcheal subjects (9.6 $\pm$ 0.55 mg, range 1.6 to 33.4 mg) was less than the LSM intake of zinc for pre-menarcheal subjects (12.2 $\pm$ 0.85 mg, range 3.0 to 34.7 mg). Intakes of zinc, total protein, animal protein, and energy were higher for pre-menarcheal subjects than for post-menarcheal subjects. The intakes of total protein, animal protein, and energy increased with increased zinc intakes.

The LSM for EZN in pre-menarcheal subjects (7.9 $\pm$ 0.18, range 3.7 to 11.0 µg/g erythrocyte) was lower than the LSM for EZN in post-menarcheal subjects (6.8 $\pm$ 0.27, range 4.0 to 13.3 µg/g). Within the post-menarcheal group, the younger subjects had lower LSM for EZN than the older subjects. There were no differences in LSM for EZN among %-RDA-zinc categories, either for pre- or post-menarcheal subjects.

The LSM for RNase of pre-menarcheal subjects (5.2 $\pm$ 0.12, range 3.6 to 7.5 units/min/ml) was greater than the LSM for RNase in

post-menarcheal subjects (4.9 ± 0.07, range 3.5 to 7.5 units/min/ml). There were no differences in LSM for RNase among %-RDA-zinc categories in either pre- or post-menarche categories.

Pre-menarcheal subjects had a greater LSM for AP (5.8 ± 0.21, range 2.3 to 9.2 Σ units/ml) than LSM for AP for post-menarcheal subjects (2.8 ± 0.14, range 1.01 to 8.9 Σ units/ml). Within the post-menarcheal group, there was an age-related difference in LSM for AP with younger subjects having greater LSM for AP than older subjects.

Multiple regression analysis resulted in equations accounting for 71% variability in zinc intake; 20% for EZN; 6% for RNase; and 61% for AP. The variable age appeared in all models, except for zinc intake, as it was not included in the list.

Zinc intakes were not related to EZN, AP, or RNase, except for AP in post-menarcheal subjects. In addition, no clear relationships were detected between AP and RNase; AP and EZN; or RNase or EZN. When these indices were "forced" into equations, the resulting relationships lost significance as other variables were freely added to the models. The major factors influencing the suggested indices of zinc status in this study were menarcheal status and age.

The biochemical determinations of EZN, RNase, and AP were possible within the limitations of this survey. As factors other than dietary zinc influenced each of them, their general use as sole indicators of zinc status cannot be recommended.

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APPENDIXES

APPENDIX A

LIST OF ABBREVIATIONS IN COMPUTER PRINTOUTS AND COMPUTER LISTS OF RAW DATA BY MENARCHEAL STATUS

List of Abbreviations in Computer Printouts

Age	- Age (months)
AP	- Serum alkaline phosphatase (Σ units/ml)
Anpro	- Animal protein intake (g)
Ca	- Calcium intake (mg)
Cu	- Copper intake (mg)
E	- Energy intake (kcal)
EZN	- Erythrocyte zinc concentration (µg/g)
Fib	- Crude fiber intake (g)
Mg	- Magnesium intake (mg)
Mens	- Menarcheal status (1=post, 2=pre)
HB	- Hemoglobin (mg/dl)
Musc	- Arm muscle mass (squared centimeters)
Pcinc	- Per capita income
Pro	- Total protein intake (g)
Race	- Race (1=white, 2=black)
RNase	- Plasma ribonuclease activity (units/min/ml)
Rht	- Height (relative to U.S. heights for age)
Skfd	- Skinfold (millimeters)
Spro	- Serum protein (g/dl)
Twoyr	- Two year age categories (12, 14, and 16)
VD	- Vitamin D (IU)
ZN	- Zinc intake (mg)

Post-Menarcheal Subjects

OBS	SUBJ	AGE	TWOYR	RACE	ZN	PRO	ANPRO	E	EZN	RNASE	AP
1	2	189	16	1	9.3	61	35.1	2178	5.7	4.73333	2.45
2	4	187	16	1	8.0	65	32.0	2605	7.7	5.33333	2.92
3	5	162	14	1	6.8	47	47.4	1170	7.6	4.86667	2.83
4	6	189	16	1	10.1	60	44.8	1622	7.7	4.93333	2.56
5	8	193	16	1	6.1	33	22.4	1078	9.6	4.73333	2.01
6	10	163	14	1	5.0	32	20.2	1195	11.7	4.93333	3.42
7	11	189	16	1	5.3	32	19.9	1109	9.8	4.73333	2.51
8	12	193	16	1	10.5	62	51.8	1271	11.0	4.40000	1.01
9	13	172	14	1	7.6	53	31.7	1572	9.1	4.66667	1.81
10	14	165	14	1	15.6	94	64.7	2368	10.2	5.26667	8.90
11	15	164	14	1	14.3	90	69.6	2124	6.8	4.53333	4.13
12	16	162	14	1	6.8	69	53.8	1132	9.9	4.53333	3.49
13	17	175	14	1	18.1	105	78.3	2188	7.0	3.53333	4.96
14	18	162	14	1	5.9	36	25.6	1005	6.8	5.33333	3.55
15	21	193	16	1	6.9	39	29.1	1291	8.5	3.73333	2.27
16	22	193	16	1	15.6	80	58.0	2035	8.2	4.80000	2.33
17	23	179	14	1	4.1	34	20.4	949	7.5	4.66667	2.73
18	24	174	14	1	5.2	34	26.9	1198	7.3	4.46667	3.08
19	25	173	14	1	5.3	36	23.2	1085	8.5	4.86667	1.44
20	26	198	16	1	8.7	85	51.3	2488	10.0	4.73333	2.60
21	27	173	14	1	5.6	42	29.3	818	8.8	5.46667	2.27
22	28	195	16	1	24.8	70	39.9	2512	7.6	5.26667	2.15
23	29	164	14	1	7.7	49	28.0	1138	5.8	5.33333	3.91
24	30	173	14	1	9.5	56	41.7	1483	7.3	4.66667	2.86
25	32	188	16	1	10.5	82	52.3	2067	8.0	5.20000	2.25
26	34	188	16	1	15.4	64	41.4	2458	4.9	5.20000	3.11
27	35	172	14	1	9.1	59	39.3	1673	7.5	4.66667	2.07
28	37	189	16	1	7.0	61	46.6	1976	6.7	5.06667	2.04
29	39	168	14	2	15.0	104	56.8	2538	8.2	5.20000	3.12
30	41	147	12	1	33.4	72	50.5	1472	6.2	4.86667	4.40
31	42	169	14	1	11.0	75	54.3	1657	7.9	5.86667	2.26
32	45	188	16	1	9.6	53	33.8	1723	7.9	5.86667	2.01
33	47	191	16	1	13.1	68	51.6	1739	7.6	4.80000	2.63
34	48	190	16	1	3.8	32	17.1	1418	11.1	6.20000	2.33
35	49	196	16	1	3.3	20	15.7	822	11.4	4.73333	1.57
36	50	174	14	1	3.5	27	17.0	948	7.3	6.73333	1.44
37	53	190	16	1	9.5	59	45.1	1551	9.8	4.93333	1.02
38	54	186	16	1	4.5	38	19.1	1081	13.2	5.53333	3.02
39	56	187	16	1	11.4	74	41.8	1747	10.0	5.60000	2.50
40	60	168	14	1	14.3	80	64.8	2298	7.6	4.20000	3.49
41	64	168	14	1	6.9	49	28.3	1647	5.6	4.80000	3.09
42	66	150	12	1	6.0	44	30.8	1429	6.7	4.73333	3.95
43	68	174	14	1	9.3	56	34.8	1749	7.0	5.20000	3.71
44	72	198	16	1	1.6	17	10.3	483	5.6	4.46667	1.68
45	74	141	12	2	7.7	49	34.9	1904	4.0	5.06667	7.18
46	75	197	16	1	6.1	60	39.0	1480	6.2	4.60000	1.88
47	77	166	14	1	3.9	42	26.6	999	5.6	4.86667	3.73
48	78	192	16	1	6.1	60	46.2	1195	7.8	5.66667	2.01

OBS	SUBJ	AGE	TWOYR	RACE	ZN	PRO	ANPRO	E	EZN	RNASE	AP
49	81	163	14	2	14.0	72	54.1	1751	8.1	7.46667	2.38
50	82	170	14	2	9.4	54	28.5	1959	8.0	4.13333	3.15
51	83	171	14	1	12.2	72	45.3	2074	7.3	4.66667	1.73
52	85	157	14	2	15.9	69	52.1	2353	8.6	4.13333	2.72
53	86	170	14	2	21.9	67	48.6	2337	8.4	4.26667	2.54
54	87	168	14	1	7.8	48	34.2	1614	5.1	4.66667	2.91
55	92	168	14	2	6.8	66	37.1	3676	6.2	4.86667	1.25
56	93	142	12	1	11.3	78	37.4	2119	6.5	5.66667	3.50
57	94	140	12	1	7.5	58	36.2	1832	5.5	5.40000	2.79
58	96	149	12	2	11.7	68	37.7	2513	5.1	5.53333	2.93
59	97	151	12	2	5.7	47	33.5	965	6.5	5.46667	1.15
60	99	170	14	2	9.0	51	32.5	1668	5.9	5.60000	3.40
61	101	170	14	1	6.0	62	42.2	1824	8.5	6.80000	2.10
62	104	168	14	2	12.6	80	60.2	2135	4.7	3.46667	2.95
63	106	165	14	1	8.5	50	35.0	1095	7.8	5.53333	1.95
64	110	168	14	1	21.0	79	58.2	2240	5.3	4.93333	3.20
65	111	164	14	2	5.0	62	46.1	2467	6.6	5.33333	4.37
66	112	171	14	1	9.1	66	29.8	2002	6.2	5.13333	3.61
67	113	168	14	2	12.6	71	31.3	2206	9.2	5.60000	2.17
68	118	153	12	2	8.0	57	43.6	1315	5.5	4.46667	2.84
69	120	193	16	2	13.2	58	31.0	2074	7.6	4.20000	2.33
70	121	190	16	2	8.9	60	48.5	1640	6.9	5.20000	1.16
71	142	196	16	2	10.6	69	46.9	1702	8.0	4.20000	2.84
72	143	162	14	2	4.4	39	27.6	1288	6.7	4.40000	4.79
73	144	180	16	2	12.3	55	35.4	2219	10.4	4.53333	3.11
74	145	158	14	2	8.8	74	41.3	2187	7.5	3.73333	2.44
75	146	166	14	2	12.5	70	51.9	2020	8.8	5.40000	1.94
76	148	175	14	2	2.2	30	11.5	1072	10.7	4.86667	3.66
77	149	167	14	2	12.7	63	46.1	2099	6.9	5.00000	1.91
78	150	169	14	1	6.0	54	35.5	2063	7.2	5.33333	2.63
79	151	170	14	1	8.1	71	56.2	1745	8.1	5.00000	2.34
80	152	172	14	2	3.6	25	11.8	748	7.0	4.66667	2.14
81	153	177	14	2	11.0	84	58.7	2825	8.1	4.80000	3.01
82	154	173	14	2	5.8	31	15.9	1214	8.1	4.06667	3.53
83	155	189	16	2	16.0	107	79.9	2482	11.5	4.53333	2.91
84	156	173	14	2	11.9	72	59.6	1908	6.7	4.93333	3.59
85	157	175	14	2	6.2	41	31.0	1331	7.2	4.93333	1.71
86	158	171	14	1	9.1	62	35.5	1852	8.5	4.73333	2.37
87	159	192	16	2	8.5	60	42.2	1753	8.5	4.06667	2.25
88	162	191	16	1	7.9	50	33.5	1556	8.4	5.26667	1.31
89	163	146	12	1	11.8	66	47.5	1807	7.5	4.60000	4.19
90	166	156	14	1	11.7	72	48.2	2375	6.7	4.73333	3.73
91	167	166	14	1	13.8	70	51.1	2007	7.7	3.60000	6.75
92	169	172	14	1	9.1	64	42.3	2349	9.7	3.66667	2.78
93	170	168	14	1	16.3	83	66.4	1638	9.4	3.86667	2.64
94	171	194	16	1	7.2	63	35.2	1844	11.3	4.33333	1.49
95	172	178	14	1	4.9	41	22.2	1481	10.4	4.00000	2.10
96	173	190	16	1	7.3	53	36.8	1330	8.6	4.20000	1.75
97	174	191	16	1	6.3	38	25.0	1205	12.9	3.93333	2.35

Pre-Menarcheal Subjects

OBS	SUBJ	AGE	TWOYR	RACE	ZN	PRO	ANPRO	E	EZN	RNASE	AP
98	1	146	12	1	13.7	84	52.4	2028	11.0	4.26667	5.74
99	3	168	14	1	13.5	90	57.2	2281	7.3	4.20000	4.44
100	9	174	14	1	10.3	67	55.1	1673	7.4	5.53333	5.06
101	19	149	12	1	8.2	59	40.2	1628	9.9	4.86667	8.32
102	20	165	14	1	14.7	102	87.2	2915	3.7	4.60000	9.24
103	31	143	12	1	18.7	97	83.2	2020	7.8	4.86667	4.72
104	33	148	12	1	16.5	96	78.4	2052	5.8	4.80000	5.96
105	38	146	12	2	11.5	74	49.9	2373	6.5	7.06667	5.30
106	40	140	12	1	7.0	50	39.5	1382	4.2	5.00000	3.65
107	43	140	12	1	16.3	98	75.7	2479	7.2	6.66667	8.25
108	44	150	12	1	12.2	98	76.3	2727	5.5	5.20000	5.02
109	51	150	12	1	18.4	60	39.4	2052	6.7	5.40000	4.24
110	52	148	12	1	34.7	163	124.9	4478	5.2	4.53333	4.42
111	57	149	12	1	8.8	62	44.4	1419	7.7	4.66667	5.84
112	58	141	12	1	12.4	70	40.6	2123	6.7	5.46667	5.52
113	59	144	12	1	8.8	50	36.9	1280	6.0	4.46667	5.72
114	63	141	12	1	6.8	42	30.2	1261	5.7	4.86667	6.06
115	65	142	12	1	11.1	104	60.8	3040	5.8	4.46667	6.45
116	71	143	12	1	12.5	71	48.4	2812	5.9	5.13333	6.44
117	76	147	12	1	8.7	71	44.6	2432	5.2	4.53333	7.07
118	79	144	12	1	6.0	45	24.9	1874	7.2	5.06667	6.03
119	80	142	12	1	11.4	57	38.8	1732	7.2	4.86667	6.19
120	84	145	12	1	32.7	184	126.2	4171	8.1	3.93333	7.44
121	88	171	14	1	6.8	45	29.8	1961	5.2	5.60000	3.69
122	89	171	14	1	8.8	68	49.5	1996	7.3	4.40000	4.76
123	91	144	12	2	11.4	99	64.5	2600	7.2	4.73333	2.28
124	95	146	12	1	6.9	53	32.9	1431	5.6	6.33333	2.58
125	98	146	12	1	19.1	96	73.6	2806	6.6	5.53333	3.61
126	100	140	12	1	16.4	93	62.7	3032	6.9	5.46667	6.86
127	102	151	12	1	15.0	85	65.4	1935	8.3	5.46667	6.82
128	103	144	12	1	11.8	70	52.2	1996	4.9	3.60000	7.68
129	105	150	12	1	15.7	113	80.4	2777	5.2	5.73333	3.70
130	107	150	12	2	7.5	66	34.1	2455	7.2	4.86667	8.02
131	108	144	12	1	3.0	19	12.8	831	7.0	6.66667	7.48
132	109	149	12	1	9.2	61	37.2	2058	5.8	7.53333	2.60
133	114	151	12	1	5.0	44	25.6	1957	6.8	5.20000	7.17
134	115	149	12	1	13.0	61	40.1	1991	7.7	6.73333	5.85
135	117	146	12	1	4.7	66	44.8	1992	8.2	5.53333	7.23
136	119	144	12	1	15.2	91	69.9	1879	9.4	4.73333	6.87
137	147	169	14	1	10.2	56	42.0	1418	8.0	4.60000	4.62
138	161	143	12	1	5.5	50	37.1	1118	8.0	4.26667	7.07

APPENDIX B

STEPWISE REGRESSION MODELS FOR ZINC INTAKE

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE ZN

STEP 1 VARIABLE PRO ENTERED R SQUARE = 0.63000001 C(P) = 41.16297590

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	2639.66316299	2639.66316299	231.57	0.0001
ERROR	136	1550.27828629	11.39910505		
TOTAL	137	4189.94144928			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-1.32165519				
PRO	0.18226314	0.01197733	2639.66316299	231.57	0.0001

THE ABOVE MODEL IS THE BEST 1 VARIABLE MODEL FOUND.

STEP 2 VARIABLE CU ENTERED R SQUARE = 0.70970113 C(P) = 5.43139561

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	2973.60616281	1486.80308140	165.02	0.0001
ERROR	135	1216.33528647	9.00989101		
TOTAL	137	4189.94144928			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-2.28910456				
PRO	0.16263424	0.01112582	1925.21731396	213.68	0.0001
CU	1.57001147	0.25788520	333.94299982	37.06	0.0001

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE ANPRO ENTERED R SQUARE = 0.72081869 C(P) = 2.16819113

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	3020.18810500	1006.72936833	115.32	0.0001
ERROR	134	1169.75334427	8.72950257		
TOTAL	137	4189.94144928			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-1.93867266				
ANPRO	0.09527132	0.04124280	46.58194219	5.34	0.0224
PRO	0.09071218	0.03300479	65.94269821	7.55	0.0068
CU	1.62619569	0.25500335	355.01225944	40.67	0.0001

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE E ENTERED R SQUARE = 0.72245603 C(P) = 3.39305283

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	3027.04845935	756.76211484	86.55	0.0001
ERROR	133	1162.89298993	8.74355632		
TOTAL	137	4189.94144928			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-2.18617962				
ANPRO	0.11043469	0.04468502	53.40417734	6.11	0.0147
PRO	0.06455534	0.04430644	18.56173942	2.12	0.1475
CU	1.62615562	0.25520853	354.99475600	40.60	0.0001
E	0.00067811	0.00076554	6.86035435	0.78	0.3773

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE FIB ENTERED R SQUARE = 0.72370222 C(P) = 4.80308996

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	3032.26992024	606.45398405	69.15	0.0001
ERROR	132	1157.67152903	8.77023886		
TOTAL	137	4189.94144928			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-2.12376285				
ANPRO	0.10237129	0.04595708	43.51740397	4.96	0.0276
PRO	0.07574063	0.04668184	23.08731044	2.63	0.1071
CU	1.64377038	0.25661512	359.85641706	41.03	0.0001
E	0.00069623	0.00076707	7.22515204	0.82	0.3657
FIB	-0.19177276	0.24854019	5.22146090	0.60	0.4417

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

STEP 6 VARIABLE CA ENTERED R SQUARE = 0.72464879 C(P) = 6.35497000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	6	3036.23600172	506.03933362	57.46	0.0001
ERROR	131	1153.70544755	8.80691181		
TOTAL	137	4189.94144928			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-2.05212185				
ANPRO	0.10470979	0.04618471	45.26908419	5.14	0.0250
PRO	0.07924418	0.04706977	24.96169982	2.83	0.0947
CU	1.61177356	0.26153408	334.48382793	37.98	0.0001
E	0.00077551	0.00077770	8.75742437	0.99	0.3205
CA	-0.00062871	0.00093687	3.96608148	0.45	0.5034
FIB	-0.18517207	0.24925344	4.86062685	0.55	0.4589

THE ABOVE MODEL IS THE BEST 6 VARIABLE MODEL FOUND.

STEP 7 VARIABLE MG ENTERED R SQUARE = 0.72539860 C(P) = 8.00000000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	7	3039.37766042	434.19680863	49.06	0.0001
ERROR	130	1150.56378886	8.85049068		
TOTAL	137	4189.94144928			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-2.05610726				
ANPRO	0.11038724	0.04726932	48.26656464	5.45	0.0211
PRO	0.07117140	0.04909296	18.60115168	2.10	0.1495
CU	1.59331018	0.26400547	322.36075008	36.42	0.0001
E	0.00068666	0.00079376	6.62324891	0.75	0.3886
CA	-0.00105786	0.00118360	7.06991405	0.80	0.3731
MG	0.00547956	0.00919707	3.14165869	0.35	0.5523
FIB	-0.31247501	0.32876948	7.99493507	0.90	0.3437

THE ABOVE MODEL IS THE BEST 7 VARIABLE MODEL FOUND.

APPENDIX C

STEPWISE REGRESSION MODELS FOR ERYTHROCYTE ZINC CONCENTRATION

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE EZN

WARNING: 3 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 1 VARIABLE AGE ENTERED R SQUARE = 0.18732483 C(P) = 1.38976833

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	81.73085059	81.73085059	30.66	0.0001
ERROR	133	354.57463090	2.66597467		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	0.10185976				
AGE	0.04479666	0.00809060	81.73085059	30.66	0.0001

THE ABOVE MODEL IS THE BEST 1 VARIABLE MODEL FOUND.

STEP 2 VARIABLE E ENTERED R SQUARE = 0.20150679 C(P) = 1.07944038

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	87.91851666	43.95925833	16.66	0.0001
ERROR	132	348.38696482	2.63929519		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	1.35372901				
AGE	0.04124978	0.00837668	64.00110354	24.25	0.0001
E	-0.00035854	0.00023417	6.18766607	2.34	0.1281

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE PRO ENTERED R SQUARE = 0.22052683 C(P) = -0.01904143

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	96.21706615	32.07235538	12.35	0.0001
ERROR	131	340.08841533	2.59609477		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	1.05407533				
AGE	0.04260776	0.00834249	67.71837063	26.08	0.0001
PRO	0.01972418	0.01103210	8.29854949	3.20	0.0761
E	-0.00100313	0.00042886	14.20406266	5.47	0.0208

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE RHT ENTERED R SQUARE = 0.23002478 C(P) = 0.43368439

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	100.36107350	25.09026838	9.71	0.0001
ERROR	130	335.94440798	2.58418775		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.59889864				
RHT	-0.05166066	0.04079545	4.14400735	1.60	0.2077
AGE	0.04049955	0.00848820	58.82924450	22.77	0.0001
PRO	0.02058772	0.01102787	9.00652104	3.49	0.0642
E	-0.00104854	0.00042937	15.41087517	5.96	0.0159

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE HB ENTERED R SQUARE = 0.23760259 C(P) = 1.19921241

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	103.66731424	20.73346285	8.04	0.0001
ERROR	129	332.63816724	2.57859044		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.46196498				
HB	-0.18175378	0.16051196	3.30624074	1.28	0.2596
RHT	-0.05271850	0.04076195	4.31319038	1.67	0.1982
AGE	0.03915728	0.00856146	53.94008637	20.92	0.0001
PRO	0.01868757	0.01114300	7.25242664	2.81	0.0960
E	-0.00101144	0.00043016	14.25622881	5.53	0.0202

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

STEP 6 VARIABLE MUSC ENTERED R SQUARE = 0.24139855 C(P) = 2.58082819

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	6	105.32350988	17.55391831	6.79	0.0001
ERROR	128	330.98197160	2.58579665		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.51947761				
HB	-0.17678909	0.16085575	3.12343237	1.21	0.2738
RHT	-0.04885293	0.04110364	3.65270825	1.41	0.2368
MUSC	-0.00015881	0.00019843	1.65619564	0.64	0.4250
AGE	0.03971773	0.00860197	55.12739263	21.32	0.0001
PRO	0.01713409	0.01132614	5.91769893	2.29	0.1328
E	-0.00102101	0.00043092	14.51630747	5.61	0.0193

THE ABOVE MODEL IS THE BEST 6 VARIABLE MODEL FOUND.

STEP 7 VARIABLE SPRO ENTERED R SQUARE = 0.24609603 C(P) = 3.81557937

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	7	107.37304757	15.33900680	5.92	0.0001
ERROR	127	328.93243391	2.59001916		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	11.53476747				
SPRO	-0.27794166	0.31244760	2.04953769	0.79	0.3754
HB	-0.17480240	0.16100252	3.05303925	1.18	0.2797
RHT	-0.04834709	0.04114112	3.57677281	1.38	0.2421
MUSC	-0.00019082	0.00020183	2.31517084	0.89	0.3462
AGE	0.04047331	0.00865079	56.69293995	21.89	0.0001
PRO	0.01681438	0.01134108	5.69319429	2.20	0.1407
E	-0.00105504	0.00043297	15.37898293	5.94	0.0162

THE ABOVE MODEL IS THE BEST 7 VARIABLE MODEL FOUND.

STEP 8 VARIABLE PCINC ENTERED R SQUARE = 0.24818060 C(P) = 5.47599062

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	8	108.28255557	13.53531945	5.20	0.0001
ERROR	126	328.02292591	2.60335655		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	11.85427537				
SPRO	-0.26341138	0.31421417	1.82957899	0.70	0.4034
HB	-0.19580150	0.16528006	3.65363159	1.40	0.2384
RHT	-0.05223176	0.04176725	4.07128223	1.56	0.2134
MUSC	-0.00019548	0.00020250	2.42602628	0.93	0.3362
AGE	0.04077871	0.00868841	57.34820634	22.03	0.0001
PCINC	0.00002905	0.00004915	0.90950801	0.35	0.5555
PRO	0.01790907	0.01152009	6.29169895	2.42	0.1226
E	-0.00107111	0.00043493	15.78918090	6.06	0.0151

THE ABOVE MODEL IS THE BEST 8 VARIABLE MODEL FOUND.

STEP 9 VARIABLE SKFD ENTERED R SQUARE = 0.25013550 C(P) = 7.15752554

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	9	109.13548883	12.12616543	4.63	0.0001
ERROR	125	327.16999265	2.61735994		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	12.34547354				
SPRO	-0.27912056	0.31625764	2.03875512	0.78	0.3792
HB	-0.20263597	0.16615588	3.89282684	1.49	0.2249
RHT	-0.05566610	0.04230935	4.53077467	1.73	0.1907
SKFD	0.01226256	0.02148104	0.85293326	0.33	0.5691
MUSC	-0.00020819	0.00020426	2.71901980	1.04	0.3101
AGE	0.03974964	0.00889630	52.25292393	19.96	0.0001
PCINC	0.00002841	0.00004929	0.86966578	0.33	0.5654
PRO	0.01671253	0.01173967	5.30440158	2.03	0.1571
E	-0.00100594	0.00045080	13.03297912	4.98	0.0274

THE ABOVE MODEL IS THE BEST 9 VARIABLE MODEL FOUND.

STEP 10 VARIABLE ZN ENTERED R SQUARE = 0.25069736 C(P) = 9.06599524

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	10	109.38063108	10.93806311	4.15	0.0001
ERROR	124	326.92485041	2.63649073		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	12.03025463				
SPRO	-0.27535837	0.31765103	1.98117243	0.75	0.3877
HB	-0.20041065	0.16692161	3.80051718	1.44	0.2322
RHT	-0.05361752	0.04299186	4.10078681	1.56	0.2147
SKFD	0.01398224	0.02228482	1.03791378	0.39	0.5315
MUSC	-0.00020911	0.00020503	2.74255812	1.04	0.3098
AGE	0.03971817	0.00892935	52.16327282	19.79	0.0001
PCINC	0.00002794	0.00004950	0.84030713	0.32	0.5734
PRO	0.01877411	0.01358443	5.03572779	1.91	0.1694
E	-0.00098545	0.00045740	12.23772031	4.64	0.0331
ZN	-0.01338311	0.04388953	0.24514225	0.09	0.7609

THE ABOVE MODEL IS THE BEST 10 VARIABLE MODEL FOUND.

APPENDIX D

STEPWISE REGRESSION MODELS FOR PLASMA RIBONUCLEASE

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE RNASE

WARNING: 3 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 1 VARIABLE AGE ENTERED R SQUARE = 0.03165186 C(P) = -2.64816226

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	2.37886921	2.37886921	4.35	0.0390
ERROR	133	72.77846412	0.54720650		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.23404040				
AGE	-0.00764254	0.00366546	2.37886921	4.35	0.0390

THE ABOVE MODEL IS THE BEST 1 VARIABLE MODEL FOUND.

STEP 2 VARIABLE PRO ENTERED R SQUARE = 0.05781569 C(P) = -4.11610500

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	4.34527305	2.17263652	4.05	0.0196
ERROR	132	70.81206029	0.53645500		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.90631499				
PRO	-0.00519948	0.00271575	1.96640383	3.67	0.0577
AGE	-0.00968145	0.00378229	3.51483128	6.55	0.0116

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE SKFD ENTERED R SQUARE = 0.06816701 C(P) = -3.48814395

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	5.12325090	1.70775030	3.19	0.0255
ERROR	131	70.03408243	0.53461132		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.99274100				
SKFD	-0.01102091	0.00913593	0.77797786	1.46	0.2299
PRO	-0.00561788	0.00273318	2.25863565	4.22	0.0418
AGE	-0.00879201	0.00384710	2.79220891	5.22	0.0239

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE MENS ENTERED R SQUARE = 0.07650946 C(P) = -2.59391169

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	5.75024682	1.43756171	2.69	0.0338
ERROR	130	69.40708651	0.53390067		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.21481792				
SKFD	-0.01041784	0.00914680	0.69259146	1.30	0.2568
PRO	-0.00618362	0.00278081	2.64000259	4.94	0.0279
AGE	-0.00555428	0.00486897	0.69477117	1.30	0.2561
MENS	0.20286110	0.18719604	0.62699592	1.17	0.2805

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE PCINC ENTERED R SQUARE = 0.08079976 C(P) = -1.16257946

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	6.07269461	1.21453892	2.27	0.0510
ERROR	129	69.08463872	0.53553984		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.95468204				
SKFD	-0.01065744	0.00916603	0.72399349	1.35	0.2471
PRO	-0.00572661	0.00284666	2.16727524	4.05	0.0463
AGE	-0.00487101	0.00495531	0.51747487	0.97	0.3275
MENS	0.22030815	0.18882666	0.72899790	1.36	0.2455
PCINC	0.00001671	0.00002153	0.32244779	0.60	0.4392

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

STEP 6 VARIABLE E ENTERED R SQUARE = 0.08271962 C(P) = 0.58294870

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	6	6.21698604	1.03616434	1.92	0.0817
ERROR	128	68.94034729	0.53859646		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.91501232				
SKFD	-0.00935281	0.00953147	0.51859426	0.96	0.3283
PRO	-0.00796847	0.00518749	1.27086172	2.36	0.1270
E	0.00010558	0.00020399	0.14429142	0.27	0.6056
AGE	-0.00498610	0.00497440	0.54113221	1.00	0.3181
MENS	0.21196789	0.19004910	0.66999571	1.24	0.2668
PCINC	0.00001566	0.00002169	0.28103772	0.52	0.4714

THE ABOVE MODEL IS THE BEST 6 VARIABLE MODEL FOUND.

STEP 7 VARIABLE SPRO ENTERED R SQUARE = 0.08466024 C(P) = 2.32572451

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	7	6.36283811	0.90897687	1.68	0.1193
ERROR	127	68.79449522	0.54168894		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.35709452				
SPRO	0.07353607	0.14171608	0.14585207	0.27	0.6047
SKFD	-0.00969349	0.00958132	0.55444636	1.02	0.3136
PRO	-0.00789212	0.00520444	1.24562759	2.30	0.1319
E	0.00011079	0.00020482	0.15848507	0.29	0.5895
AGE	-0.00500805	0.00498884	0.54586798	1.01	0.3174
MENS	0.21823953	0.19097677	0.70738481	1.31	0.2553
PCINC	0.00001674	0.00002185	0.31804642	0.59	0.4449

THE ABOVE MODEL IS THE BEST 7 VARIABLE MODEL FOUND.

STEP 8 VARIABLE EZN ENTERED R SQUARE = 0.08569294 C(P) = 4.18884347

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	8	6.44045283	0.80505660	1.48	0.1723
ERROR	126	68.71688051	0.54537207		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.39133004				
SPRO	0.07016895	0.14247690	0.13227992	0.24	0.6232
EZN	-0.01514192	0.04013798	0.07761472	0.14	0.7066
SKFD	-0.00963695	0.00961500	0.54786406	1.00	0.3181
PRO	-0.00759597	0.00528078	1.12840102	2.07	0.1528
E	0.00009538	0.00020954	0.11300497	0.21	0.6497
AGE	-0.00433914	0.00531053	0.36410275	0.67	0.4154
MENS	0.21937676	0.19164864	0.71459944	1.31	0.2545
PCINC	0.00001682	0.00002192	0.32104895	0.59	0.4444

THE ABOVE MODEL IS THE BEST 8 VARIABLE MODEL FOUND.

STEP 9 VARIABLE RACE ENTERED R SQUARE = 0.08610398 C(P) = 6.13436169

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	9	6.47134526	0.71903836	1.31	0.2384
ERROR	125	68.68598808	0.54948790		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.52052677				
SPRO	0.07106805	0.14306377	0.13559624	0.25	0.6202
EZN	-0.01497628	0.04029521	0.07590304	0.14	0.7108
SKFD	-0.00977999	0.00967005	0.56205275	1.02	0.3138
PRO	-0.00787695	0.00543152	1.15566580	2.10	0.1495
E	0.00010805	0.00021701	0.13623008	0.25	0.6194
AGE	-0.00466161	0.00550129	0.39454850	0.72	0.3984
MENS	0.20020909	0.20866571	0.50585210	0.92	0.3392
RACE	-0.04126564	0.17403700	0.03089243	0.06	0.8130
PCINC	0.00001507	0.00002321	0.23145892	0.42	0.5175

THE ABOVE MODEL IS THE BEST 9 VARIABLE MODEL FOUND.

STEP 10 VARIABLE MUSC ENTERED R SQUARE = 0.08662198 C(P) = 8.06570208

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	10	6.51027685	0.65102768	1.18	0.3133
ERROR	124	68.64705648	0.55360529		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.37711025				
SPRO	0.07928413	0.14690306	0.16125421	0.29	0.5904
EZN	-0.01381711	0.04068141	0.06386205	0.12	0.7347
SKFD	-0.01014593	0.00980382	0.59291638	1.07	0.3027
MUSC	0.00002591	0.00009770	0.03893159	0.07	0.7913
PRO	-0.00770653	0.00548957	1.09104056	1.97	0.1629
E	0.00011262	0.00021850	0.14706070	0.27	0.6072
AGE	-0.00470265	0.00552403	0.40121222	0.72	0.3962
MENS	0.20451404	0.21007421	0.52468788	0.95	0.3322
RACE	-0.05148212	0.17888562	0.04585241	0.08	0.7740
PCINC	0.00001442	0.00002343	0.20987708	0.38	0.5392

THE ABOVE MODEL IS THE BEST 10 VARIABLE MODEL FOUND.

APPENDIX E

STEPWISE REGRESSION MODELS FOR SERUM ALKALINE PHOSPHATASE

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE AP

WARNING: 2 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 1 VARIABLE MENS ENTERED R SQUARE = 0.48804309 C(P) = 36.53371346

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	240.60115103	240.60115103	127.74	0.0001
ERROR	134	252.39046000	1.88351090		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	-0.10850000				
MENS	2.91912500	0.25827829	240.60115103	127.74	0.0001

THE ABOVE MODEL IS THE BEST 1 VARIABLE MODEL FOUND.

STEP 2 VARIABLE AGE ENTERED R SQUARE = 0.55334807 C(P) = 17.03563504

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	272.79595520	136.39797760	82.39	0.0001
ERROR	133	220.19565583	1.65560643		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	7.33223659				
AGE	-0.03737264	0.00847499	32.19480417	19.45	0.0001
MENS	1.98395258	0.32188407	62.89566489	37.99	0.0001

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE VD ENTERED R SQUARE = 0.58411735 C(P) = 8.90653867

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	287.96495160	95.98831720	61.80	0.0001
ERROR	132	205.02665943	1.55323227		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.78759193				
VD	0.00182768	0.00058484	15.16899639	9.77	0.0022
AGE	-0.03602750	0.00822007	29.83692150	19.21	0.0001
MENS	1.89757134	0.31299638	57.08920715	36.76	0.0001

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE RNASE ENTERED R SQUARE = 0.60761785 C(P) = 3.17028821

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	299.55050189	74.88762547	50.71	0.0001
ERROR	131	193.44110914	1.47664969		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.05912057				
RNASE	-0.39993724	0.14278160	11.58555030	7.85	0.0059
VD	0.00178881	0.00057041	14.52210536	9.83	0.0021
AGE	-0.03814729	0.00805051	33.15571051	22.45	0.0001
MENS	1.95511924	0.30587343	60.33099173	40.86	0.0001

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE MUSC ENTERED R SQUARE = 0.62087504 C(P) = 0.80608588

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	306.08618552	61.21723710	42.58	0.0001
ERROR	130	186.90542551	1.43773404		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	10.28539247				
RNASE	-0.38234427	0.14112903	10.55249082	7.34	0.0077
VD	0.00149617	0.00057934	9.58902552	6.67	0.0109
MUSC	-0.00031006	0.00014543	6.53568363	4.55	0.0349
AGE	-0.03879111	0.00794946	34.23483193	23.81	0.0001
MENS	1.81367477	0.30902103	49.52463890	34.45	0.0001

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

STEP 6 VARIABLE PCINC ENTERED R SQUARE = 0.62793745 C(P) = 0.48117335

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	6	309.56789687	51.59464948	36.29	0.0001
ERROR	129	183.42371416	1.42188926		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.79483353				
RNASE	-0.40316252	0.14097835	11.62843336	8.18	0.0049
VD	0.00145443	0.00057676	9.04210548	6.36	0.0129
MUSC	-0.00033726	0.00014566	7.62251469	5.36	0.0222
AGE	-0.03709719	0.00797930	30.73393479	21.61	0.0001
MENS	1.87986566	0.31021095	52.21618837	36.72	0.0001
PCINC	0.00005410	0.00003457	3.48171135	2.45	0.1201

THE ABOVE MODEL IS THE BEST 6 VARIABLE MODEL FOUND.

STEP 7 VARIABLE RHT ENTERED R SQUARE = 0.62976132 C(P) = 1.88076516

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	7	310.46704813	44.35243545	31.10	0.0001
ERROR	128	182.52456290	1.42597315		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	12.67636586				
RNASE	-0.40464662	0.14119304	11.71215014	8.21	0.0049
VD	0.00148928	0.00057925	9.42617706	6.61	0.0113
RHT	-0.02513115	0.03164839	0.89915126	0.63	0.4286
MUSC	-0.00032978	0.00014618	7.25743537	5.09	0.0258
AGE	-0.03908996	0.00837556	31.06091519	21.78	0.0001
MENS	1.81976372	0.31974351	46.18892592	32.39	0.0001
PCINC	0.00005697	0.00003481	3.81904109	2.68	0.1042

THE ABOVE MODEL IS THE BEST 7 VARIABLE MODEL FOUND.

STEP 8 VARIABLE SKFD ENTERED R SQUARE = 0.63082150 C(P) = 3.53175797

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	8	310.98970964	38.87371370	27.13	0.0001
ERROR	127	182.00190139	1.43308584		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	12.40932434				
RNASE	-0.41260742	0.14215723	12.07281090	8.42	0.0044
VD	0.00143154	0.00058851	8.47954408	5.92	0.0164
RHT	-0.02196809	0.03215663	0.66883014	0.47	0.4958
SKFD	-0.00930873	0.01541404	0.52266150	0.36	0.5470
MUSC	-0.00032180	0.00014714	6.85482986	4.78	0.0306
AGE	-0.03822253	0.00851839	28.85332360	20.13	0.0001
MENS	1.82275625	0.32057825	46.32989052	32.33	0.0001
PCINC	0.00005799	0.00003494	3.94858378	2.76	0.0994

THE ABOVE MODEL IS THE BEST 8 VARIABLE MODEL FOUND.

STEP 9 VARIABLE E ENTERED R SQUARE = 0.63209799 C(P) = 5.11154526

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	9	311.61900615	34.62433402	24.05	0.0001
ERROR	126	181.37260487	1.43946512		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	12.76258588				
RNASE	-0.42241114	0.14324275	12.51775909	8.70	0.0038
E	-0.00012733	0.00019258	0.62929652	0.44	0.5097
VD	0.00155497	0.00061865	9.09387326	6.32	0.0132
RHT	-0.02149951	0.03223591	0.64029241	0.44	0.5060
SKFD	-0.01137838	0.01576224	0.75011169	0.52	0.4717
MUSC	-0.00033737	0.00014933	7.34692793	5.10	0.0256
AGE	-0.03864758	0.00856150	29.33230179	20.38	0.0001
MENS	1.84699429	0.32337550	46.95891262	32.62	0.0001
PCINC	0.00005494	0.00003532	3.48332801	2.42	0.1223

THE ABOVE MODEL IS THE BEST 9 VARIABLE MODEL FOUND.

STEP 10 VARIABLE EZN ENTERED R SQUARE = 0.63222401 C(P) = 7.07005976

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	10	311.68113345	31.16811334	21.49	0.0001
ERROR	125	181.31047758	1.45048382		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	12.64625347				
EZN	0.01360977	0.06576062	0.06212729	0.04	0.8364
RNASE	-0.42081974	0.14399540	12.38818909	8.54	0.0041
E	-0.00011987	0.00019664	0.53897020	0.37	0.5433
VD	0.00154099	0.00062468	8.82666246	6.09	0.0150
RHT	-0.02077210	0.03254938	0.59072923	0.41	0.5245
SKFD	-0.01159301	0.01585640	0.77534728	0.53	0.4661
MUSC	-0.00033457	0.00015051	7.16691373	4.94	0.0280
AGE	-0.03914759	0.00892733	27.89201879	19.23	0.0001
MENS	1.84792579	0.32464201	46.99725524	32.40	0.0001
PCINC	0.00005492	0.00003545	3.48038970	2.40	0.1239

THE ABOVE MODEL IS THE BEST 10 VARIABLE MODEL FOUND.

APPENDIX F

FIGURES 3 THROUGH 13

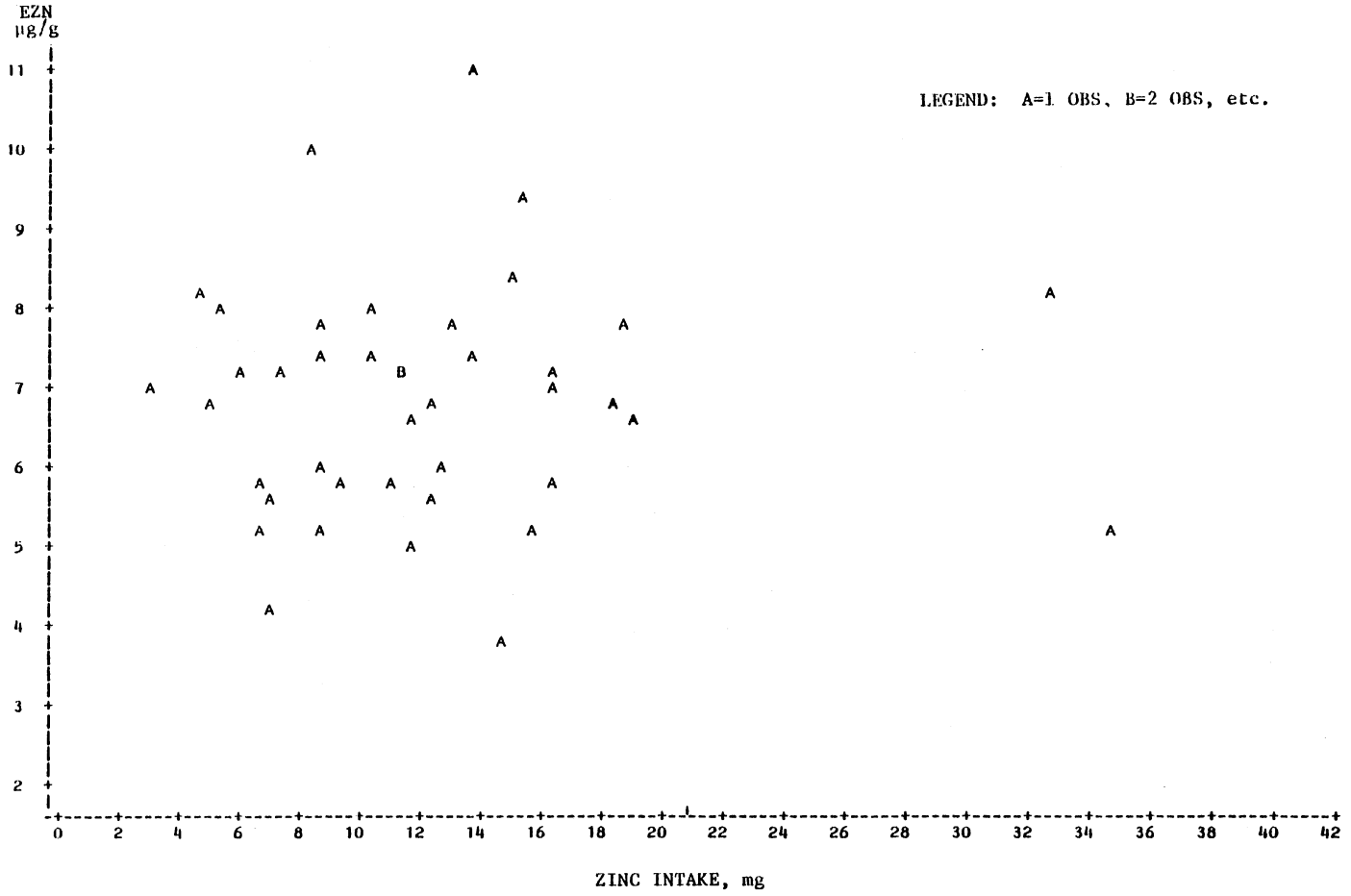

Figure 3. Scattergram of Erythrocyte Zinc Concentration and Zinc Intake for Pre-Menarcheal Subjects

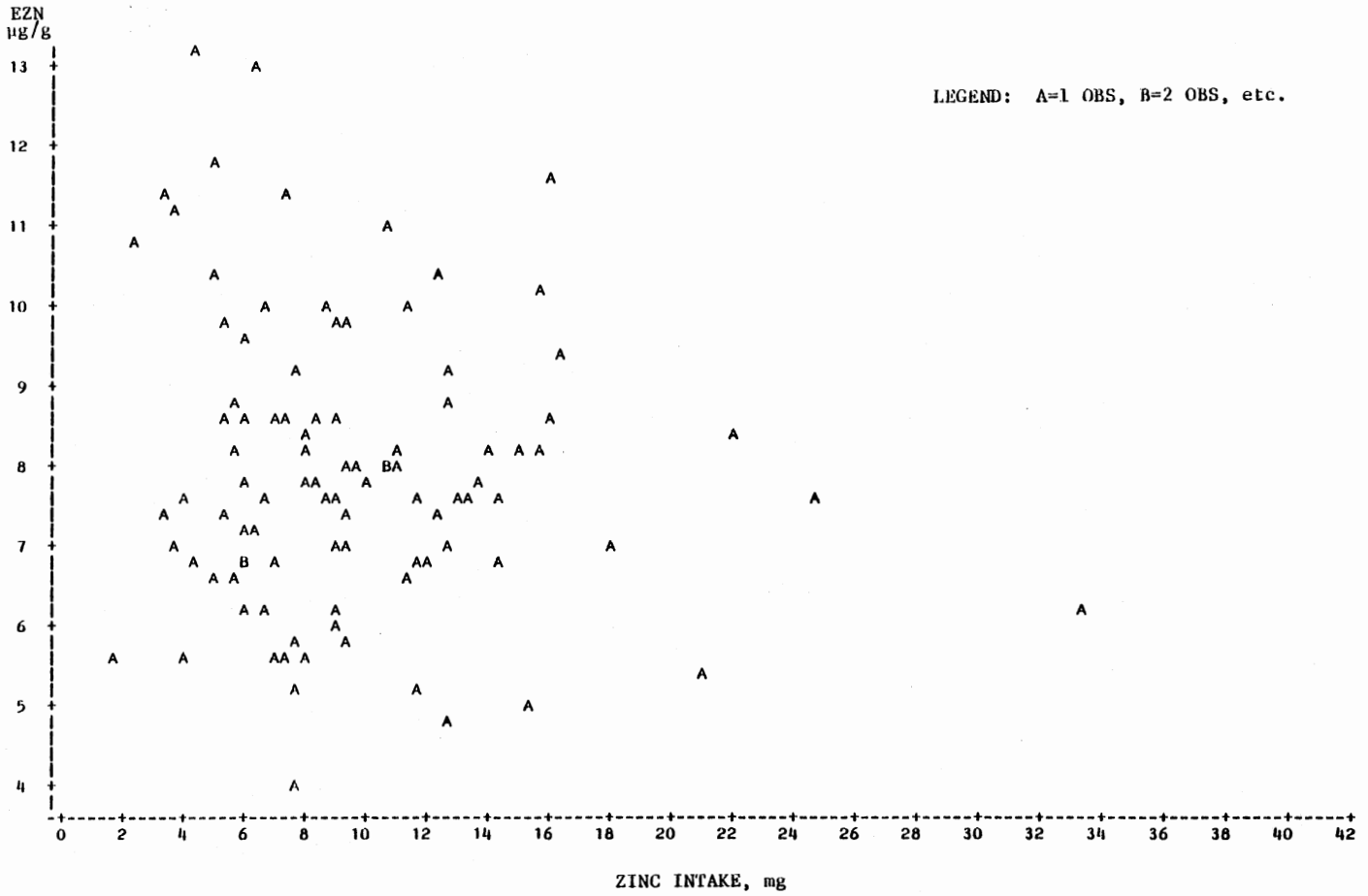

Figure 4. Scattergram of Erythrocyte Zinc Concentration and Zinc Intake for Post-Menarcheal Subjects

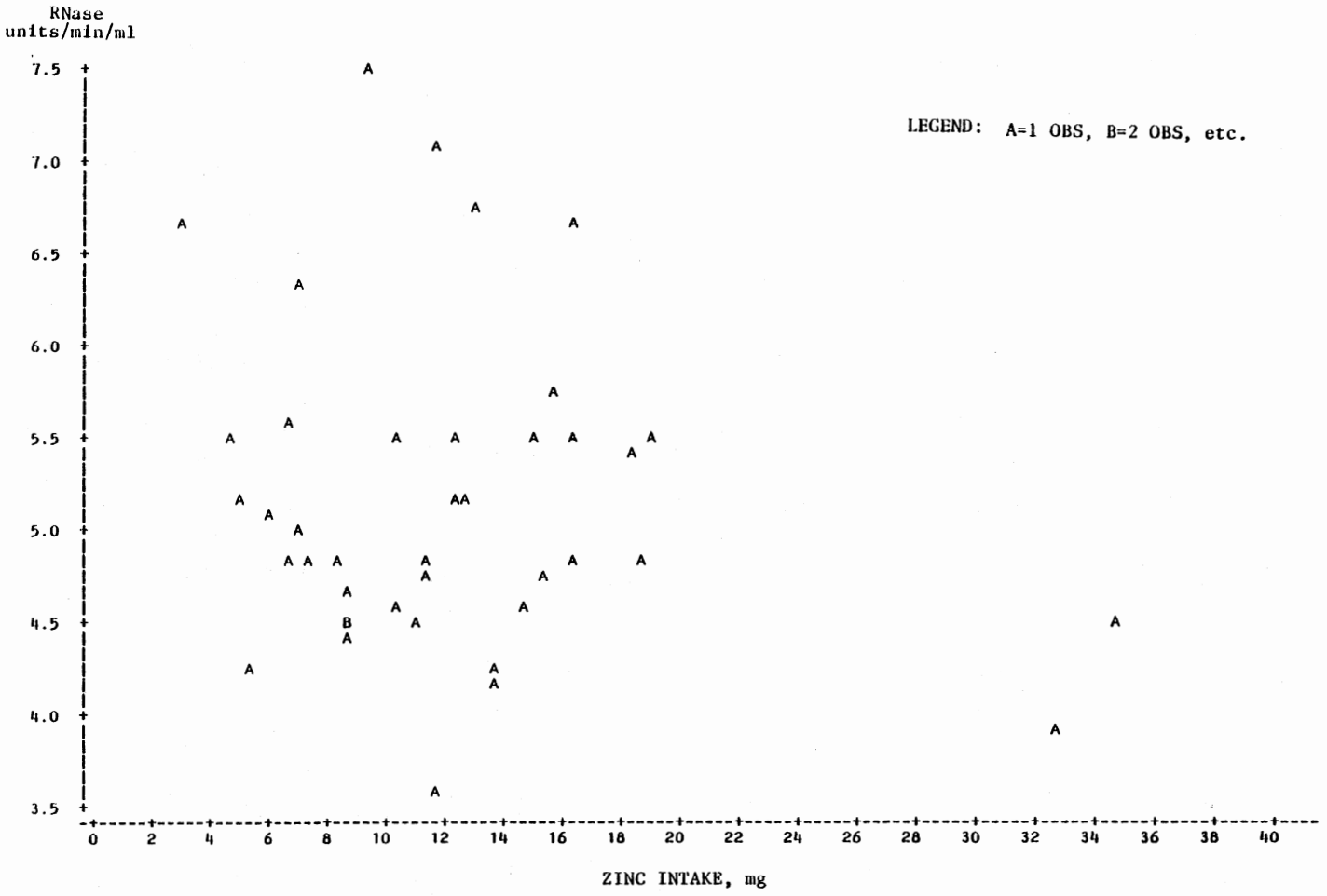

Figure 5. Scattergram of Plasma Ribonuclease and Zinc Intake for Pre-Menarcheal Subjects

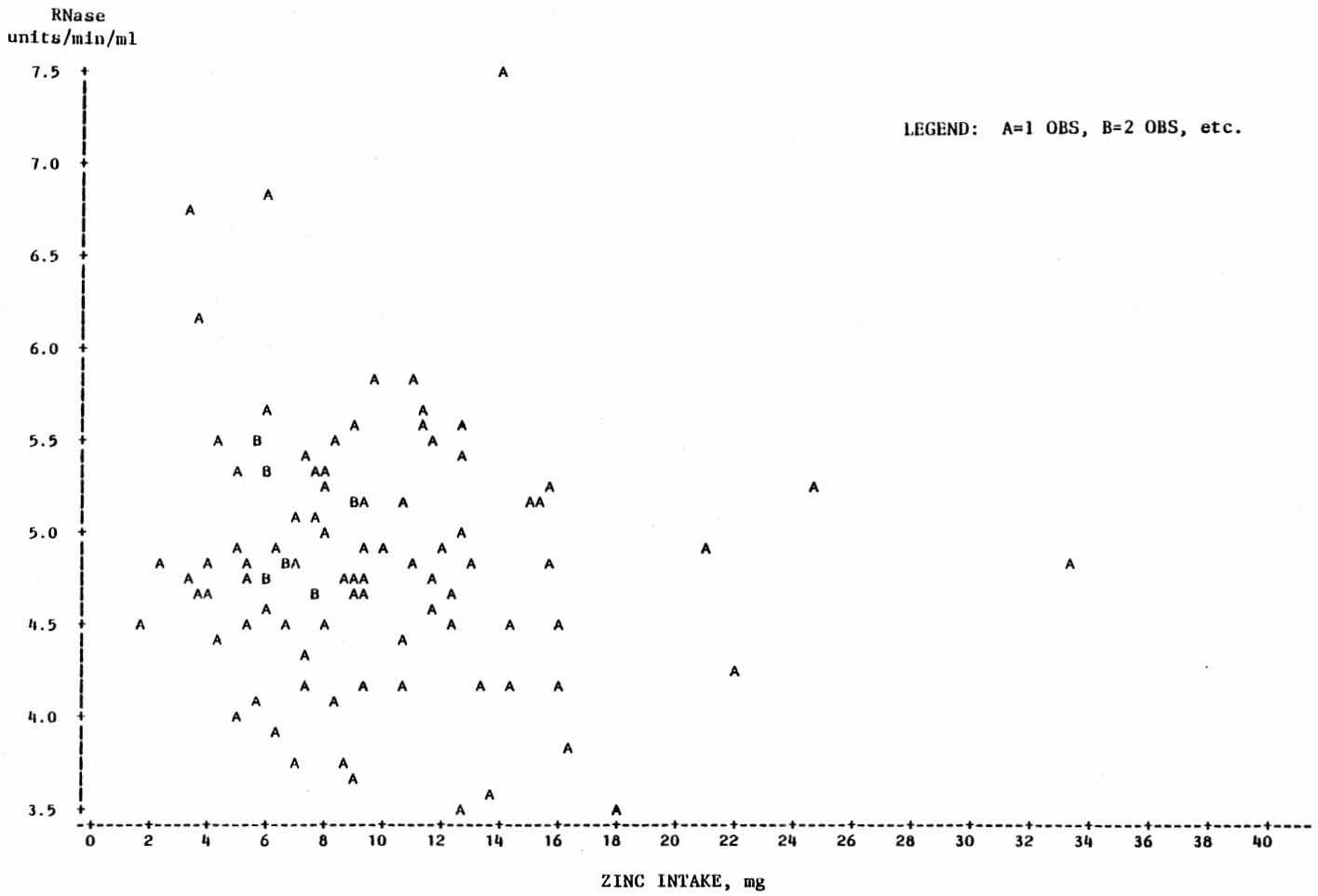

Figure 6. Scattergram of Plasma Ribonuclease and Zinc Intake for Post-Menarcheal Subjects

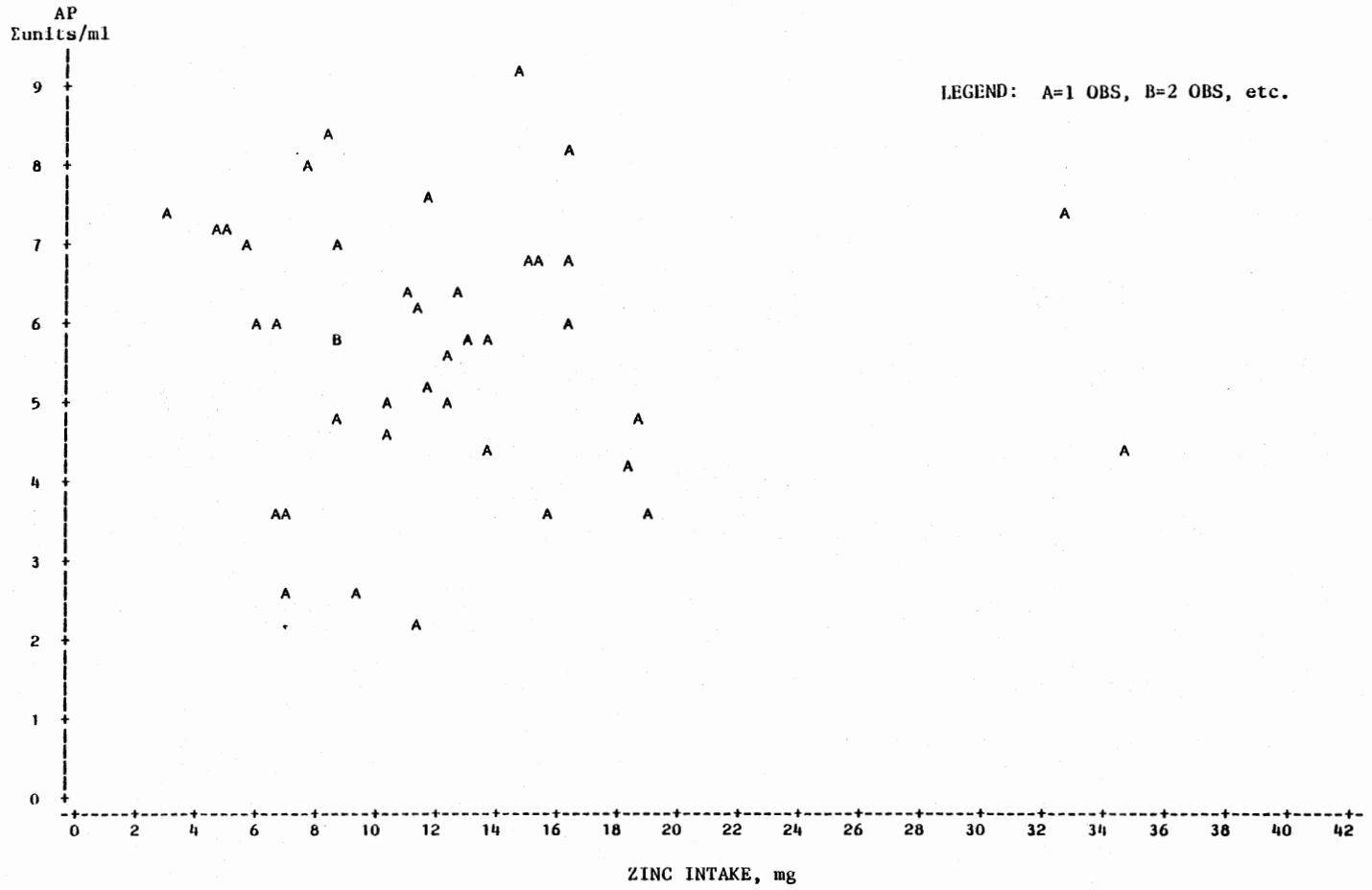

Figure 7. Scattergram of Serum Alkaline Phosphatase and Zinc Intake for Pre-Menarcheal Subjects

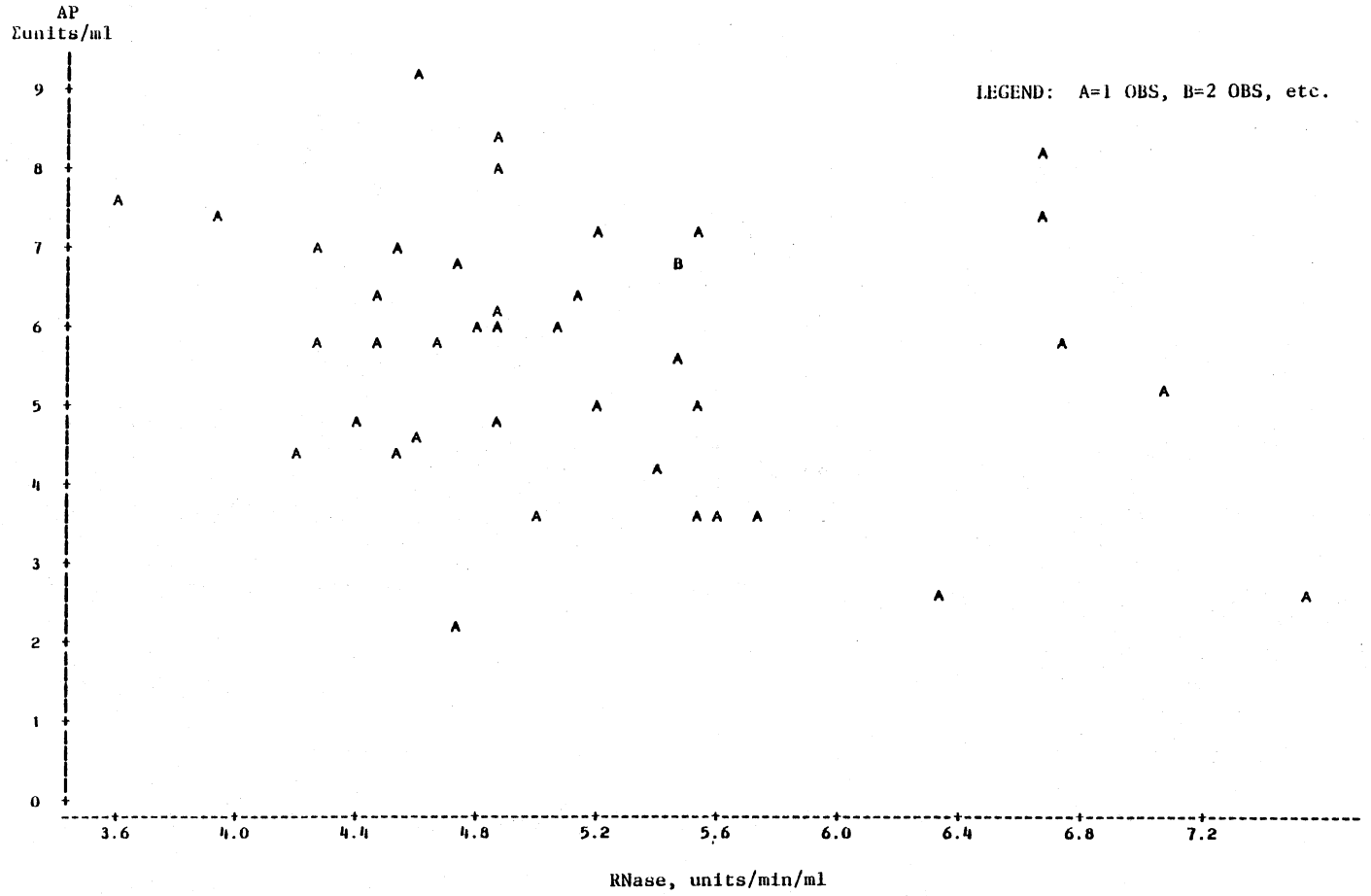

Figure 8. Scattergram of Serum Alkaline Phosphatase and Plasma Ribonuclease for Pre-Menarcheal Subjects

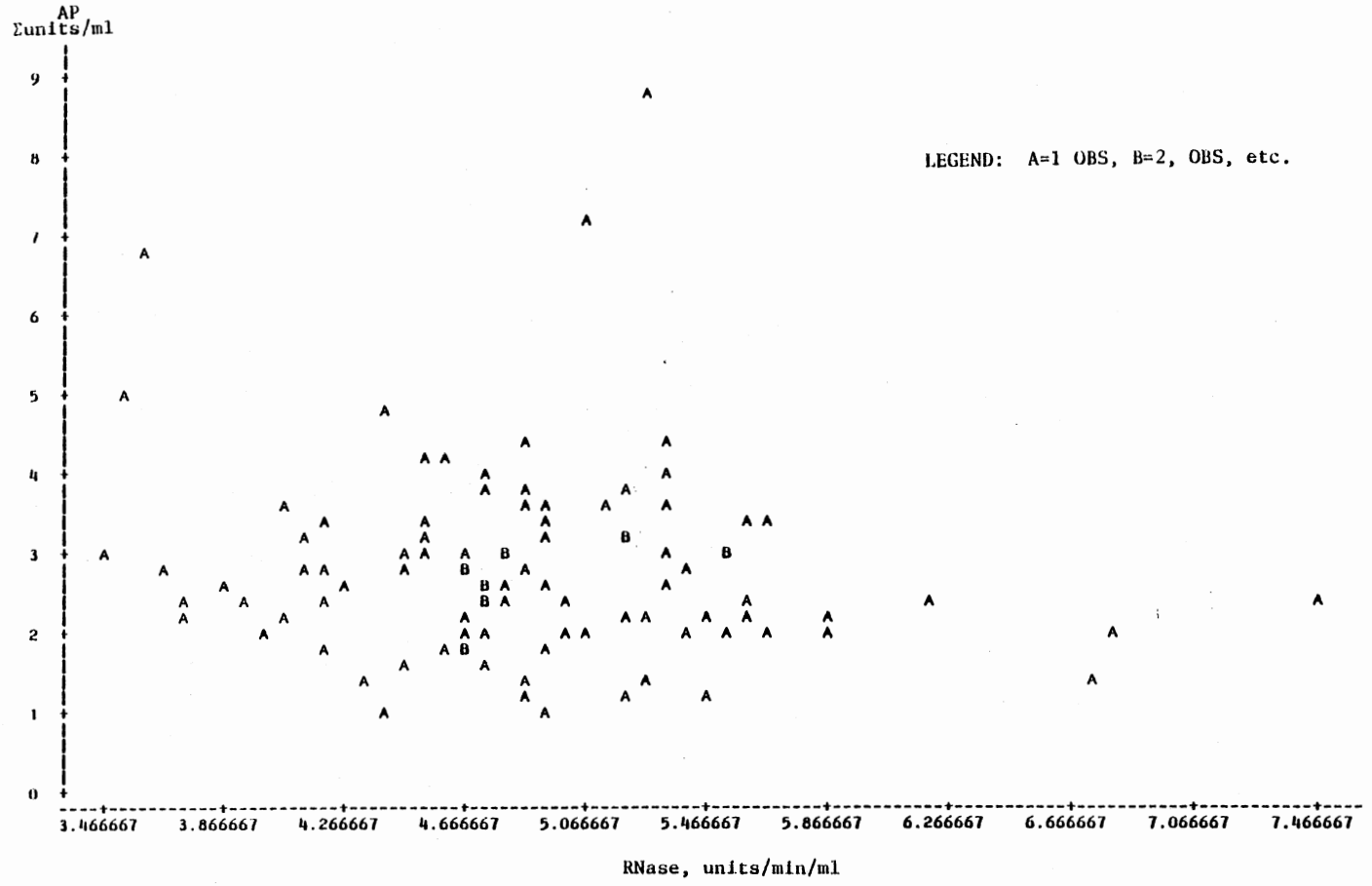

Figure 9. Scattergram of Serum Alkaline Phosphatase and Plasma Ribonuclease for Post-Menarcheal Subjects

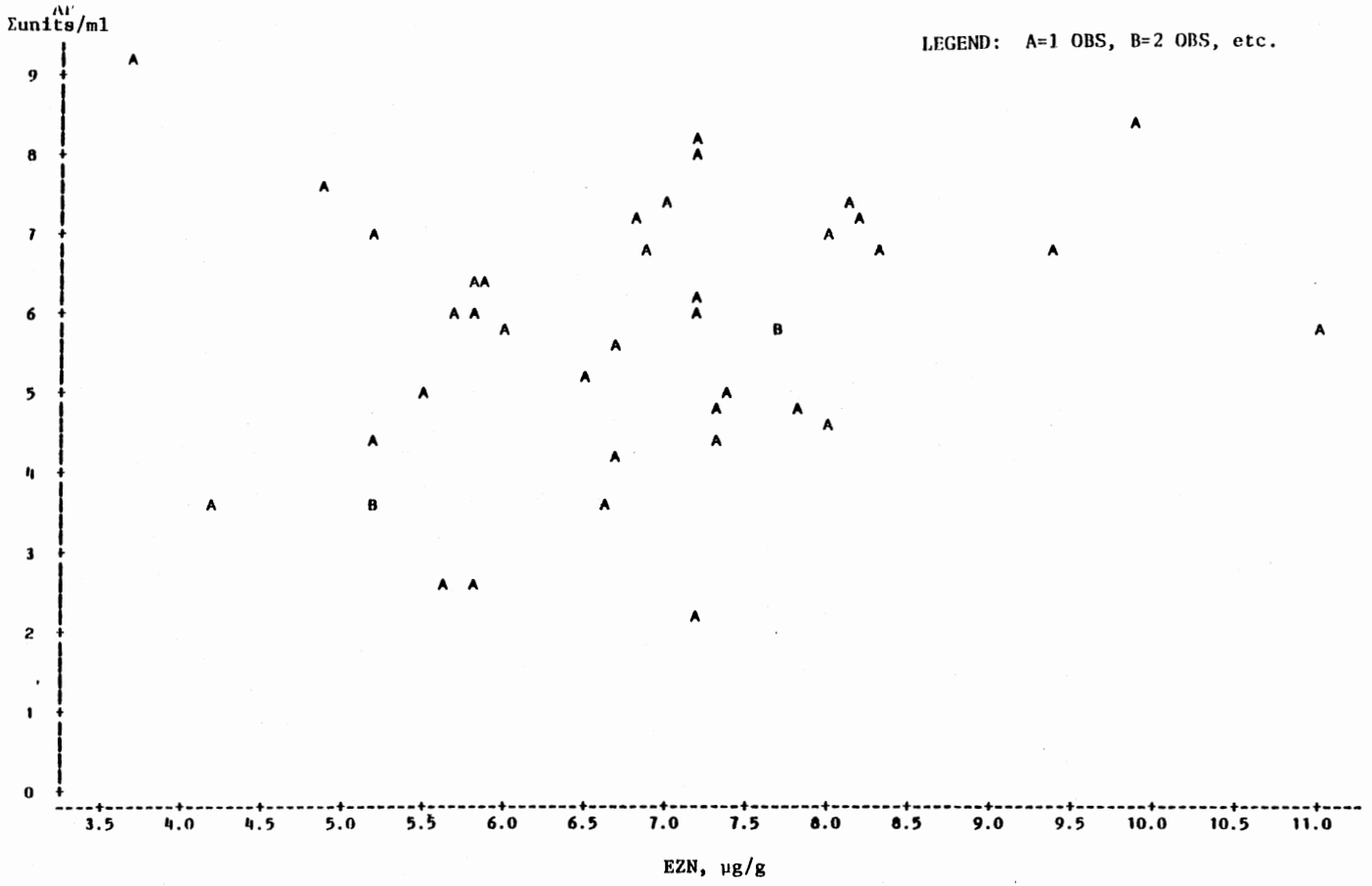

Figure 10. Scattergram of Serum Alkaline Phosphatase and Erythrocyte Zinc Concentration for Pre-Menarcheal Subjects

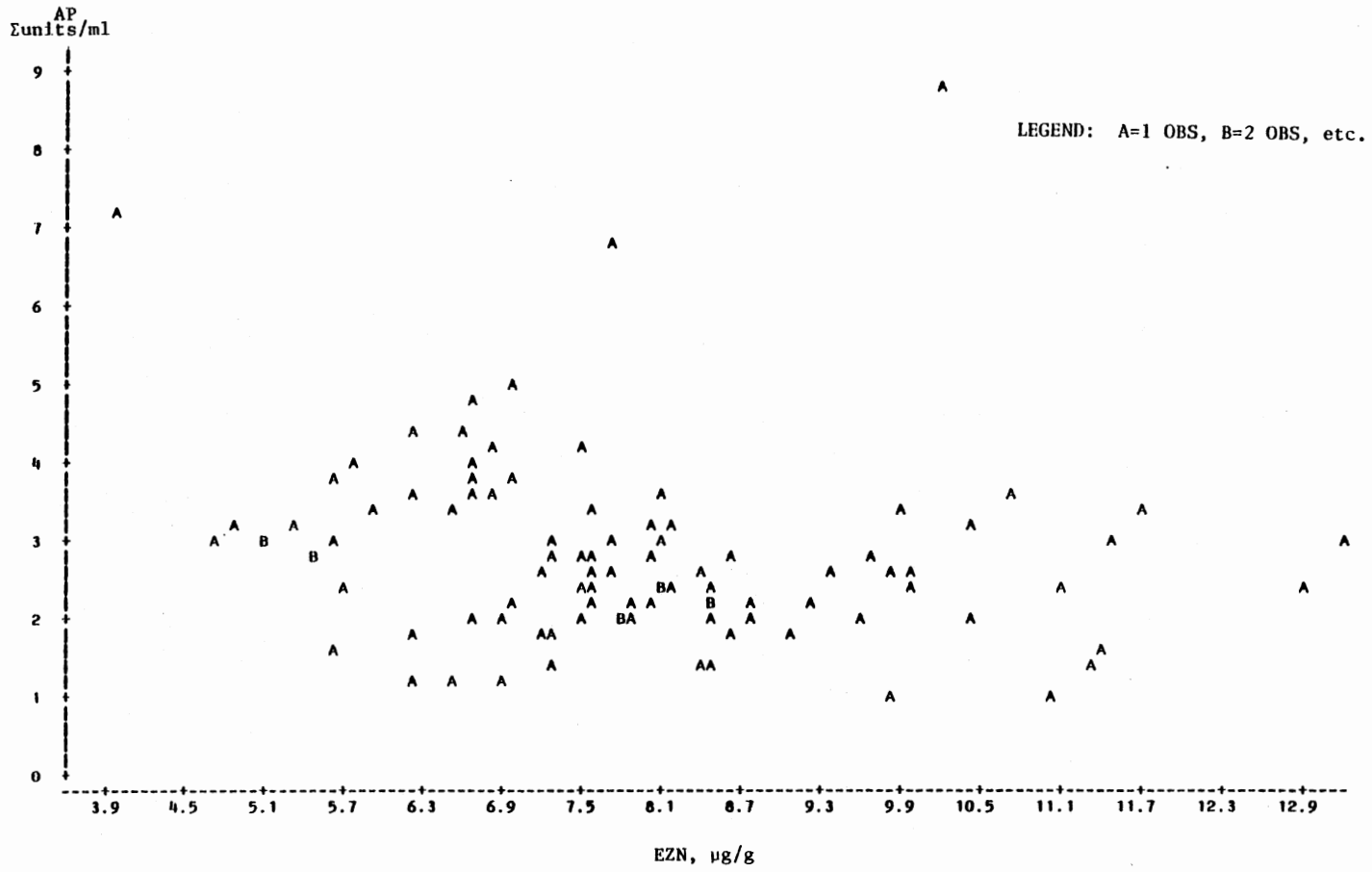

Figure 11. Scattergram of Serum Alkaline Phosphatase and Erythrocyte Zinc Concentration for Post-Menarcheal Subjects

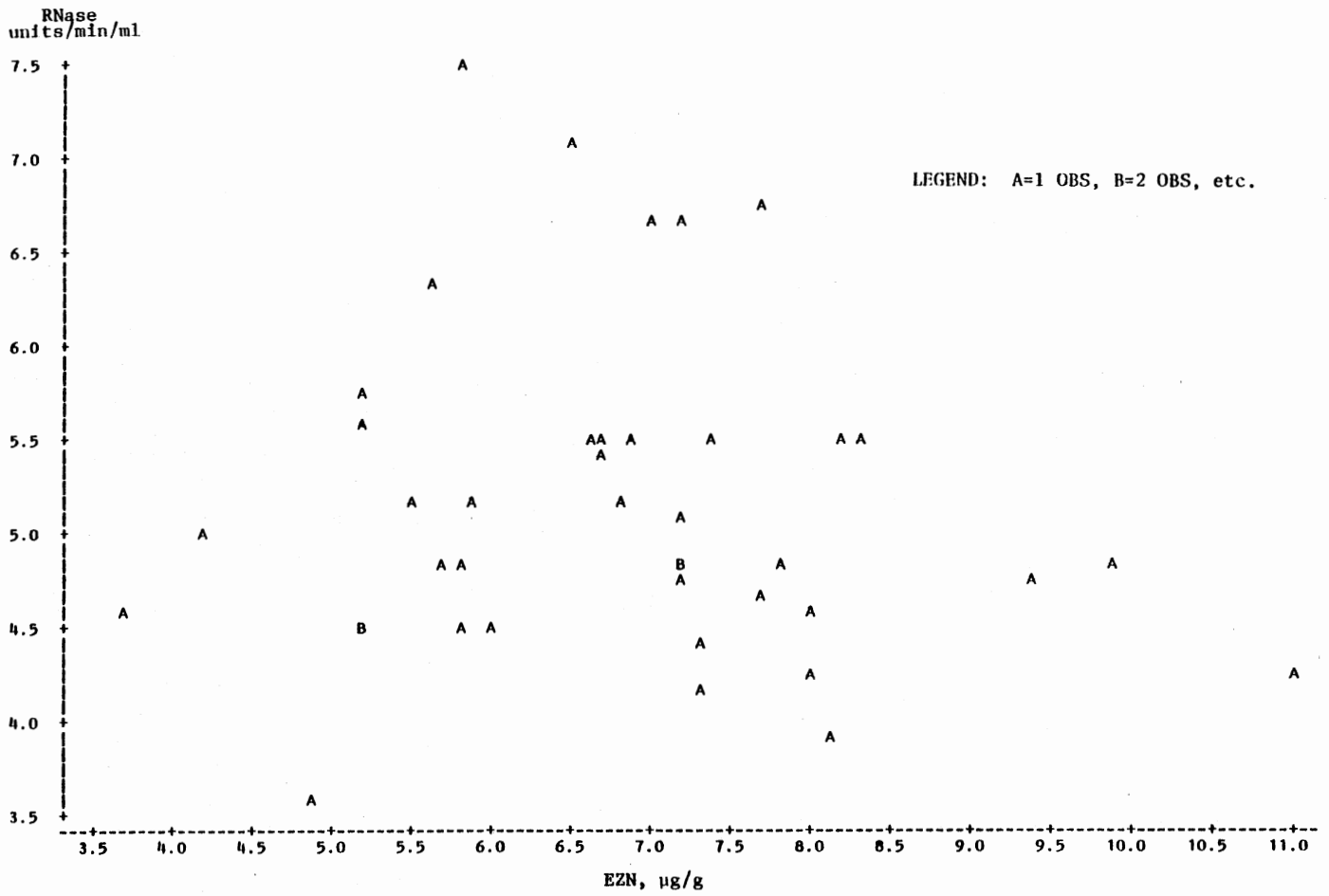

Figure 12. Scattergram of Plasma Ribonuclease and Erythrocyte Zinc Concentration for Pre-Menarcheal Subjects

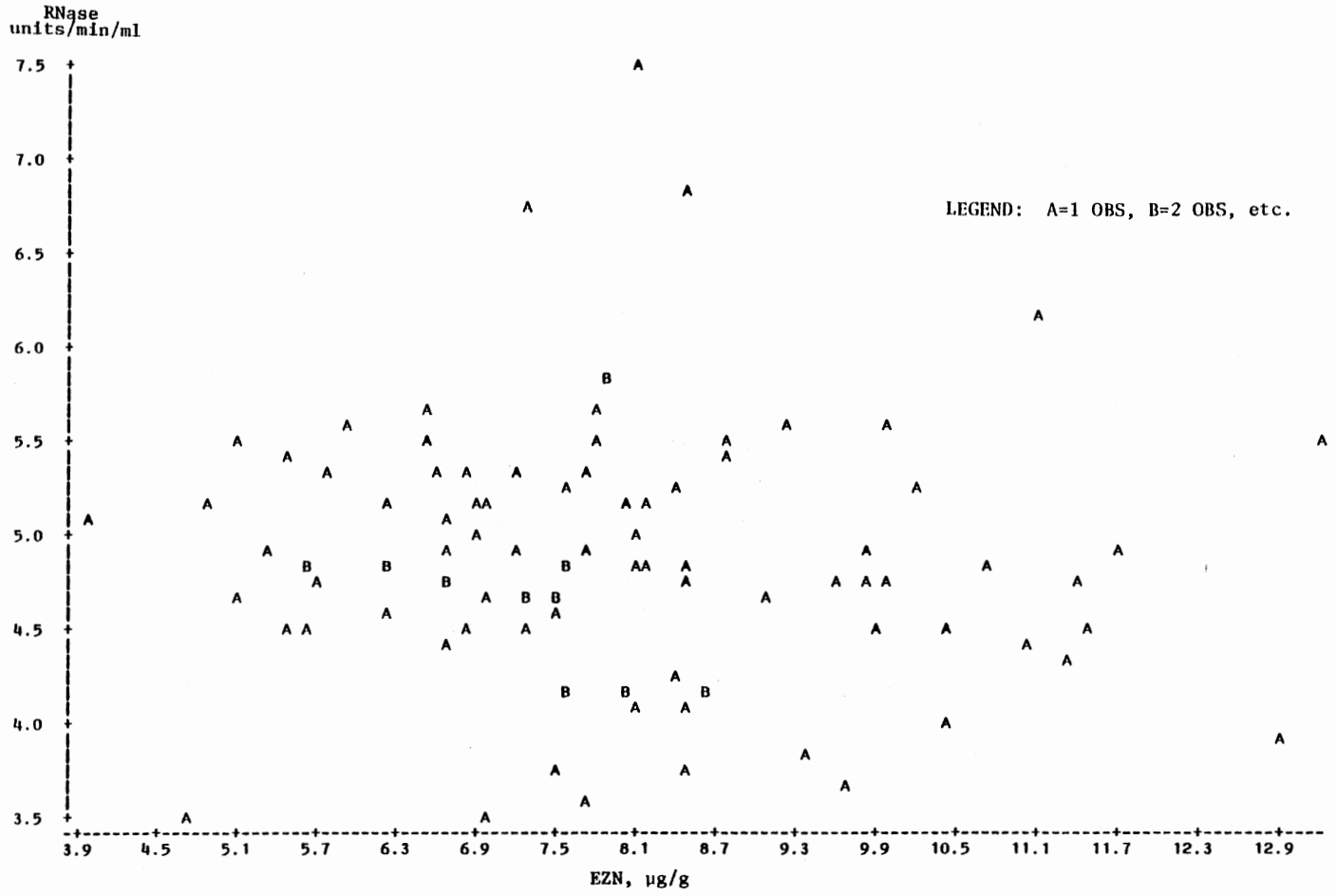

Figure 13. Scattergram of Plasma Ribonuclease and Erythrocyte Zinc Concentration for Post-Menarcheal Subjects

APPENDIX G

STEPWISE REGRESSION MODELS WITH "FORCED" VARIABLES

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE EZN

THE FIRST 1 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

WARNING: 3 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 INCLUDED VARIABLE ENTERED R SQUARE = 0.02173409 C(P) = 28.36551612

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	9.48270078	9.48270078	2.95	0.0879
ERROR	133	426.82278070	3.20919384		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	8.06475299				
ZN	-0.04778841	0.02780062	9.48270078	2.95	0.0879

STEP 2 VARIABLE AGE ENTERED R SQUARE = 0.19001068 C(P) = 2.95222674

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	82.90270208	41.45135104	15.48	0.0001
ERROR	132	353.40277941	2.67729378		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	0.48589599				
ZN	-0.01723706	0.02605404	1.17185149	0.44	0.5094
AGE	0.04356425	0.00831900	73.42000130	27.42	0.0001

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE E ENTERED R SQUARE = 0.20306463 C(P) = 2.82565776

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	88.59821331	29.53273777	11.13	0.0001
ERROR	131	347.70726817	2.65425396		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	1.33887058				
ZN	0.01787301	0.03531924	0.67969665	0.26	0.6137
AGE	0.04144154	0.00840893	64.46634845	24.29	0.0001
E	-0.00046834	0.00031971	5.69551124	2.15	0.1454

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE EZN

THE FIRST 1 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

STEP 4 VARIABLE PRO ENTERED R SQUARE = 0.22212090 C(P) = 1.72127487

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	96.91256744	24.22814186	9.28	0.0001
ERROR	130	339.39291404	2.61071472		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	1.01739631				
ZN	-0.02134285	0.04135074	0.69550128	0.27	0.6066
AGE	0.04262540	0.00836602	67.77334080	25.96	0.0001
PRO	0.02330642	0.01305993	8.31435413	3.18	0.0767
E	-0.00098909	0.00043092	13.75422057	5.27	0.0233

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE RHT ENTERED R SQUARE = 0.23051476 C(P) = 2.35386461

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	100.57485187	20.11497037	7.73	0.0001
ERROR	129	335.73062962	2.60256302		
TOTAL	134	436.30548148			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.33908710				
ZN	-0.01204413	0.04202369	0.21377836	0.08	0.7749
RHT	-0.04943286	0.04167162	3.66228443	1.41	0.2377
AGE	0.04060042	0.00852559	59.02190131	22.68	0.0001
PRO	0.02257200	0.01305422	7.78107580	2.99	0.0862
E	-0.00103866	0.00043227	15.02559897	5.77	0.0177

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE RNASE

THE FIRST 1 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

WARNING: 3 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 INCLUDED VARIABLE ENTERED R SQUARE = 0.01366807 C(P) = 7.36365375

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	1.02725532	1.02725532	1.84	0.1769
ERROR	133	74.13007801	0.55736901		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.32729794				
EZN	-0.04852258	0.03574177	1.02725532	1.84	0.1769

STEP 2 VARIABLE AGE ENTERED R SQUARE = 0.03361175 C(P) = 6.56593330

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	2.52616945	1.26308472	2.30	0.1047
ERROR	132	72.63116389	0.55023609		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.23611651				
EZN	-0.02038205	0.03939315	0.14730023	0.27	0.6057
AGE	-0.00672950	0.00407726	1.49891413	2.72	0.1012

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE AP ENTERED R SQUARE = 0.06427967 C(P) = 4.26380651

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	4.83108830	1.61036277	3.00	0.0326
ERROR	131	70.32624503	0.53684157		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	7.66235204				
EZN	-0.01684205	0.03894821	0.10038319	0.19	0.6661
AP	-0.09120264	0.04401520	2.30491886	4.29	0.0402
AGE	-0.01342855	0.00516448	3.62954127	6.76	0.0104

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE RNASL

THE FIRST 1 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

STEP 3 AGE REPLACED BY MENS R SQUARE = 0.06881905 C(P) = 3.62701761

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	5.17225601	1.72408534	3.23	0.0244
ERROR	131	69.98507732	0.53423723		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	4.94195306				
EZN	-0.03756729	0.03666334	0.56090555	1.05	0.3074
AP	-0.10479346	0.04636692	2.72889006	5.11	0.0255
MENS	0.53136195	0.19490523	3.97070898	7.43	0.0073

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE PRO ENTERED R SQUARE = 0.09140218 C(P) = 2.45903257

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	6.86954408	1.71738602	3.27	0.0137
ERROR	130	68.28778925	0.52529069		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.17628674				
EZN	-0.04069270	0.03639661	0.65661505	1.25	0.2656
AP	-0.09441332	0.04633827	2.18065373	4.15	0.0436
PRO	-0.00495181	0.00275478	1.69728807	3.23	0.0746
MENS	0.58351124	0.19543171	4.68282649	8.91	0.0034

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE AGE ENTERED R SQUARE = 0.11564189 C(P) = 1.05866118

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	8.69133607	1.73826721	3.37	0.0069
ERROR	129	66.46599726	0.51524029		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.98497379				
EZN	-0.01694656	0.03819482	0.10142949	0.20	0.6580
AP	-0.12704845	0.04906496	3.45466264	6.70	0.0107
PRO	-0.00513624	0.00273006	1.82371806	3.54	0.0622
AGE	-0.01013106	0.00538779	1.82179199	3.54	0.0623
MENS	0.45434952	0.20538019	2.52158058	4.89	0.0287

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE RNASE

THE FIRST 2 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

WARNING: 3 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 INCLUDED VARIABLES ENTERED R SQUARE = 0.02929632 C(P) = 7.17130601

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	2.20183336	1.10091668	1.99	0.1405
ERROR	132	72.95549998	0.55269318		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.56202058				
EZN	-0.05625629	0.03598473	1.35079585	2.44	0.1204
ZN	-0.01700470	0.01166461	1.17457804	2.13	0.1473

STEP 3 VARIABLE AGE ENTERED R SQUARE = 0.05697722 C(P) = 5.28820114

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	4.28225592	1.42741864	2.64	0.0514
ERROR	131	70.87507741	0.54103113		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.70742823				
EZN	-0.02443453	0.03912696	0.21099775	0.39	0.5334
ZN	-0.02113581	0.01173159	1.75608648	3.25	0.0739
AGE	-0.00805912	0.00410982	2.08042256	3.85	0.0520

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE AP ENTERED R SQUARE = 0.08285401 C(P) = 3.65817866

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	6.22708639	1.55677160	2.94	0.0231
ERROR	130	68.93024694	0.53023267		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	7.97487460				
EZN	-0.02074525	0.03878240	0.15171707	0.29	0.5936
ZN	-0.01893655	0.01167056	1.39599809	2.63	0.1071
AP	-0.08418468	0.04395674	1.94483047	3.67	0.0577
AGE	-0.01410434	0.00514946	3.97785113	7.50	0.0070

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE RNASE

THE FIRST 2 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

STEP 4 AGE REPLACED BY MENS R SQUARE = 0.08653425 C(P) = 3.14191061

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	6.50368353	1.62592088	3.08	0.0185
ERROR	130	68.65364980	0.52810500		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.11913858				
EZN	-0.04258362	0.03658896	0.71532809	1.35	0.2466
ZN	-0.01847094	0.01163296	1.33142751	2.52	0.1148
AP	-0.09760644	0.04632172	2.34481113	4.44	0.0370
MENS	0.55115644	0.19418398	4.25444827	8.06	0.0053

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE AGE ENTERED R SQUARE = 0.11177335 C(P) = 1.60134401

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	8.40058688	1.68011738	3.25	0.0087
ERROR	129	66.75674646	0.51749416		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.97159119				
EZN	-0.01857862	0.03832829	0.12158884	0.23	0.6287
ZN	-0.01985893	0.01153830	1.53296887	2.96	0.0876
AP	-0.13080688	0.04902346	3.68433140	7.12	0.0086
AGE	-0.01035158	0.00540676	1.89690335	3.67	0.0578
MENS	0.41868616	0.20429675	2.17350049	4.20	0.0424

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE RNASE

THE FIRST 3 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

WARNING: 3 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 INCLUDED VARIABLES ENTERED R SQUARE = 0.02992702 C(P) = 9.08283045

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	2.24923526	0.74974509	1.35	0.2611
ERROR	131	72.90809807	0.55655037		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.61238030				
EZN	-0.05874556	0.03710379	1.39514067	2.51	0.1158
ZN	-0.01635119	0.01191751	1.04768823	1.88	0.1724
AP	-0.01038421	0.03558179	0.04740190	0.09	0.7709

STEP 4 VARIABLE MENS ENTERED R SQUARE = 0.08653425 C(P) = 3.14191061

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	6.50368353	1.62592088	3.08	0.0185
ERROR	130	68.65364980	0.52810500		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.11913858				
EZN	-0.04258362	0.03658896	0.71532809	1.35	0.2466
ZN	-0.01847094	0.01163296	1.33142751	2.52	0.1148
AP	-0.09760644	0.04632172	2.34481113	4.44	0.0370
MENS	0.55115644	0.19418398	4.25444827	8.06	0.0053

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE AGE ENTERED R SQUARE = 0.11177335 C(P) = 1.60134401

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	8.40058688	1.68011738	3.25	0.0087
ERROR	129	66.75674646	0.51749416		
TOTAL	134	75.15733333			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.97159119				
EZN	-0.01857862	0.03832829	0.12158884	0.23	0.6287
ZN	-0.01985893	0.01153830	1.53296887	2.96	0.0876
AP	-0.13080688	0.04902346	3.68433140	7.12	0.0086
AGE	-0.01035158	0.00540676	1.89690335	3.67	0.0578
MENS	0.41868616	0.20429675	2.17350049	4.20	0.0424

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE AP

THE FIRST 1 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

WARNING: 2 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 INCLUDED VARIABLE ENTERED R SQUARE = 0.05991470 C(P) = 177.47149092

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	29.53744305	29.53744305	8.54	0.0041
ERROR	134	463.45416798	3.45861319		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.63193122				
EZN	-0.25963685	0.08884457	29.53744305	8.54	0.0041

STEP 2 VARIABLE MENS ENTERED R SQUARE = 0.49032448 C(P) = 37.78269193

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	241.72585579	120.86292790	63.98	0.0001
ERROR	133	251.26575524	1.88921620		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	0.36779734				
EZN	-0.05285341	0.06850063	1.12470476	0.60	0.4417
MENS	2.85981907	0.26984760	212.18841274	112.32	0.0001

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE AGE ENTERED R SQUARE = 0.55503320 C(P) = 18.48089713

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	273.62671218	91.20890406	54.88	0.0001
ERROR	132	219.36489885	1.66185529		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	7.32665391				
EZN	0.04826672	0.06826646	0.83075698	0.50	0.4808
AGE	-0.03952929	0.00902224	31.90085639	19.20	0.0001
MENS	1.98414608	0.32249107	62.90788879	37.85	0.0001

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE AP

THE FIRST 1 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

STEP 4 VARIABLE VD ENTERED R SQUARE = 0.58519832 C(P) = 10.55068733

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	288.49786269	72.12446567	46.20	0.0001
ERROR	131	204.49374834	1.56102098		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.78789795				
EZN	0.03870028	0.06623555	0.53291109	0.34	0.5600
VD	0.00181163	0.00058695	14.87115051	9.53	0.0025
AGE	-0.03776852	0.00876283	28.99876739	18.58	0.0001
MENS	1.89848495	0.31378405	57.14277411	36.61	0.0001

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE RNASE ENTERED R SQUARE = 0.60830942 C(P) = 4.94262688

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	299.89143989	59.97828798	40.38	0.0001
ERROR	130	193.10017114	1.48538593		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.04256101				
EZN	0.03098332	0.06467105	0.34093800	0.23	0.6327
RNASE	-0.39697854	0.14333644	11.39357720	7.67	0.0064
VD	0.00177625	0.00057270	14.28887800	9.62	0.0024
AGE	-0.03952546	0.00857141	31.58553165	21.26	0.0001
MENS	1.95542494	0.30677757	60.34959868	40.63	0.0001

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE AP

THE FIRST 2 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

WARNING: 2 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 INCLUDED VARIABLES ENTERED R SQUARE = 0.06187657 C(P) = 178.82565279

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	30.50462865	15.25231432	4.39	0.0143
ERROR	133	462.48698238	3.47734573		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	6.23977115				
EZN	-0.26512173	0.08968986	30.38448281	8.74	0.0037
RNASE	-0.11420464	0.21654731	0.96718560	0.28	0.5988

STEP 3 VARIABLE MENS ENTERED R SQUARE = 0.50908041 C(P) = 33.60833280

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	250.97236980	83.65745660	45.63	0.0001
ERROR	132	242.01924123	1.83347910		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	2.11121911				
EZN	-0.06391119	0.06766200	1.63583761	0.89	0.3466
RNASE	-0.35658824	0.15878738	9.24651400	5.04	0.0264
MENS	2.94373967	0.26845089	220.46774115	120.25	0.0001

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE AP

THE FIRST 2 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

STEP 4 VARIABLE AGE ENTERED R SQUARE = 0.57932540 C(P) = 12.48402425

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	285.60256189	71.40064047	45.10	0.0001
ERROR	131	207.38904914	1.58312251		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.62685259				
EZN	0.04016550	0.06669480	0.57416535	0.36	0.5481
RNASE	-0.40689474	0.14794019	11.97584971	7.56	0.0068
AGE	-0.04129488	0.00882930	34.63019209	21.87	0.0001
MENS	2.04079374	0.31543227	66.26748750	41.86	0.0001

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE VD ENTERED R SQUARE = 0.60830942 C(P) = 4.94262688

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	299.89143989	59.97828798	40.38	0.0001
ERROR	130	193.10017114	1.48538593		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.04256101				
EZN	0.03098332	0.06467105	0.34093800	0.23	0.6327
RNASE	-0.39697854	0.14333644	11.39357720	7.67	0.0064
VD	0.00177625	0.00057270	14.28887800	9.62	0.0024
AGE	-0.03952546	0.00857141	31.58553165	21.26	0.0001
MENS	1.95542494	0.30677757	60.34959868	40.63	0.0001

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE AP

THE FIRST 3 VARIABLES IN EACH MODEL ARE INCLUDED VARIABLES.

WARNING: 2 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 0 INCLUDED VARIABLES ENTERED R SQUARE = 0.09586715 C(P) = 169.63611813

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	47.26170202	15.75390067	4.67	0.0041
ERROR	132	445.72990901	3.37674173		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	5.03931456				
EZN	-0.23372112	0.08949989	23.02760638	6.82	0.0101
RNASE	-0.05461288	0.21506202	0.21775119	0.06	0.7999
ZN	0.06459372	0.02899614	16.75707337	4.96	0.0276

STEP 4 VARIABLE MENS ENTERED R SQUARE = 0.51187641 C(P) = 34.68790165

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	252.35077674	63.08769418	34.34	0.0001
ERROR	131	240.64083429	1.83695293		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	1.82596038				
EZN	-0.05794003	0.06807595	1.33066271	0.72	0.3963
RNASE	-0.33527572	0.16083075	7.98296152	4.35	0.0390
ZN	0.01890086	0.02181935	1.37840694	0.75	0.3879
MENS	2.89667429	0.27414316	205.08907472	111.65	0.0001

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE AGE ENTERED R SQUARE = 0.57995525 C(P) = 14.27667882

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	285.91307548	57.18261510	35.90	0.0001
ERROR	130	207.07853554	1.59291181		
TOTAL	135	492.99161103			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	9.41531464				
EZN	0.04197144	0.06702561	0.62462280	0.39	0.5323
RNASE	-0.39621804	0.15035427	11.06187426	6.94	0.0094
ZN	0.00902103	0.02043204	0.31051360	0.19	0.6596
AGE	-0.04088065	0.00890611	33.56229875	21.07	0.0001
MENS	2.02738773	0.31785959	64.80294119	40.68	0.0001

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

VITA

Wendy McGovern Sandoval

Candidate for the Degree of

Doctor of Philosophy

Thesis: INDICES OF ZINC STATUS IN ADOLESCENT FEMALES

Major Field: Home Economics

Biographical:

Personal Data: Born in Albuquerque, New Mexico, October 29, 1948, the daughter of James E. and Edith M. McGovern.

Education: Graduated from St. Pius X High School, Albuquerque, New Mexico, in May, 1966; received Bachelor of Science in Home Economics degree from University of New Mexico in 1971; received Master of Science degree in Home Economics from University of Nevada-Reno in 1972; completed Administrative Dietetic Internship, University of California, Berkeley, 1973; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1982.

Professional Experience: Food and Nutrition Specialist, Cooperative Extension Service, University of Nevada-Reno, 1/71 to 7/71; Graduate Research Assistant, University of Nevada-Reno, 8/71 to 12/72; Vocational Home Economics Teacher, Valley High School, Albuquerque, New Mexico, 1/73 to 6/73; Director, Dietetic Technician Program and Instructor, Oscar Rose Junior College, Midwest City, Oklahoma, 7/74 to 7/75; Nutritionist, Maternity and Infant Care Project, Albuquerque, New Mexico, 8/75 to 8/78; faculty member in Home Economics Department, College of Education, University of New Mexico, Albuquerque, New Mexico, 8/78 to present.

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