EFFECT OF ZINC SUPPLEMENTATION ON IRON, COPPER AND ZINC STATUS IN PREMENOPAUSAL WOMEN

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INTRODUCTION

Trace mineral interactions clearly exist in biological systems. The status of several trace minerals may be affected by use of zinc supplements. Zinc has been shown to decrease absorption and utilization of other trace minerals.

The effects of trace mineral supplements on the status of other minerals has been incompletely studied. Solomons (1,2) found that iron supplements reduced plasma zinc concentration. The effect of zinc supplementation on copper status also has been researched. Greger et al (3) found that subjects on two different levels of dietary zinc had higher fecal copper losses on the higher level than on the lower zinc diet. These differences were not significant, but the levels of zinc were not high. Yadrick et al (4) also found an interaction between iron and zinc when supplementing subjects with zinc or with a combination of zinc and iron.

Vitamin and mineral supplement use is widespread in the United States, with women as the primary supplement users. Medeiros et al (5) reported greater frequency of supplement usage with multiple vitamin and/or mineral supplements in women users than in men. Among female supplement users in seven western states, 11 percent took iron

supplements and 12 percent took zinc supplements. Stewart et al (6) assessed vitamin and mineral supplement usage of adults aged 16 years and older via a national telephone survey and found that women reported greater use of iron supplements than men. Individuals reported using single vitamin supplements over multivitamin complexes. Approximately five percent of subjects reported using single nutrient supplements. One-third of those female subjects aged 25-64 years old used copper and zinc supplements and two-thirds took iron supplements (6). Median reported intakes for folic acid and iron were between 100 to 200 percent of the RDAs. In contrast, median dietary intakes of zinc and copper were two-thirds or less of the RDAs or upper Estimated Safe and Adequate Daily Dietary Intakes (ESADDI) levels, respectively. Looker et al (7) compared dietary intake and biochemical indices of iron status of supplement users and non-users from the NHANES II data. Between supplement users and non-users, no significant difference in iron status was observed in children and adolescents, 1 to 19 years of age. The NHANES II data did not identify those in need of iron supplements, amount and bioavailability of supplemental iron and the possible presence of antecedent infections. Iron and zinc supplements exceeded the RDA for more than half of those using these supplements. Nevertheless, the effect of single mineral supplements on the status of other minerals has not been completely investigated. Due to a widely

available supply, self-selected supplementation increases the risk of artificially creating mineral deficiencies.

Mineral supplement research has primarily focused on subjects fed experimental diets. Limited research has been conducted on the effects of mineral supplements on blood mineral concentrations. Greger et al (3) reported that female adolescent subjects fed experimental diets containing two different levels of zinc (11.5 mg and 14.7 mg) resulted in significantly increased fecal copper losses with the higher level of zinc. Copper and iron retention were lower, but not significantly, with the higher zinc diet. Yadrick et al (4) studied the effects of supplementation with 50 mg zinc daily for 10 weeks on iron, copper and zinc status of adult women. Serum ferritin and hematocrit were significantly reduced with zinc supplementation compared with a Zn-Fe combination. Copper erythrocyte superoxide dismutase (ESOD) concentrations also decreased after six weeks of supplementation and were significantly decreased from baseline at ten weeks. Serum zinc was significantly increased after six and ten weeks of supplementation compared to baseline concentrations. Colin et al (8) studied the effect of two levels of zinc supplementation on zinc and copper retention in young adult women. The levels of zinc were 9.5 mg and 19.9 mg per day. The subjects were provided with pre-selected meals in a metabolic unit. Zinc fecal excretion paralleled zinc intake; however, copper fecal excretion was not significantly affected.

Thus, 20 mg of zinc a day may not be high enough to affect the absorption and retention of copper. Plasma zinc increased in all subjects on the low zinc diets. Mean plasma copper concentrations were within normal levels. Plasma copper levels were higher at the conclusion of the study, but these changes were not significant.

The effects of zinc supplements on mineral status in healthy individuals consuming self-selected diets has not been thoroughly studied. This research will investigate the effects of zinc supplementation on iron, copper and zinc status in apparently healthy non-institutionalized premenopausal women.

PURPOSE

The purpose of this study was to investigate whether moderate zinc supplementation had a significant effect on iron, copper, or zinc status in apparently healthy premenopausal women.

OBJECTIVES

The following list includes objectives formulated for this study:

1. to determine the erythrocyte and plasma concentrations of iron, copper and zinc in apparently healthy premenopausal women;

2. to determine the effect of zinc supplementation on iron, copper and zinc status indicators in apparently healthy premenopausal women;

3. to determine the relationship between dietary intake and iron, copper and zinc status in apparently healthy premenopausal women; and

4. to determine the relationship between body composition and mineral status in apparently healthy premenopausal women.

HYPOTHESES

The hypotheses postulated in this study were:

H_1: There will be no significant effect of zinc supplementation on iron status as measured by plasma and erythrocyte iron concentrations and plasma ferritin in apparently healthy premenopausal women.

H_2: There will be no significant effect of zinc supplementation on copper status as measured by plasma and erythrocyte copper concentrations in apparently healthy premenopausal women.

H_3: There will be no significant difference in mineral status of individuals differing in body composition measures.

ASSUMPTIONS

1. Eight weeks is adequate time to see effects of zinc supplementation on measures of iron, copper and zinc status.
2. Dietary intake was accurately reported and analyzed.
3. Phlebotomy procedures were accurately followed and storage of blood samples was adequate.
4. Subjects fasted for 12 hours before data collection periods.
5. Subjects consistently took the zinc supplements and reported any missed supplements.

LIMITATIONS

1. The collected data is limited to those willing to participate.
2. The collected data is limited to premenopausal women over the age of 18 years not using oral contraceptives.
3. The subjects were not randomly selected; therefore, the results are limited to this study group.
4. The dietary data collection methods were limited in terms of the database for this study.

DEFINITION OF TERMS

Apparently Healthy	no obvious signs of disease and no chronic disease.
Bioelectrical Impedance	assessment of fat-free mass involving the measurement of the impedance to flow of electricity introduced into a subject; highly correlated to total body water. (9)
Body Composition	estimation of specific components of the body, including fat, water, protein, and bone mineral. (10)
Body Mass Index	an index for estimating obesity. The weight in kilograms is divided by the height in meters squared. BMI = kg/m^2. (10)
ESADDI	Estimated Safe and Adequate Daily Dietary Intake. Established by the Food and Nutrition Board. A category of safe and adequate intakes for essential nutrients when data were sufficient to estimate a range of requirements, but insufficient for developing an RDA. (11)
Premenopausal	a female still experiencing menses with childbearing capabilities.
RDA	Recommended Dietary Allowance. The RDAs have been prepared by the Food and Nutrition Board. They are the levels of intake of essential nutrients that, on the basis of scientific knowledge, are judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of practically all healthy persons. (11)

Chapter II

LITERATURE REVIEW

Supplement Usage in the United States

The second National Health and Nutrition Examination Study (NHANES II) data indicated that 39.8 percent of women and 30 percent of men aged 18 to 74 years old reported taking vitamin or mineral supplements (12). Individuals with higher nutrient intakes were more frequent users of mineral or vitamin supplements than those with lower intakes. Bender et al (13) studied trends in prevalence of vitamin and mineral usage and correlation with health status, and reported supplement usage is highest among populations that perceive themselves as healthy. Another study (14) showed that those individuals taking supplements rated their health lower than non-supplement users. More recently, Neuhouser (15) surveyed 104 women regarding supplement usage and found that almost half of the respondents reported using multi-vitamin supplements because they found it difficult to eat a balanced diet (15).

Looker et al (7,16) used NHANES II data to examine the dietary intake and biochemical measures of iron status of supplement users and non-supplement users between the ages of 1 and 19 years and again in a separate study of adults. In these groups, supplement users consumed

more fruits, vegetables and vitamin C on average than non-users, potentially affecting iron status by increasing non-heme iron absorption. In addition, supplement users consumed an iron-containing supplement as their major supplement. Supplement usage among women was highest in the 25 to 44 year old age group. Looker et al (7) found no significant differences between users and non-users in iron status as measured by several indices: hemoglobin concentration, mean corpuscular volume (MCV), erythrocyte protophorphyrin, transferrin saturation, and serum ferritin. In general, iron status was not associated with supplement use.

The Food and Drug Administration (FDA) conducted a random nationwide telephone survey to determine the prevalence and levels of vitamin and mineral supplement use in the United States (6). Stewart et al (6) found that female subjects had a higher percentage of supplement use than the male subjects. Female subjects between the ages of 25 years and 64 years reported the greatest supplement usage (47%). They also reported that the most widely consumed supplement was the single vitamin/miscellaneous dietary component supplements. Median supplement intakes for iron, vitamin D, vitamin A, and folic acid fell within 100 to 200 percent of the RDA's with 95 percent taking no more than 500 percent of the RDA's. The median dietary intakes for minerals (zinc, copper, and magnesium) were two-thirds or less of the RDA (or

upper ESADDI levels) with 95 percent of the users consuming less than 300 percent of the RDA's (or upper ESADDI). A recent survey of adult women found that 63 percent of multi-vitamin users used at least one of the single nutrient supplements (15). Schutz et al (17) collected data from 2451 adults in seven Western states on food supplement usage. They reported that 66.6 percent of the respondents used some type of food supplements and 40 percent consumed up to three supplements per day. Of these subjects 26.3 percent took multiple vitamins with iron, 8.4 percent used zinc supplements and 7 percent took iron supplements. Medeiros et al (5) conducted a survey to determine the dosage level of single supplements in addition to the prevalence of supplementation in seven Western states. They found that 17.6 percent of the respondents took five or more supplements a day.

Biological Roles of Minerals

Zinc and Zinc Supplementation

The trace mineral, zinc, is essential to several physiologic functions: cell growth and replication, sexual maturation, fertility and reproduction, night vision, immune diseases and taste and appetite (18). An early study by Sandstead (19) reported that many American diets are marginal or even deficient in zinc, especially those of some infants, pregnant women, teenage and college women and some people on low

income diets. The U.S. food supply provides between 11 and 13 mg/day/person, but women select diets that have less than 81 percent of the Recommended Daily Allowance (RDA) for zinc which is 12 mg per day (20). NHANES III data reported similar findings. Women 16 to 49 years old reported dietary zinc intakes less than 80 percent of the RDA for zinc (21). In addition, the 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) also reported that adult females failed to meet the RDA for zinc (22). Zinc is prevalent in today's food supply in sources such as liver, beef, lamb, pork, chicken, fortified breads and cereals as well as colostrum and breastmilk for infants (19, 22-24). Conversely, there are several factors involved which reduce zinc uptake by the body: a diet high in phytate, low zinc intake, diarrhea, heavy metals in the water supply, fasting, prolonged use of intravenous fluids, infections, blood loss, chelating agents, burns, surgery and shock (24). Dietary factors may include: intrinsic factors which may relate to zinc chemistry; and extrinsic factors, which relate to the intake of nonheme iron, dietary fiber, phytic acid, calcium, copper and certain foods such as cow's milk, cheese, coffee, eggs, celery and lemon; all shown to decrease bioavailability of zinc (24).

In a review article, Solomons (24) wrote that the site of zinc absorption along the gastrointestinal tract was not determined, but in vitro studies have shown the ileum to have the greatest absorption of

zinc. However, in vivo studies show the duodenum to be the site for zinc absorption. The most commonly used index of zinc nutritional status is plasma/serum zinc even though it does not always reflect total body zinc nutriture (25). It is important to determine whether decreased plasma zinc concentration indicates clinical zinc deficiency or rather a redistribution of zinc in body compartments (25).

The adequacy of a diet in terms of zinc is dependent not only on the quantity of the mineral in the diet but also its availability to the body (2). Johnson et al (26) conducted an animal study on zinc absorption and found that current dietary zinc intake influenced zinc absorption; endogenous zinc excretion was affected by current as well as past zinc intake when past zinc intake was insufficient for growth. Plasma zinc has been reported previously to be sensitive to changes in dietary intake (27). There is a diurnal variation in concentration as well as a decline following food consumption (28). Baer and King (29) found that plasma zinc decreased significantly in males during experimental zinc depletion and responded promptly to increased dietary zinc intakes. Golden and Golden (30) describe human zinc deficiency features as similar to protein and calorie malnutrition. The features include: anorexia, diarrhea, stunting, wasting, skin desquamation and ulceration, hair fragility and dyspigmentation, reduction in lymphoid tissue and increased susceptibility to infection. Henkin et al (31) found similar characteristics

such as anorexia, taste and smell dysfunction, organic mental and psychiatric symptoms and cerebellar dysfunction with histidine-induced zinc depletion in patients with progressive systemic sclerosis (PSS). They found these symptoms to correlate with clinical zinc depletion. The symptoms ceased when the participants received treatment with zinc sulfate. Halsted and Smith (32) looked at plasma zinc concentrations in children and adults with a variety of disease states and physical conditions: indolent ulcers, alcoholic cirrhosis, other types of liver disease, tuberculosis, non-tuberculous pulmonary infection, myocardial infarction, uremia, hemodialysis, pregnancy, oral contraceptives, cystic fibrosis, Down's syndrome and growth retardation. They found low plasma zinc levels in all these disease states and conditions except in hemodialysis and cystic fibrosis. Plasma zinc levels were unaffected in those undergoing hemodialysis and were normal in children with cystic fibrosis. Halsted and Smith (32) stated that an abnormally low plasma zinc concentration suggests zinc deficiency, but only a clinical response to zinc therapy under controlled conditions would provide conclusive evidence of deficiency. Todd et al (33) investigated whether or not zinc exerted a beneficial role in the nutrition of the rat and found that rats fed low levels of zinc had lower growth than did those on zinc-fortified diets. Prasad (34) described studies in Egypt that showed the rate of growth was greater in patients receiving supplemental zinc than in those

receiving iron or an adequate animal protein diet. He concluded that nutritional as well as conditioned deficiencies of zinc may complicate many disease states in human subjects. Growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation and delayed wound healing are some of the manifestations of moderate zinc deficiency in humans (34). The importance of zinc in healing may be its essential role in protein synthesis and in cellular growth and replication (35).

Walravens and Hambidge (36) found improved growth in 6-month-old male infants fed zinc-supplemented formulas from birth. Goldenberg et al (23) support this finding with a study that investigated infants of mothers who received daily vitamins and 25 mg zinc. These mothers delivered heavier infants than those who took vitamins without zinc due to a decrease in premature deliveries among the mothers taking zinc.

Zinc may also play a role in the biologic activity of hormones in the menstrual cycle, specifically the regulation of progesterone and prolactin (37). Deuster et al (37) evaluated changes in the plasma concentrations of zinc as a function of menstrual cycle phase in women across three cycles. They found that there are specific phase related patterns for plasma zinc during the menstrual cycle despite day to day variation. It was discovered that mean plasma zinc concentration was highest during menses and the follicular phase and significantly decreased during the

ovulatory and luteal phases. Reasons for these changes remain unclear but may be related to hormonal changes or to specific carrier proteins.

Iron

Iron is an essential nutrient for all humans as a component of hemoglobin, myoglobin and several enzymes (38, 39). It is also required for the prevention of anemia which is fairly common among American women (40, 41). Excellent food sources of iron include meat, legumes, and enriched and fortified grain products (42). The Recommended Dietary Allowance (RDA) for women 20 to 50 years old is 15 milligrams (11). However, dietary intakes calculated from NHANES III data showed that women 16 to 49 years old consumed only 83 percent of the RDA for iron (21). Previously, NHANES I and II surveys reported similar findings of low dietary iron intakes in women (43, 12). Impaired iron status occurs in 10 to 20 percent of females of childbearing age (39). Iron functions in the human body as part of oxidation reactions, aerobic metabolism, enzymatic processes, oxygen and electron transport, hemoglobin synthesis, and physical growth (39). Iron deficiency has been associated with inadequate growth, increased prevalence of certain infectious diseases, and decreased physical and intellectual performance (44).

In the United States, iron absorption is often inadequate in premenopausal women because of menstrual iron losses and in pregnant women due to an increase in blood volume, the demands of the fetus and possible blood losses during childbirth (45). Iron absorption is affected by several factors: the intestinal mucosa, the amount and bioavailability of iron in food, and a number of dietary factors (45). Still other substances have been known to decrease nonheme iron absorption: calcium phosphate, phytates, tannins and antacids (45).

Fricker et al (46) addressed the issue of obesity in determining iron status in menstruating women. They concluded that obese women consumed more iron that did non-obese women, but the differences were due more to an increase in overall caloric intake than to a high intake of iron dense foods. Serum ferritin concentrations were higher in obese women than non-obese women. The prevalence of increased iron stores in obese women would appear to place them at low risk of iron deficiency.

Copper

Research indicates that U.S. diets may supply less than 2.0 mg of copper per day (47, 22). More specifically, NHANES III researchers stated that women aged 16 to 49 years of age reported dietary copper intakes less than 76 percent of the lower Estimated Safe and Adequate Daily

Dietary Intake (ESADDI) range for copper (21). An RDA has not been established for copper because it is has not been determined how much copper humans require, but it is recommended that adults consume 1.5 to 3 mg a day which is the ESADDI range for copper (11). Excellent food sources of copper include oysters, shellfish, and legumes (47, 22, 18).

Copper has several physiologic functions in humans including erythropoiesis, leukopoiesis, skeletal mineralization, connective tissue synthesis, melanin and myelin synthesis, oxidative phosphorylation, thermal regulation, antioxidant protection, cholesterol metabolism, immune and cardiac function, and glucose metabolic regulation (18). Copper deficiency is rare in the U.S., but symptoms include neutropenia and microcytic hypochromic anemia (18).

Iron and zinc are two inhibitory factors which affect the bioavailability of copper (48). Copper uptake by the gastrointestinal tract is affected by dietary factors as well, including phytate, fiber, ascorbic acid and amino acids (48). Some copper is stored in the liver as ceruloplasmin. When copper is mobilized from the liver, it is carried by plasma albumin into tissues (48).

Mineral Interactions

Mineral interactions are a well known factor contributing to inadequate trace mineral status often compounded by low nutrient

intakes. Higher levels of one mineral can interfere with the absorption of another mineral (49, 50). Because many trace minerals have similar chemical properties, there may be an increased risk for mineral interactions that can cause problems in absorption and utilization of competing trace minerals. This may be true of iron, copper and zinc.

Zinc-Iron Interactions

Zinc-iron interactions have a measurable effect on human nutrition. Zinc and iron have identical outer electron shell configurations in the first transition series of the periodic table of elements (51). Therefore, an increased iron intake may reduce the absorption of zinc as well as high zinc intakes affecting iron absorption. It is known that animal foods are better sources of zinc and iron than foods of plant origin (19, 22, 24, 42). Recommendations for the U. S. dietary intakes have shifted towards a decreased intake of red meat and a higher intake of fiber which can be an issue in terms of adequate zinc and iron nutriture (11, 52). Already low intakes of both minerals have been reported in premenopausal women (21). When nutrient status of pregnant plantation workers in Sri Lanka was assessed, depleted iron stores and low serum zinc levels were found in one-third of subjects. Iron deficiency persisted up to 36 weeks postpartum but zinc stores were not found to be depleted after pregnancy (53).

Research has reported interactions occurring between zinc and iron in humans. Solomons reviewed studies showing competitive interactions between zinc and iron that justified the need for a balanced ratio in regard to zinc and iron supplementation (1). He stated that if an individual is taking chronic, high levels of iron, than zinc supplementation could be justified to prevent zinc depletion (1). Yadrick et al (4) studied the effect of zinc and zinc with iron supplements on 18 women aged 25-40 years old and found decreased serum ferritin concentrations, but no change in hemoglobin concentration with zinc supplementation. When subjects were given zinc and iron combined, serum ferritin increased; thus, indicating that zinc supplementation alone could be detrimental to the iron status of adult women. Crofton (54) found that inorganic zinc inhibited iron absorption when a group of healthy subjects was given a solution of 842 umol ferrous sulphate and another dose of 842 umol ferrous sulphate plus 344 umol zinc given 35 days later. They reported that the iron with zinc solution inhibited plasma iron appearance and, by implication, iron absorption. They suggested that it is possible that mineral interactions occur in the intestinal mucosa when high levels or ratios of minerals are administered. Furthermore, it is possible that dietary factors could play a role in modifying the form and absorption of iron and zinc, thereby minimizing the risk for interaction between large doses of iron and zinc

(54). Another study reported that serum iron concentrations were significantly elevated in zinc and iron deficient female endurance runners after iron supplementation and iron with zinc supplementation (55). Valberg et al (56) studied the effect of high levels of iron on zinc absorption. When subjects were given meals containing 92 umol zinc chloride and 920 umol iron, zinc absorption was significantly reduced. When subjects were fed a turkey meal with either 306 or 610 umol of inorganic iron, zinc absorption was not significantly affected. The researchers concluded that the administration of iron in the presence of a meal has no effect on zinc absorption in humans. Similarly, Rossander-Hultén et al (57) found that the administration of 15 mg and 45 mg zinc given in a water solution significantly decreased absorption of iron to less than half of baseline measures. When 15 mg zinc was provided in a meal, iron absorption remained constant. The reduction in iron absorption observed with moderate levels of zinc suggests an interaction occurring in the intestinal lumen (57). In support of this, zinc did not inhibit iron when it was given in a meal suggesting that there is no interaction between iron and zinc when dietary ligands are present (57, 58). Other studies have found similar results with high levels of iron negatively affecting absorption of zinc (2, 58). Solomons (2) found that 50 mg of ferrous iron with 25 mg of zinc reduced uptake of zinc for absorption. Zinc supplements or high dietary levels of zinc do not appear

to affect iron absorption when combined with a meal. The propensity for mineral interactions seems to occur when taking multi-mineral supplements between meals (57, 54).

Zinc-Copper Interactions

Dietary intakes of zinc and copper may be marginal in entire population groups (21, 22). Moreover, zinc and copper have been shown to have biological interactivity, which may impact already deficient zinc and copper nutriture (48). Metallothionein may be involved in zinc-copper interactions (48). High zinc intakes have been shown to impair copper status due to increased synthesis of intestinal metallothionein which binds copper and prevents its absorption (48). It is possible that both minerals are transported by shared ligands and therefore, they may compete for transport across the basolateral membrane affecting absorption of either mineral (48).

Fischer et al (49) reported decreased copper erythrocyte superoxide dismutase (ESOD) levels after supplementation with 50 mg zinc over a 6 week period. Yadrick et al (4) noted similar results when subjects took 50 mg zinc over a 10 week supplementation period. Copper ESOD decreased with zinc supplementation after 6 weeks, but there was no change in serum ceruloplasmin throughout the duration of the study. Copper ESOD concentrations at 10 weeks supplementation were significantly

decreased from baseline concentrations (4). Early research by Van Campen (59) evaluated the effects of high and low levels of zinc on copper in rats in ratios of zinc:copper ranging from 0 to 600. They found that higher doses of zinc significantly decreased the uptake of copper whereas lower levels of zinc did not affect copper absorption. Colin et al (8) studied the effect of two levels of zinc on zinc and copper retention in young adult women. The levels of zinc ranged from 9.5 mg to 19.9 mg. The subjects were provided with pre-selected meals by the metabolic unit. Zinc supplements, in the form of zinc sulfate, were added to juice daily. Zinc fecal excretion paralleled zinc intake. Zinc intake did not affect fecal copper excretion and retention. They stated that 20 mg of zinc a day may not be high enough to affect the absorption and retention of copper. Plasma zinc increased in all subjects on the low zinc diets. Mean plasma copper was within normal levels. Plasma copper levels were higher at the conclusion of the study, but these changes were not significant. Superoxide dismutase was not studied. In an earlier study, Greger et al (3) investigated the effects of low to moderate zinc supplementation on copper status in adolescent girls. The effects of low zinc levels (11.5 mg) on copper status produced no significant effect on copper absorption, retention or utilization. However, when the higher level of zinc (14.7 mg) was given, there were higher fecal losses of copper possibly due to competition occurring through the gastrointestinal tract.

Taper et al (60) reported similar findings with varying levels of zinc on copper status in premenopausal women. At low to moderate levels (8 mg, 16 mg, 24 mg), zinc did not have a significant effect on copper retention. Another study in males supports the findings that zinc supplementation at various low to moderate levels did decrease plasma copper concentrations but not outside the normal range (61). However, a significant reduction in plasma copper was seen in subjects when they were given the higher level (18.5 mg) of zinc. Copper retention may only be affected when there is a high ratio of zinc to copper. L'Abbé et al (62) fed diets to rats containing varying amounts of zinc as zinc sulfate per kg diet: 15 mg, 30 mg, 60 mg, 120 mg, or 240 mg in addition to 6 mg/kg diet copper for a 6 week period. Serum ceruloplasmin did not decrease linearly with any of the zinc levels. However, an increasing number of rats did exhibit extremely low levels of ceruloplasmin as the amount of zinc in the diet increased indicating decreased copper stores.

Method Justification

Dietary Data Collection

Researchers have utilized various methods to collect dietary data including food frequency questionnaires, dietary recall methods, diet histories, and food records. Each of these methods has particular uses, strengths and limitations; all are dependent on the type of research being

conducted (63). No standard method for assessing dietary intake of a population has been found. Researchers must use a method for evaluating dietary intake that best matches the purpose and objectives of the study (63). One of the most commonly used dietary collection methods has been the 3-day dietary or food record in which the amount of food consumed is weighed or estimated and recorded (64, 65). A common problem in dietary assessment is determining the number of days of food records necessary to accurately estimate nutrient intake (66). Schlundt (66) recommended keeping food records for 3 to 7 days and stated that there are few instances when keeping food records more than 2 to 3 weeks is advantageous. Payette (64) found that for most nutrients, there was no advantage to using 7 day food records over 3 day food records (64). Tinker et al (67) supported the use of 3 to 4 day food records because of the high cost in terms of time and personnel in analyzing dietary data from food records kept for an entire study period. They found that multiple food records served several purposes in addition to serving as a research tool for data collection and analysis. For example, food records also serve as a motivational tool to remind subjects of their responsibility within the research protocol and as a teaching tool for nutrition assessment and counseling for subjects post-study (67). Multiple food records have been considered to yield the most valid estimates of individuals' usual intake (68).

Bergman et al (69) indicated that the food frequency questionnaire gave higher estimates of group nutrient intake than did the three-day diet record analyses. They stated that a diet record may provide a more accurate method of assessing nutrient intakes for moderate to large groups of subjects. Tinker et al (67) used food records when assessing the intake of 41 subjects over an 8-week period and found food records to provide a reasonable estimate of intake for studies of similar design and sample size.

Greater variances have been found in nutrient intake for the 24-hour recall estimates than for the three-day food records (65, 70). Buzzard et al (71) compared dietary recall to food records and found a higher incidence of underreporting intake using dietary recall.

When comparing four-day food records to the dietary history method, Peterson et al (72) found that food intake was overestimated when using the diet history method. The diet history method was not recommended to measure micronutrient intake because certain foods with different nutrient contents were grouped together with common codes used in nutrient analyses.

Therefore, the food record method for dietary data collection seems to be advantageous in analyzing micronutrient content in moderate size groups of subjects. It is a suitable method for accurately assessing nutrient intakes (65). This form of dietary assessment has major

strengths including its greater precision in determining food amounts and less reliance on memory (71). Larkin et al (73) found that random day food records provided closer estimates of dietary intake than consecutive records, but consecutive records are less expensive and more convenient in terms of scheduling and use of interviewers' time. Therefore, they speculate that convenience can lead to improved compliance and participation in a study. Karvetti et al (65) found that nutrient intake according to food records was accurate when compared to that of observed intake for groups. It has been well established that a single day food record provides poor accuracy in estimates of an individual's usual nutrient intake and several days yields better precision of a person's usual nutritional intake (68). Another strength to consider when using food records was that Jorgenson found women were more willing than men to complete seven-day food records (74). These points were considered when choosing three-day food records as the dietary data collection method in the present zinc supplementation study.

Body Composition

Body composition is defined as the estimation of specific components of the body, including fat, water, protein, and bone mineral (10). Most body composition methods measure two separate components, fat and fat free mass (75, 76). Lukaski (75) classifies body composition

methods into two categories: traditional or new. Traditional refers to more established methods whereas new methods are those that reflect contemporary hypotheses and technology. Anthropometric measurements, including skinfolds and arm circumference, have been established as traditional methods. Electrical conductance includes bioelectrical impedance which is considered a new method for assessing body composition (75). All three methods: tricep skinfolds, mid arm circumference and bioelectrical impedance measure body composition indirectly (76). Baumgartner et al (77) found BIA estimates to be improved with the use of anthropometric variables including arm circumference and skinfold measures.

Each method has advantages and disadvantages depending on the type of research conducted and size and health status of subjects. Several factors to consider when selecting methods for body composition include the health status of the study sample, type of survey, type of data collected, cost, subject cooperation, skill and expertise of researcher and the ease with which the method may be used (75).

Skinfold measures are commonly used to estimate body fat without the use of sophisticated equipment and substantial resources (78). Triceps skinfold (TSF) is determined to be non-invasive, inexpensive with minimal training required by the researcher (79, 80, 81). One advantage of using the skinfold measurements is the ability to assess

body composition outside of the laboratory setting. Another advantage is that precision within 5 percent can be obtained by properly trained individuals (75). Mid-arm circumference and triceps skinfold measurements combined, are much more precise as indicators of nutritional status than if each method is used individually. Mid arm circumference gives an indication of the body's muscle mass (75).

Bioelectrical impedance analysis (BIA) measures fat-free mass rather than adipose tissue or fat mass using the measurement of the impedance to a flow of electricity introduced into a subject (9, 79, 80). Electrical conductivity is greater in fat free mass than fat (82). The assumption is that fat free tissues conduct a low level of alternating electric current better than adipose tissues (77). An excitation current is introduced at distal electrodes on the hands and feet, and the resistance is measured by proximal electrodes (83). BIA offers a convenient, rapid, safe and noninvasive method of collecting body composition data in apparently healthy individuals (9, 79-82, 84). Research has shown BIA values to be highly correlated to total body water which is associated with percentage of lean body weight and therefore, body fatness (9, 77, 82). Lukaski et al (84) found that the BIA method had reliability in repeated measurements when taken on five consecutive days. They went on to state that this technique may be useful for routine assessment of body composition and suited for nutritional surveys and

epidemiological studies. BIA could prove invaluable in the field assessment of nutritional status. Kushner et al (85) found that BIA accurately measures body composition in weight stable subjects as well as changes in body composition due to weight loss in subjects.

Chapter III

METHODS AND PROCEDURES

Sample Selection

Subjects were recruited via flyers (Appendix A) to the faculty and students of the college of Human Environmental Sciences and by word of mouth in Stillwater, Oklahoma. To participate in the twelve-week study, subjects met the following criteria: over the age of 18 and premenopausal, not pregnant or planning to be pregnant in the next six months, not using oral contraceptives, nor taking estrogen replacement therapy and no knowledge of having a chronic disease. Ten adult women, ages 18 to 48 years, volunteered to participate in the zinc supplementation study. Supplementation lasted eight weeks. Measurement periods were baseline (M1), at four weeks supplementation (M2), at eight weeks supplementation (M3), and four weeks post-supplementation (M4). Supplementation began immediately after baseline data collection. This study was approved by the Institutional Review Board at Oklahoma State University (Appendix B).

Procedures

All subjects were provided with and signed an informed consent form prior to the study (Appendix C). Prior to data collection, subjects

were instructed and provided with written guidelines for recording dietary intake, menstrual cycle, and directions for fasting prior to phlebotomy (Appendix D). A health history questionnaire was also completed prior to the study (Appendix E).

Zinc Supplement

The zinc supplement used in the study was in the form of zinc sulfate ($ZnSO_4 \bullet 7H_20$). The supplement was made by grinding and mixing 110 g of $ZnSO_4 \bullet 7H_20$ with 470.45 g lactose. Capsules were filled to a weight of 320 mg/capsule calculated to provide 15 mg Zn. By analysis, the first month of supplements provided a mean of 14.675 mg Zn and the second month of supplements provided a mean of 16.52 mg Zn. Subjects were asked to take one supplement each morning and one supplement each night with meals during the eight week supplementation period. Subjects were instructed to return the bottles with any missed supplements at the subsequent data collection.

Anthropometrics

Following a 12-hour fast subjects were scheduled for all data collection measures. Anthropometric measurements were taken at each collection period prior to phlebotomy and included height, weight, mid-arm circumference, tricep skinfold and bioelectrical impedance. Height in inches was measured against the wall using a rigid steel tape perpendicular to the floor and a right angle head board constructed for

this project. Weight was measured in pounds to the nearest one-sixteenth of a pound on a calibrated beam balance scale.

Mid-arm Circumference

Mid-arm circumference (MAC) was measured using the technique described by Grant and DeHoog (86). Each subject stood with her right arm exposed and bent at a 90 degree angle with the palm facing upward. The end of the nonstretchable tape was placed on the lateral tip of the acromial process and extended along the side of the upper arm below the elbow to the olecranon process. The subject's upper arm was marked at the midpoint. Each subject then dropped the arm at the elbow to hang loosely at the side of the body. The tape was placed, without compressing the skin, around the arm at the marked midpoint and parallel to the floor. The measurement was recorded to the nearest 0.1 cm.

Triceps Skinfold

Triceps skinfold was measured in addition to mid-arm circumference (MAC) using calibrated Lange calipers. The same midpoint which was used for measuring MAC was used to obtain the triceps skinfold. Each subject stood with her arm hanging loosely at the side of the body. The measurement was taken using the skinfold at the back of the arm over the tricep muscle. The skinfold was picked up firmly between the thumb and index finger of the left hand 1.0 inch above the marked level. The calipers were placed over the fatfold at the

marked level at a point where the sides of the skinfold are approximately parallel. The caliper was held parallel to the floor and released without releasing the pinch. After waiting three to four seconds, the reading was recorded to the nearest 0.1 mm. Three measurements were taken and the values averaged.

Bioelectrical Impedance

Bioelectrical impedance was measured using a Biodynamics Model 310 Bioelectrical Impedance analyzer (Chatanooga Corporation, Chatanooga, TN) using the manufacturer's instructions. The analyzer was charged to 100 percent and turned on. Each subject was instructed to lay down on a horizontal surface facing up. Subjects were positioned with hands, palms down, at least six inches from the body. The feet were also positioned six inches apart. The right hand and foot were exposed for the placement of the electrodes. Each subject was instructed to place her hand in the dorsiflexion position and an electrode was placed near the subject's dorsal metacarpals of the hands and the distal prominence of the radius and ulna. The same procedure was used to place the electrodes on the right foot of each subject near the distal metatarsals and between the medial and lateral malleoli of the ankle. The subject was told to relax and remain motionless for the duration of the test which lasted for approximately 10 seconds. The test was performed a second time for reliability using a new set of electrodes and the two values were averaged for analysis.

Upon completion of the anthropometric measurements, a basic health questionnaire was administered to obtain information on any recent illness (i.e. colds, flu, etc.), supplement intake, and menstrual cycle at each measurement period (Appendix F).

Dietary Data

Each subject received a set of graduated non-biasing food models (87) and was trained to keep food records before the initial measurement period. Subjects kept food records on the three days prior to each data collection period to ensure that food intake was similar on the days prior to phlebotomy days. Subjects were asked to avoid consuming oysters and zinc-fortified cereals, both high zinc sources, throughout the duration of the study. Food records were analyzed using the Food Processor II Computer Software Program (Version 3.12, ESHA Research, Salem, OR). Mean nutrient intakes over each three day period were used in the analysis.

Phlebotomy

Following a 12 hour fast, blood samples were drawn between 8:00 and 9:00 AM using stainless steel butterfly needles and plastic syringes (Sarstedt, Newton, NC). The anticoagulant used was sodium citrate (100 ul of 300 g/L in physiological saline). Aliquots of whole blood were removed with a plastic transfer pipet for total hemoglobin determinations before separation of plasma by centrifugation. Immediately after

centrifugation, plasma was removed and stored frozen in trace element free plastic tubes (Falcon) for analysis. All preliminary sample handling, preparation for ashing and dilution of ash were performed under a clean air hood.

Biochemical Analyses

Lipid Analyses

Total cholesterol was measured using a quantitative enzymatic determination (Sigma procedure 352, St. Louis, MO) in plasma at 500 nm. The Cholesterol Reagant (1.0 mL) was added to the sample, mixed by inversion, and incubated at 37°C for 5 minutes. The tubes were read at 500 nm and the absorbances recorded. Dextran sulfate was the HDL Cholesterol Reagent (Sigma procedure 352-3, St. Louis, MO) used to isolate HDL cholesterol in the supernatant. Triglycerides were assayed using a quantitative enzymatic determination (Sigma procedure 339, St. Louis, MO). The Triglyceride (GPO-Trinder) reagent was added to the sample, mixed by inversion, and incubated at 37°C for 5 minutes. Tubes were read at 540 nm and absorbances recorded. LDL Cholesterol was calculated by subtracting HDL cholesterol and triglycerides from total cholesterol using the following equation: LDL Cholesterol = Total Cholesterol – HDL Cholesterol – (Tg/5) (Sigma Procedure 352, St. Louis, MO).

Hemoglobin

The cyanmethoglobin method (Sigma procedure 525-modified for volumes, St. Louis, MO) was used to determine hemoglobin in whole blood and erythrocytes. Drabkin's solution (2.5 ml) was added to the labeled tubes, to which 10 ul of whole blood or lysed erythrocytes was added, mixed and allowed to stand at room temperature for 15 minutes. Tubes were read at 540 nm and the absorbances recorded.

Plasma Albumin

Albumin was analyzed using the procedure for the quantitative, colorimetric determination in plasma at 600 nm (Sigma procedure 625, St. Louis, MO). Bromcresol purple forms a stable blue-purple color complex with human plasma albumin at an absorption maximum of 600 nm. The intensity of the color is proportional to the concentration of plasma albumin in the sample.

Plasma Ferritin

Plasma ferritin was measured using the double antibody radioimmunoassay procedure (Diagnostic Products Corp., Los Angeles, CA). In this procedure, (^{125}I)-labeled ferritin was used to compete with ferritin in the samples for sites on ferritin-specific antibody. The polyethylene glycol (PEG)-accelerated double-antibody method was used for separation of bound from free ferritin after a fixed incubation time.

The precipitate was then counted in a gamma counter with the counts being inversely related to the amount of ferritin in the sample.

Plasma Estradiol and Progesterone

Plasma estradiol and progesterone were measured using double antibody radioimmunoassay kits (Diagnostic Systems Laboratories, Webster, TX). These assays are reproducible and require only a small amount of sample per test (200 ul for estradiol and 100 ul for progesterone). The RIA procedure follows the principle of a competition between a radioactive and non-reactive antigen for a fixed number of antibody binding sites. The amount of ^{125}I-labeled estradiol bound to the antibody is inversely proportional to the concentration of estradiol present in the plasma. A pre-reacted double antibody system is used to separate the free from the bound antigen. This principle for progesterone is the same as for estradiol. All reagents used in the assay were allowed to reach room temperature and mixed thoroughly by inversion before use.

Mineral Analyses

Plasma and erythrocytes were wet and dry ashed using a modification of the Hill et al method (88) and analyzed by atomic absorption spectrophotometry (AAS). Samples were wet ashed with concentrated double distilled HNO_3 (GFS Chemicals, Powell, OH), dry ashed for 16 hours at 375° C, again wet ashed with concentrated HNO_3 and ultrapure H_2O_2. Samples were again dry ashed followed by a final

wet ashing period. Care was taken throughout these procedures to prevent contamination. Samples were dissolved and diluted with 0.5% HNO_3 for immediate analysis. Plasma zinc and erythrocyte iron and zinc were analyzed by flame AAS with deuterium background correction. Plasma iron and plasma and erythrocyte copper were analyzed by graphite furnace AAS with Zeeman background correction using a Perkin Elmer 5100PC AAS. Erythrocyte mineral concentrations were expressed per gram erythrocyte hemoglobin.

Statistical Analysis

Data were analyzed by the General Linear Model for repeated measures using SAS version 6.07. Significance level was set at $p<0.05$.

Format

Chapter IV was written in journal article format following the Guidelines for Authors according to the *Journal of the American Dietetic Association*.

Chapter IV

Effect of Zinc Supplements on Iron, Copper and Zinc Status in Premenopausal Women.*

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ABSTRACT Ten non-pregnant women between the ages of 18 and 48 years volunteered to participate in a zinc supplementation study designed to determine the effects of supplemental zinc on indicators of trace mineral status. Dietary intake was estimated by three-day food records kept by each subject during each period of the study. Nutrient intake was calculated by Food Processor II (ESHA Research, Salem, OR) dietary analysis software. Bioelectrical impedance, triceps skinfold and mid-arm circumference were used to assess body composition. Zinc supplements (15 mg zinc as zinc sulfate) were taken two times a day with meals for eight weeks. Data were collected at baseline (M1), four weeks supplementation (M2), eight weeks supplementation (M3), and four weeks post supplementation (M4). Plasma and erythrocyte minerals, hemoglobin, and ferritin were evaluated for the effect of zinc supplementation. Dietary intake, body composition, plasma albumin and hemoglobin measures did not differ significantly between any of the measurement periods. Plasma ferritin decreased, but not significantly,

from baseline to post supplementation. There was an overall significant quadratic effect for plasma iron and plasma zinc. Plasma iron at M4 was significantly lower than at the other measurement periods. Erythrocyte iron was significantly increased at M3 compared to baseline. There was no significant difference in plasma copper over time. Erythrocyte copper at M1 was significantly different than M3 and M4 and M2 was different than M3.

KEY WORDS zinc, supplementation, minerals, trace mineral status, women

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INTRODUCTION

Supplement usage is widely practiced in the United States. Schutz et al (1), using survey research, reported that 67 percent of adults in seven Western states consumed some type of food supplement. Data from the Second National Health and Nutrition Examination Survey (NHANES II) showed that 40 percent of women and 30 percent of men reported taking vitamins or minerals. Of the 35 percent that took supplements, 21 percent took them daily and 14 percent took them at least once a week with multivitamins used most frequently (2). A telephone survey conducted by the Food and Drug Administration (FDA)

found women had higher supplement usage than men. Among supplement users, the FDA found that the single vitamin/miscellaneous dietary component supplements were consumed most often (3). A second survey of adults in seven Western states addressed mineral supplementation and reported 11 percent and 12 percent of respondents supplemented with iron and zinc, respectively, with 50 percent of the respondents exceeding the Recommended Daily Allowance (RDA) by up to five times the recommendations (4).

Complex interactions occur between trace minerals which can pose risks for some consistent supplement users. Solomons concluded that the interaction of iron with zinc seems to have a measurable effect on human nutrition (5). Zinc supplements have been shown to depress copper superoxide dismutase (ESOD) when given in 50 mg doses (6, 7) and to also depress serum ferritin (7), but no changes were found in hemoglobin (6, 8). When subjects were given zinc and iron combined, serum ferritin increased indicating that zinc supplementation alone could be detrimental to the iron status of adult women (6). When zinc supplements were given at low to moderate levels, there was decreased iron retention (9) and absorption (8), but they had no effect on copper retention (10, 11) or absorption (10). However, iron absorption remained constant when zinc was provided with meals (8). They suggested

competitive iron-zinc and copper-zinc interactions occurred with zinc supplementation.

The present study examined the effects of moderate zinc supplementation on iron status measures of hemoglobin, ferritin, and erythrocyte and plasma concentrations of iron, copper, and zinc and the effects of withdrawal of that supplement.

METHODS

Subjects

Ten adult women volunteered to participate in a zinc supplementation study upon meeting the required criteria: premenopausal, non-pregnant, not oral contraceptive, not nutrient supplement users, and weight stable. Supplementation twice a day with 15 milligrams of zinc as zinc sulfate lasted eight weeks. Measurement periods were baseline (M1), at four weeks supplementation (M2), at eight weeks supplementation (M3), and four weeks post-supplementation (M4). Supplementation began immediately after baseline data collection.

Nutritional Status

Nutritional status was assessed using hemoglobin, albumin, total cholesterol, HDL and LDL cholesterol. Comparisons were made to normal or desirable values as published (12, 13).

Anthropometric and dietary measures

Anthropometric measurements were taken at each collection period prior to phlebotomy and included height, weight, mid-arm circumference, triceps skinfolds and bioelectrical impedance. Body mass index (BMI) was calculated (kg/m^2) (14). Three-day food records were kept by subjects prior to each measurement period. Each subject received a set of graduated non-biasing food models and was trained to keep food records before the initial measurement period (15). Food records were analyzed using the Food Processor II Computer Software Program (version 3.12, ESHA Research, Salem, OR). Means of each three-day period were used in these analyses.

Phlebotomy

Fasting blood samples were drawn between 8:00 and 9:00 AM using siliconized stainless steel butterfly needles and trace mineral free plastic syringes (Sarstedt, Newton, NC). A sample of whole blood was removed for hemoglobin analyses before centrifugation. Immediately after centrifugation, plasma was removed and stored frozen in trace element free plastic tubes for analysis. Erythrocytes were washed three times in 9 g/L saline, lysed with two volumes of glass distilled water, and stored frozen until digestion and analysis. The cyanmethemoglobin method was used to determine hemoglobin in whole blood and erythrocytes (Sigma

Proc. No. 525, St. Louis, MO). All preliminary sample handling, preparation for ashing and dilution of ash were done in a laminar flow hood. Plasma estradiol, progesterone, and ferritin were measured using double antibody radioimmunoassay kits.

Mineral analyses

Plasma and erythrocytes were wet and dry ashed (16) and analyzed by atomic absorption spectrophotometry (AAS). Dilutions of the same ashed sample were used for all analyses. Plasma zinc and erythrocyte iron and zinc were analyzed by flame AAS with deuterium background correction. Plasma iron and copper and erythrocyte copper were analyzed by graphite furnace AAS with Zeeman background correction using a Perkin Elmer 5100 PC AAS. Erythrocyte minerals were expressed per gram erythrocyte hemoglobin. Plasma minerals were compared to normal range for adults (13).

Statistical analysis

Data were analyzed by the General Linear Model for repeated measures using SAS version 6.07. Significance was set at $p \leq 0.05$.

RESULTS

Anthropometric measurements

The premenopausal female volunteers were within acceptable parameters for body composition measures (Table 1). Mean body mass index (kg/m^2) and body fat percentage were both mid-range within normal limits. None of the subjects were at risk for low body fat, and only one subject was above the appropriate range for adults. The mean triceps skinfold measurement was between the 25th and 75th percentile for women ages 18 to 54 (17). There were no significant changes over time for any of the body composition measures.

Dietary intakes

Calculated mean dietary intakes of energy, protein and fat were adequate, but calculated intakes of the selected trace elements did not meet the RDA (18)(Table 2). Mean iron and zinc intakes were 82.6 and 76 percent of the RDA, respectively. Mean copper intake was 76 percent of the lower limit of the ESADDI. While food selection varied during the study, there were no significant changes in mean nutrient intakes at any of the collection periods. Mean total zinc intake, which includes dietary zinc and the zinc supplement, was 36.5 mg during M2 and 40.1 mg during M3.

Biochemical analyses

Biochemical indicators of nutritional status reflect normal apparently healthy women (Table 1). Total cholesterol, plasma albumin, and total hemoglobin were within acceptable limits at baseline. There were no significant changes in total cholesterol and plasma albumin during this study. Hemoglobin at M2 was significantly lower than at all other periods (Figure 1). Baseline mean plasma iron and copper were within expected values (13). Mean plasma zinc was slightly low at baseline (13).

Estradiol did not differ significantly from baseline at any of the remaining three collection periods. However, there was a significant difference in estradiol between measurement periods M3 and M4. Progesterone was significantly different at M3 from baseline. These hormones did not have any significant effects on any mineral status parameters examined in this study (data not shown).

There was an overall significant quadratic effect for plasma iron and plasma zinc (Figure 2). Erythrocyte iron increased during zinc supplementation, but only at M3 was it significantly greater than baseline (Figure 3). Four weeks following supplementation, erythrocyte iron concentration decreased but not to baseline levels or below as occurred with plasma iron. There was no significant change in plasma ferritin over time (Figure 4).

There was no significant change in plasma copper over the duration of the study (Figure 2). However, there was a linear effect on erythrocyte copper (Figure 5).

As expected the response to zinc supplementation was most obvious in the plasma zinc concentrations at M2 and M3 (Figure 2). Erythrocyte zinc increased slightly, but not significantly, during supplementation. However, at M4 erythrocyte zinc fell below baseline concentration, but was not significantly different from other measurement periods (Figure 6).

Body Fat and Trace Mineral Status

When subjects were divided into body fat groups using the median body fat of 22 percent as the divider, significant differences were found in plasma and erythrocyte mineral concentrations. Lean subjects are described as those subjects with body fat below 22 percent and less lean subjects as those with body fat above 22 percent. Lean subjects had significantly decreased hemoglobin concentration at M2 that was significantly different from all other periods (Figure 7). In less lean subjects, hemoglobin concentration was decreased at M2 as well and was significantly different than M1 and M4, but not M3. Between the two groups, hemoglobin concentration was significantly higher in lean subjects at 8 weeks supplementation (M3) and post-supplementation

(M4) than in less lean subjects. Plasma ferritin concentrations did not change significantly within lean or less lean subjects or between the two groups at any time during the study (data not shown). Plasma iron concentrations differed significantly in lean subjects but not in less lean subjects (Figure 8). In lean subjects, plasma iron at post supplementation was significantly decreased from the three previous collection periods and fell below baseline. Between the two groups, plasma iron concentration was significantly different at M1, M2 and M3, but not at M4. Less lean subjects had lower plasma iron concentration than lean subjects at baseline and during supplementation but not at four weeks post supplementation.

Erythrocyte iron concentrations did not differ significantly in lean subjects, but M3 differed significantly from M4 in less lean subjects (Figure 9). There were no significant differences between fat groups throughout the study for erythrocyte iron.

Plasma copper concentrations did not differ within lean subjects or less lean subjects over time. However, plasma copper was significantly higher in less lean subjects at four weeks supplementation than in lean subjects (Figure 10). The pattern of erythrocyte copper concentrations changes during supplementation was similar in both groups with no significant differences between groups (Figure 11).

In lean subjects, there was an overall quadratic effect for plasma zinc (Figure 12). Plasma zinc among less lean subjects significantly increased from baseline at M2, and then decreased, but not significantly, below baseline at four weeks post supplementation. There were no significant differences between lean and less lean subjects for plasma zinc.

DISCUSSION

Plasma iron concentration increased during zinc supplementation and plasma ferritin decreased over time with supplementation indicating declining iron stores. The fact that plasma ferritin continued to decrease below baseline concentration after cessation of supplementation cannot be explained from these data, but similar results were found by Yadrick et al in subjects given 50 mg zinc supplements (6). These women were at risk for iron inadequacy at baseline based on dietary intake of iron although their mean hemoglobin and plasma ferritin concentrations were within lower normal values. Thus, the effect of zinc supplementation for this group may have been to further reduce marginal stores of iron. Other studies have shown that both iron and zinc supplements inhibit absorption of the other mineral (8, 19, 20). We did not conduct absorption studies although increases in total iron in plasma may indicate that absorption actually increased or iron was mobilized from

stores. Mobilization is likely as ferritin decreased from baseline to the end of the study. These findings do not reflect that of other research. Rossander-Hultén et al (8) reported decreased iron absorption with both 15 and 45 mg zinc but only when given in the fasted state. Crofton (20) also found iron absorption to be inhibited in subjects, but when given elemental iron (421 umol) in addition to elemental zinc (1048 umol) in solution after an overnight fast.

During supplementation with zinc, erythrocyte iron increased significantly at M3 compared to baseline. One would suspect from the significant change in erythrocyte iron and from the increases in plasma iron that the increased iron was incorporated into body tissues. However, in spite of the increases in both plasma and erythrocyte iron, hemoglobin did not increase significantly in whole blood. Further research is needed to explain the mechanisms for increased absorption and to explain the long-term effect of zinc supplementation on iron status measures.

In this study, plasma copper did not significantly change with zinc supplementation. The level of zinc provided may not have been high enough or the supplementation period long enough to see a change in copper status. Research has indicated that low to moderate levels of zinc supplementation do not affect the retention of copper (9, 21, 11). Other research studies have reported decreases in copper ESOD and

ceruloplasmin with zinc supplementation at levels similar to the level provided in this study (6, 7). However, neither copper ESOD nor ceruloplasmin was measured during this study and warrant investigation in further research studies to better explain the changes in copper status occurring with zinc supplementation.

Plasma zinc has been reported previously to be sensitive to changes in intake (22). There is a diurnal variation in concentration as well as a decline following food consumption (23). Thus in this study, subjects fasted for 12 or more hours prior to phlebotomy and all blood was collected between 8 and 9 AM. Our data show the increased concentration in plasma zinc as a response to supplementation fell to baseline when the supplement was withdrawn. Erythrocyte zinc also increased during supplementation and fell below baseline during the four weeks following supplementation.

Neither estradiol, progesterone nor their interactions had significant effects on plasma or erythrocyte iron or copper. Others have reported that estradiol affects plasma zinc concentrations (19), however, this study was designed to minimize the potential for that effect by collecting all samples at four week intervals.

Body Fat and Trace Mineral Status

Significant differences in certain measures of trace mineral status were found when subjects were divided based on body fat percentages. Significant differences occurred between body fat groups and within each body fat group.

Little research examining trace element status in individuals of differing body composition has been reported. In lean subjects, plasma iron increased from baseline with zinc supplementation and decreased below baseline after supplementation. In less lean subjects, plasma iron increased at eight weeks supplementation but the change was not significant as in lean subjects. Erythrocyte iron did not change significantly in less lean subjects, but there was a significant decrease in less lean subjects from eight weeks supplementation to post-supplementation. Fricker et al (24) addressed the issue of obesity in determining iron status in menstruating women. Hemoglobin and serum ferritin concentrations were higher in obese women than non-obese women, but no significant differences in serum iron were found. Hemoglobin and ferritin, in the current study, were not significantly different in either body fat group, but plasma iron was significantly higher in lean subjects at baseline. However, our less lean group was not obese. Fricker attributed the increase in hemoglobin and ferritin in obese women due to a higher caloric intake and a higher dietary iron intake.

Obese subjects had dietary iron intakes above the RDA whereas non-obese subjects reported iron intakes less than the RDA for iron. This is similar to the dietary intakes of iron for the lean subjects in this study as intakes were below the recommendation.

As suggested by other research (5, 9, 11, 10), low to moderate levels of multi-mineral supplementation in balanced ratios seems to prevent the depletion of one or more minerals. Taking supplements in the presence of a meal appears to prevent marked decreases in mineral status as well (8, 20, 25, 26).

Nevertheless, moderate zinc supplementation taken with meals did have a significant effect on iron, copper, and zinc status in these apparently healthy premenopausal women.

Table 1
Anthropometric and Biochemical Characteristics of Subjects[1]

VARIABLE	MEAN	RANGE	DESIRABLE VALUE[2]
Age, y	32.8	18-48	
Anthropometric Measures			
Weight, kg	62.1	52.5 - 85.5	
BMI, kg/m^2	22.7	18.3 - 32.8	
Triceps, mm	17.7	10.3 - 25.3	
Mid-Arm Circumference, cm	27.5	24.0 – 33.0	
Body Fat, %	23.4	14.7 - 38.2	
Biochemical Evaluation			
Hemoglobin, g/L	132	123 - 142	115 – 155
Plasma Ferritin, ng/ml	45.3	9.7 - 102	11 – 120
Plasma Iron, ug/L	1084	605 - 1731	600 – 1600
Plasma Copper, ug/L	823	603 - 1081	700 – 1400
Plasma Zinc, ug/L	628	566 - 720	750 – 1200
Plasma Albumin, g/dl	4.39	4.0 - 4.67	4.0 – 6.0
Total Cholesterol, mg/dl	161	139 - 214	<200
HDL, mg/dl	47	24 - 84	>35
LDL, mg/dl	133	81 - 204	<130
Triglycerides, mg/dl	91	37 - 216	30 – 190

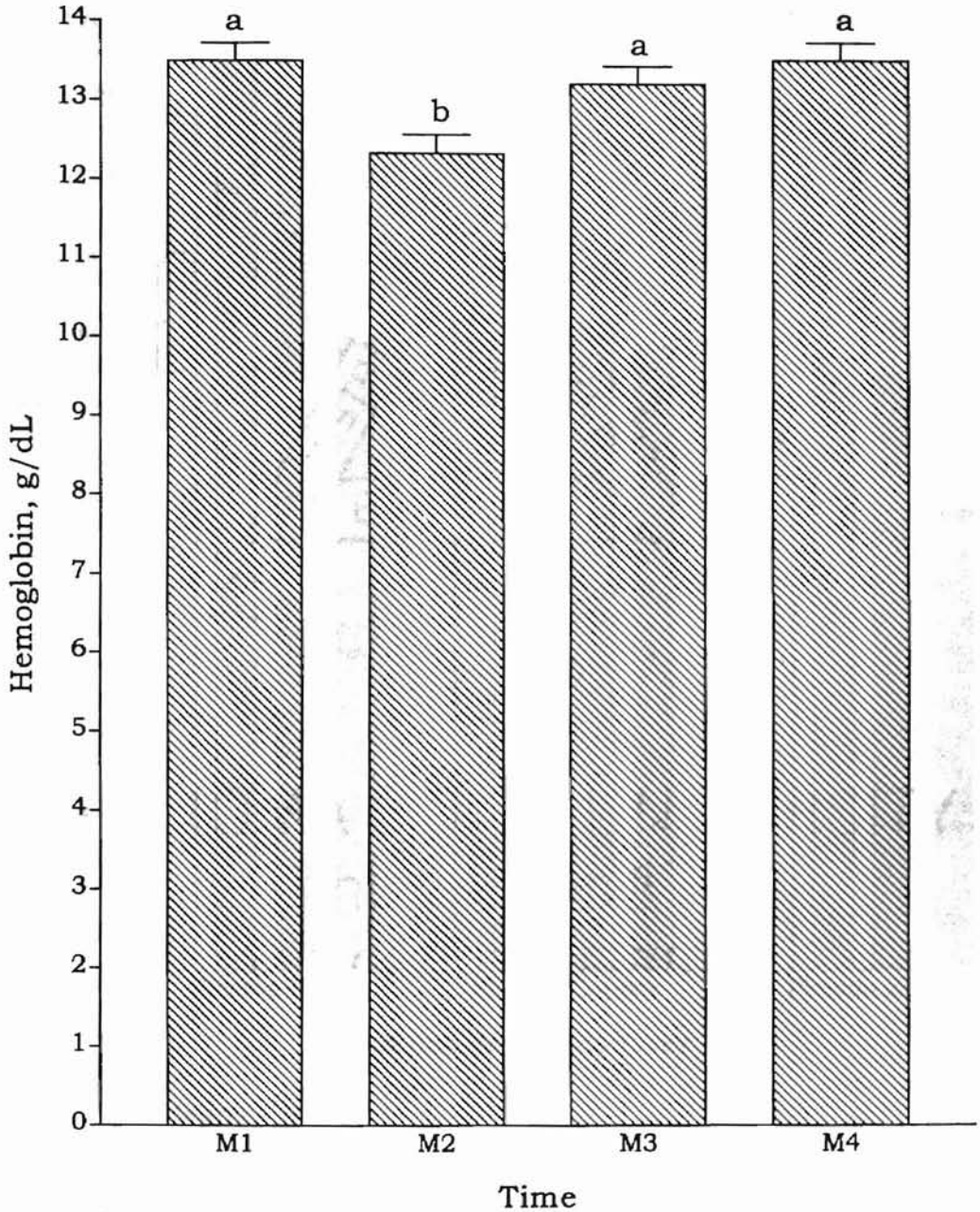
[1]Baseline measures used.
[2] References 12, 13

Table 2
Dietary Characteristics of Subjects at Baseline

VARIABLE	MEAN	RANGE	1989 RDA[1]	ESADDI[1]
Energy	1787	854 - 3009		
Protein, g	74	42 - 111	45 - 50	
Fat, g	69	19 - 137		
Cholesterol, mg	215	106 - 373		
Iron, mg	12.4	6.29 - 28.9	15	
Zinc, mg	8.9	3.8 - 19.5	12	
Copper, mg	1.14	.539 – 2.26		1.5 – 3.0
Source of Energy			Dietary Goals	
Carbohydrate, %	49.5	38 - 60	58	
Protein, %	16.8	11 - 24	12	
Fat, %	33.5	21 - 49	30	

[1] Reference 18

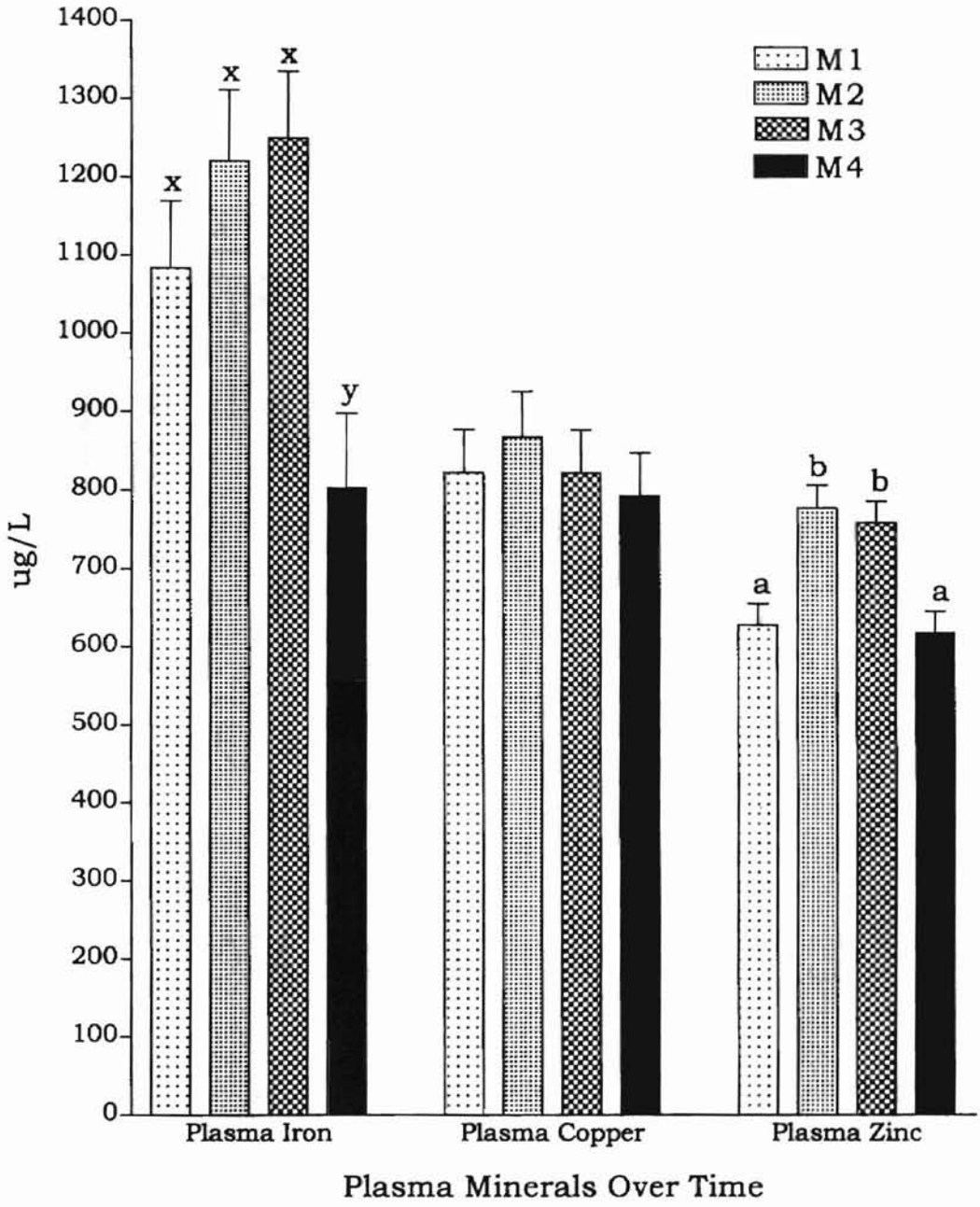
Fig 1. Effect of Zinc Supplementation on Hemoglobin Over Time[1-2]



[1]Means ± SEM.

[2]Bars not sharing common letters are significantly different ($p<0.05$).

Fig 2. Effect of Zinc Supplementation on Plasma Minerals Over Time[1-4]



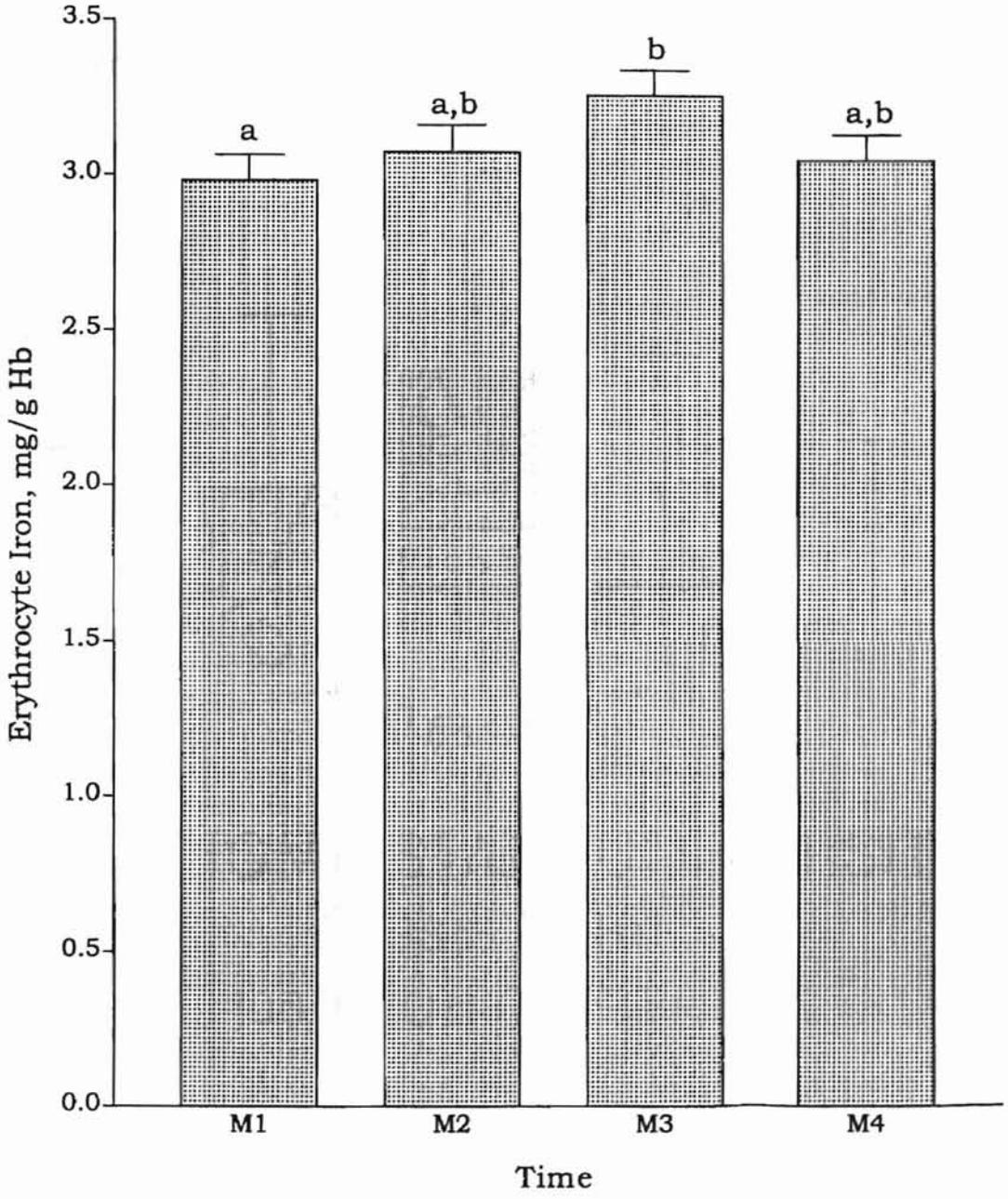
[1]Means ± SEM.

[2]Bars within minerals not sharing common letters are significantly different ($p<0.05$).

[3]Overall quadratic effect ($p<0.001$) for plasma zinc.

[4]Overall quadratic effect ($p = 0.001$) for plasma iron.

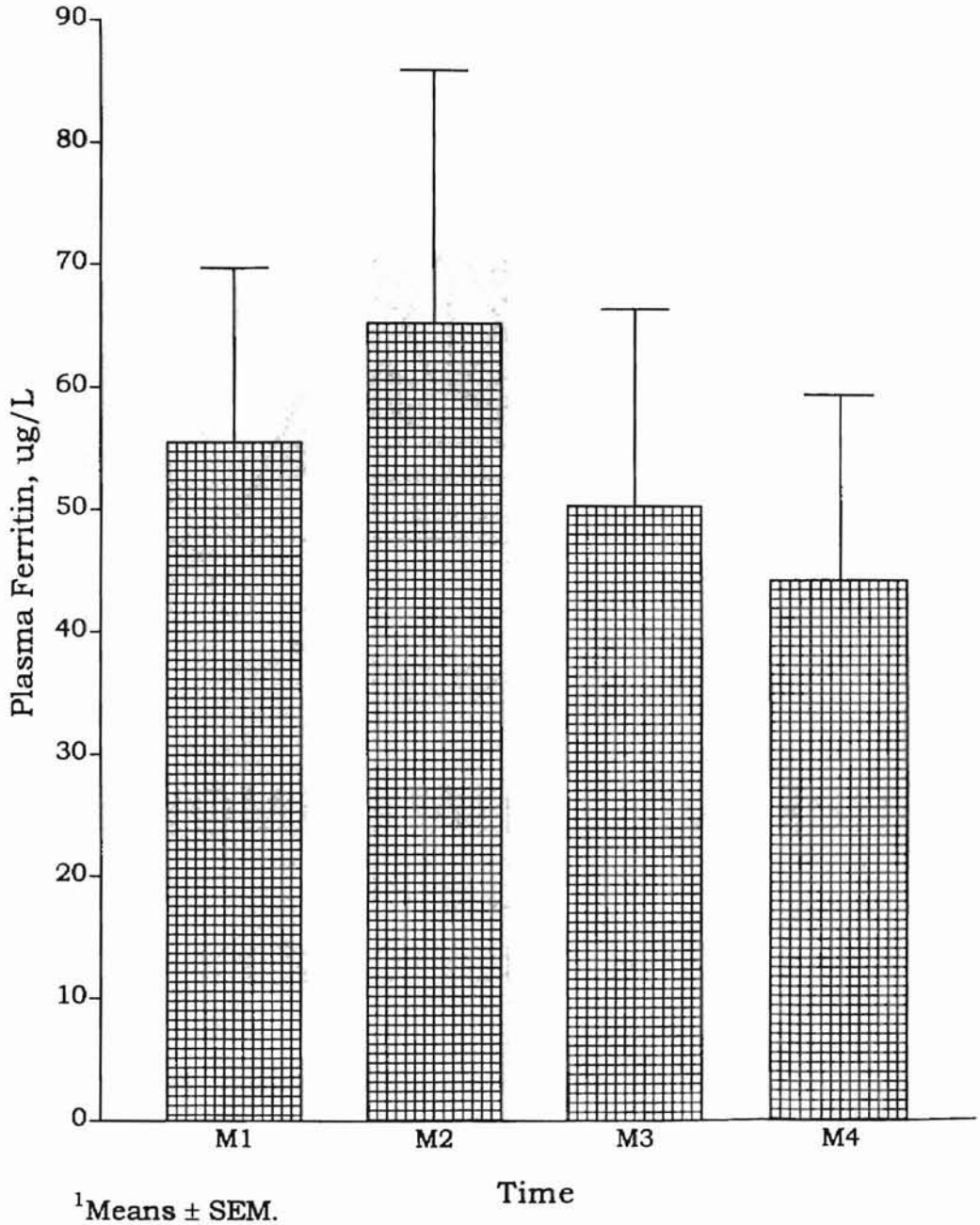
Fig 3. Effect of Zinc Supplementation on Erythrocyte Iron Over Time[1-2]



[1]Means + - SEM.

[2]Bars not sharing common letters are significantly different ($p<0.05$).

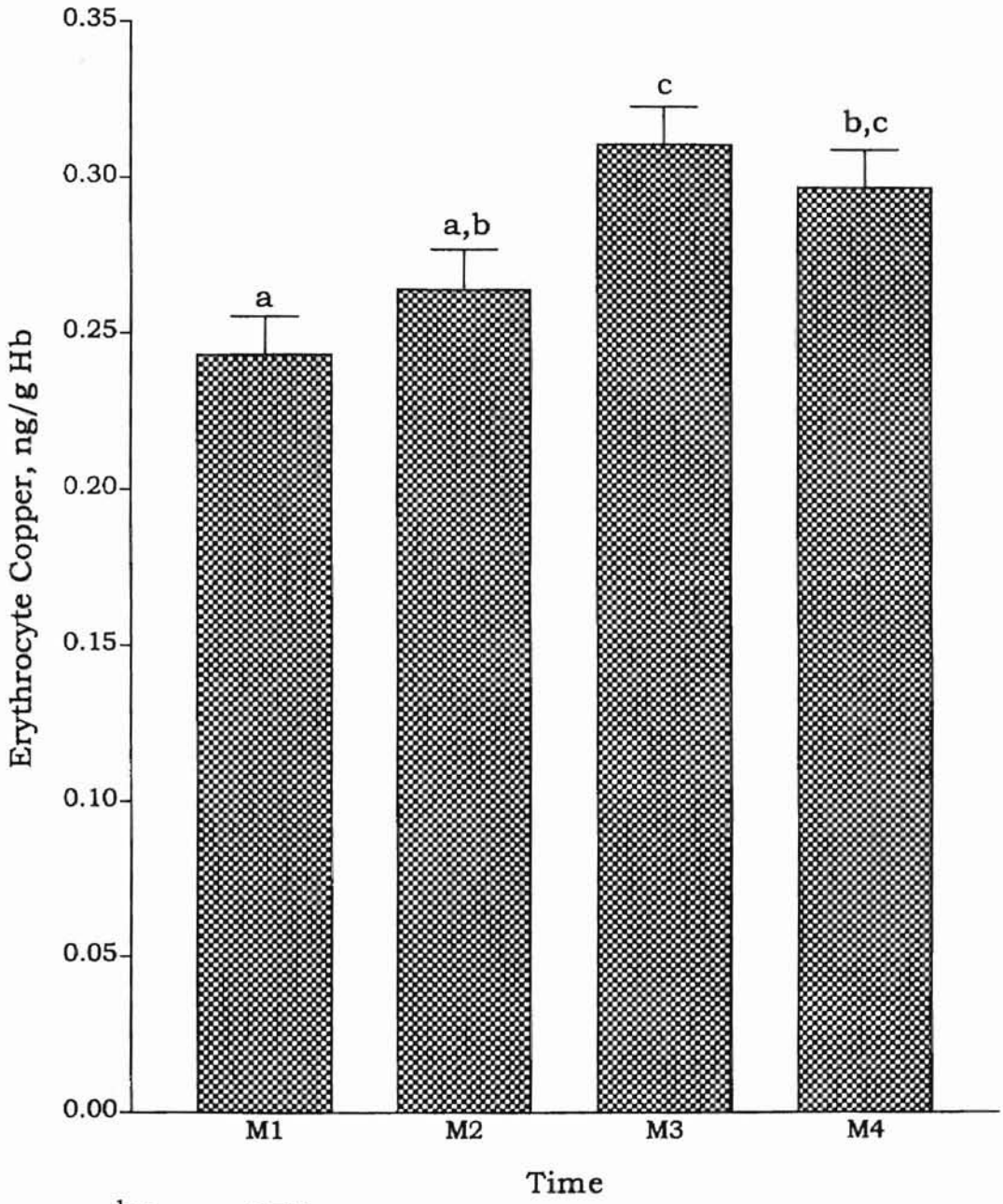
Fig 4. Effect of Zinc Supplementation on Plasma Ferritin Over Time[1-2]



[1]Means ± SEM.

[2]There were no significant differences in plasma ferritin over time.

Fig 5. Effect of Zinc Supplementation on Erythrocyte Copper Over Time[1-3]

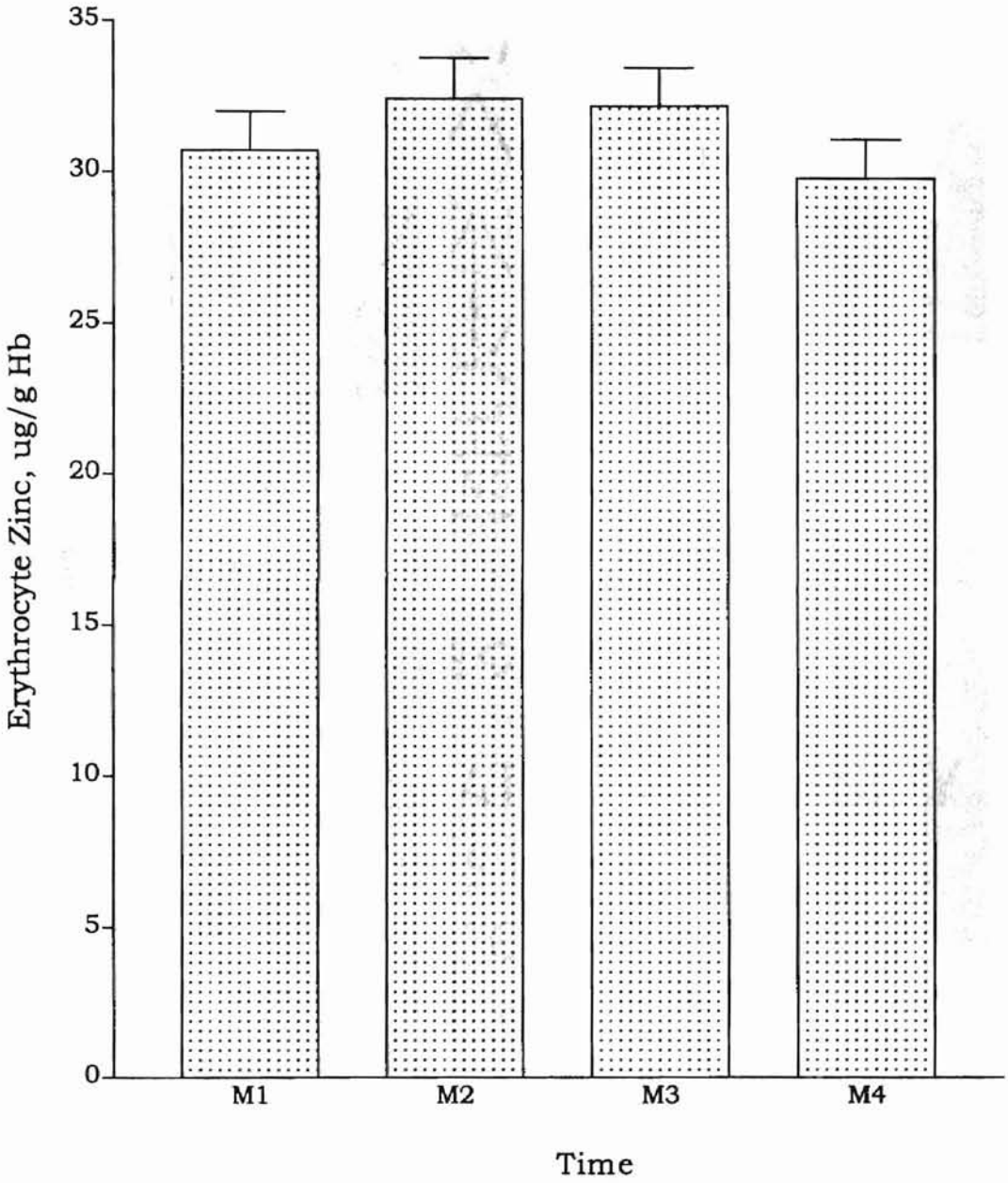


[1]Means ± SEM.

[2]Bars not sharing common letters are significantly different ($p<0.05$).

[3]Linear effect $<p = 0.05$.

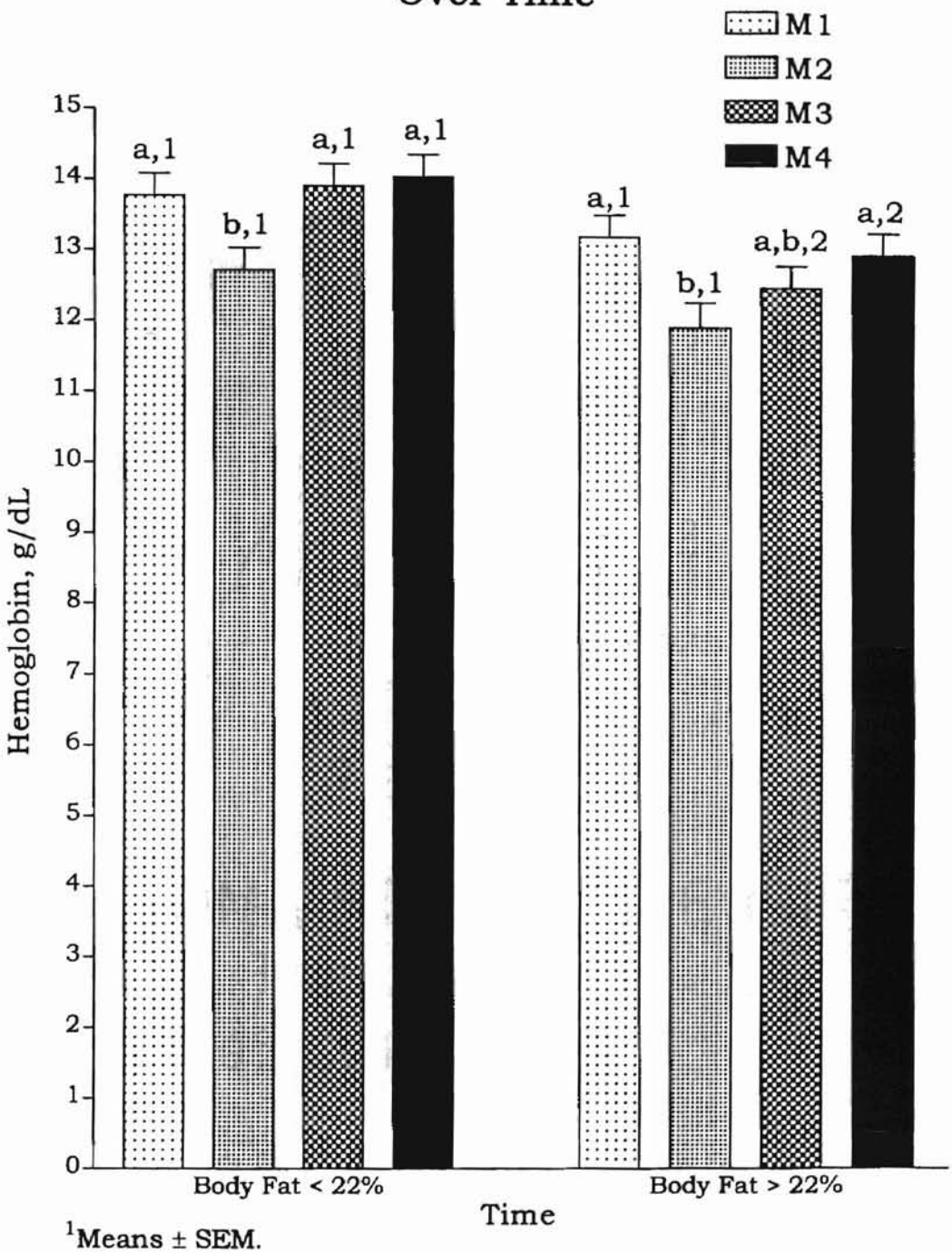
Fig 6. Effect of Zinc Supplementation on Erythrocyte Zinc Over Time[1-2]



[1]Means ± SEM.

[2]There were no significant differences in erythrocyte zinc over time.

Fig 7. Difference in Hemoglobin Concentration by Body Fat Group and Over Time[1-3]

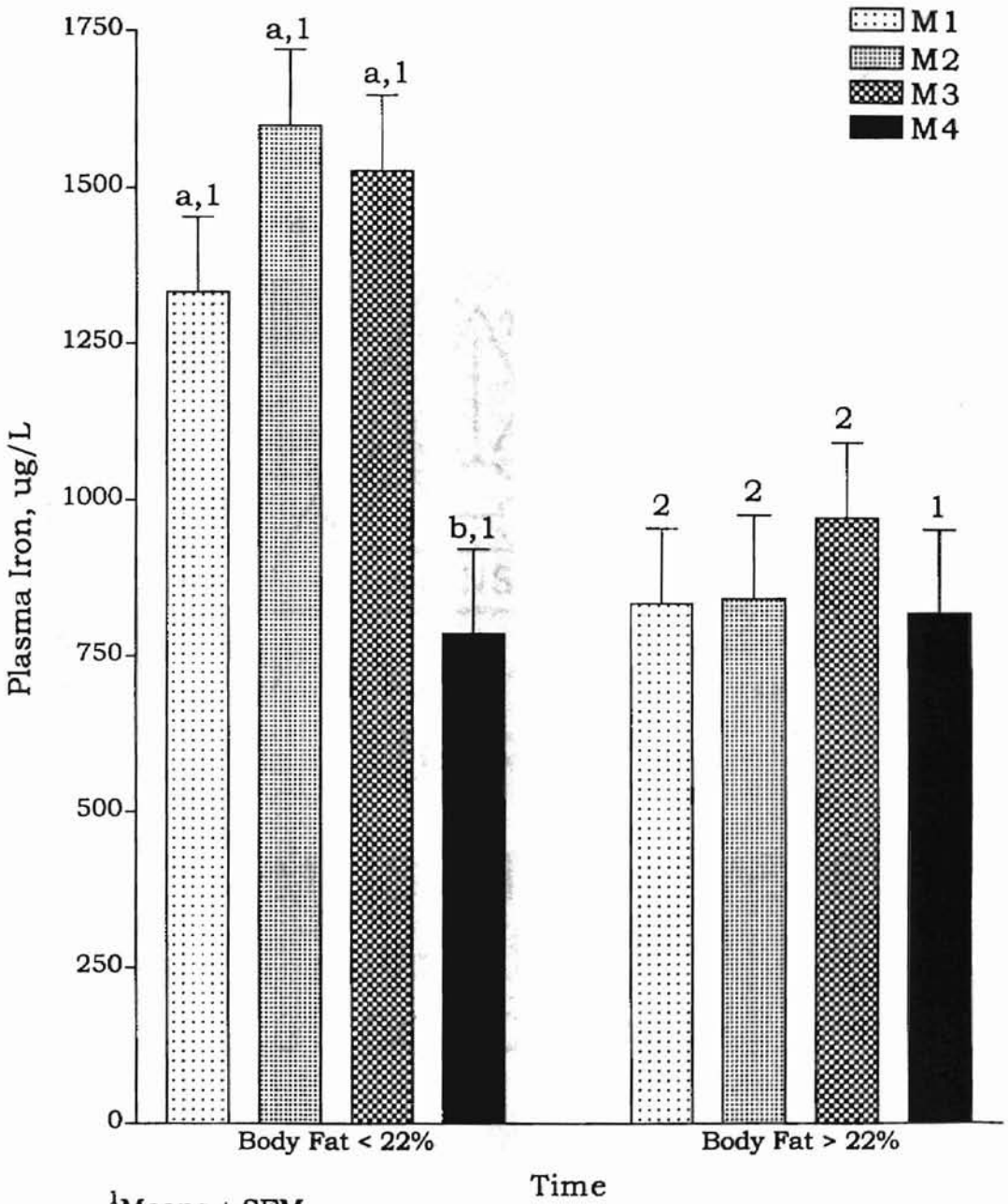


[1]Means ± SEM.

[2]Within groups, bars not sharing common letters are significantly different ($p<0.05$).

[3]Between groups, at the same measurement period, bars not sharing common numbers are significantly different ($p<0.05$).

Fig 8. Difference in Plasma Iron by Body Fat Group and Over Time[1-3]

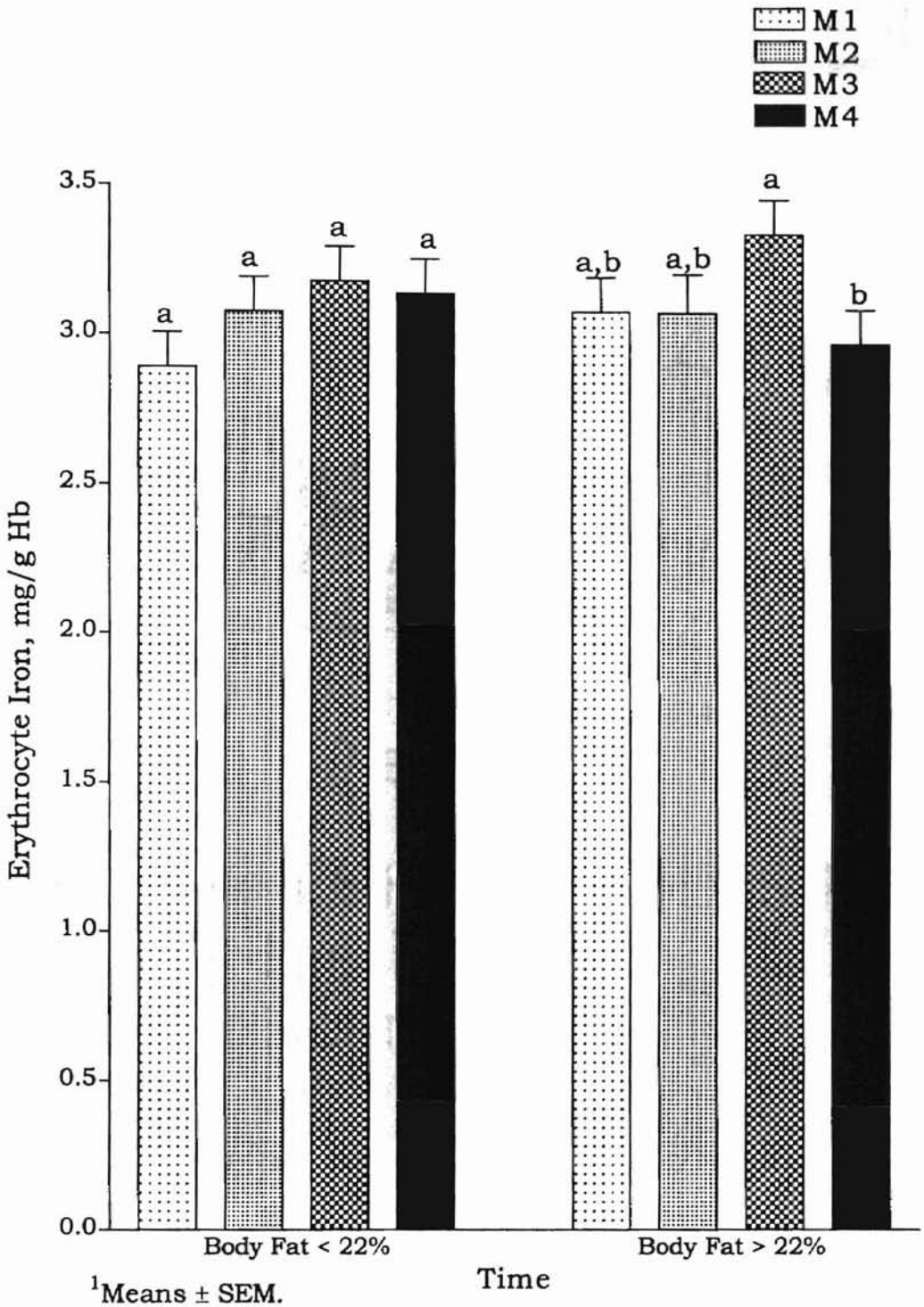


[1]Means ± SEM.

[2]Within groups, bars not sharing common letters are significantly different ($p<0.05$).

[3]Between groups, at the same measurement period, bars not sharing common numbers are significantly different ($p<0.05$)

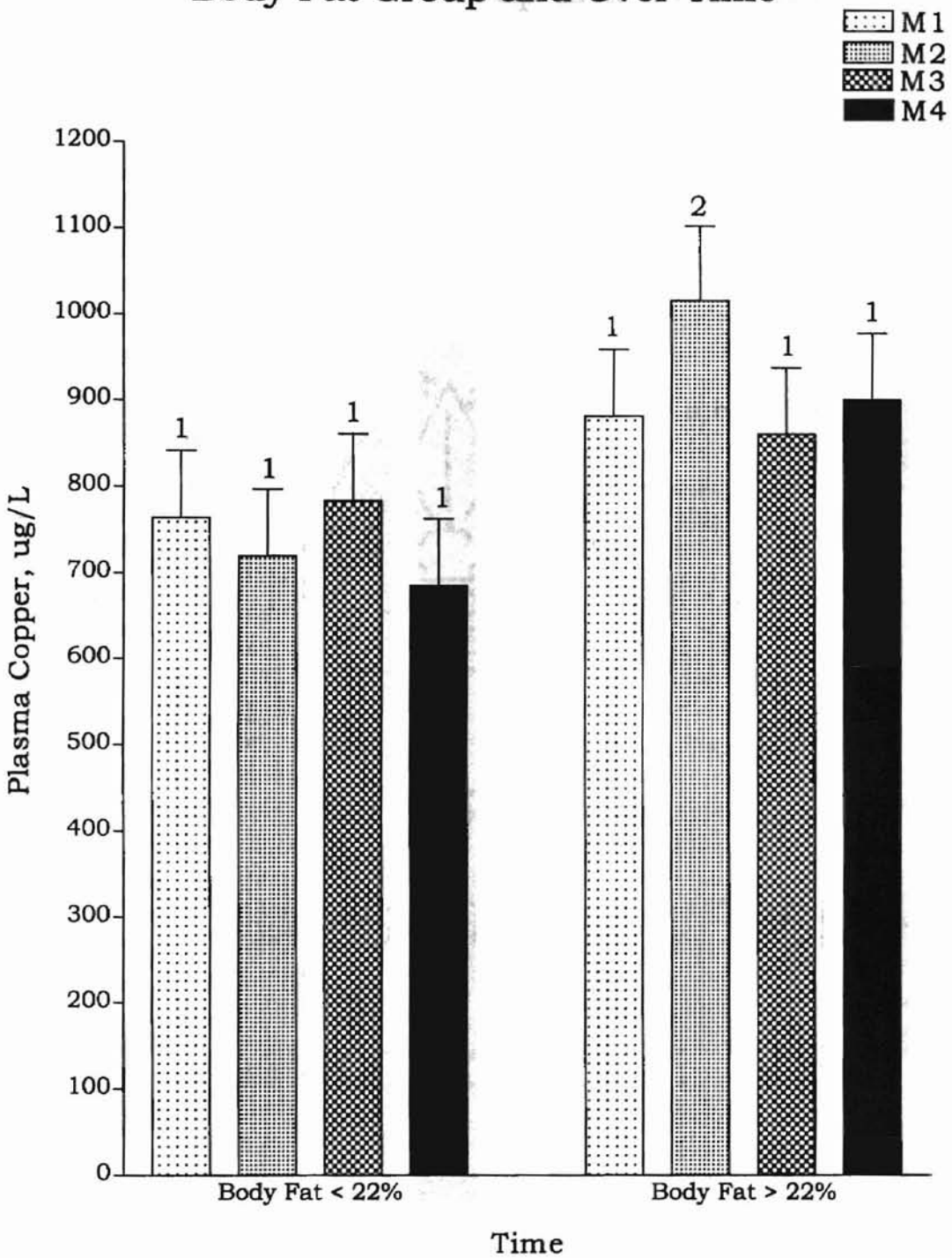
Fig 9. Difference in Erythrocyte Iron by Body Fat Group and Over Time[1-2]



[1]Means ± SEM.

[2]Within groups, bars not sharing common letters are significantly different ($p<0.05$).

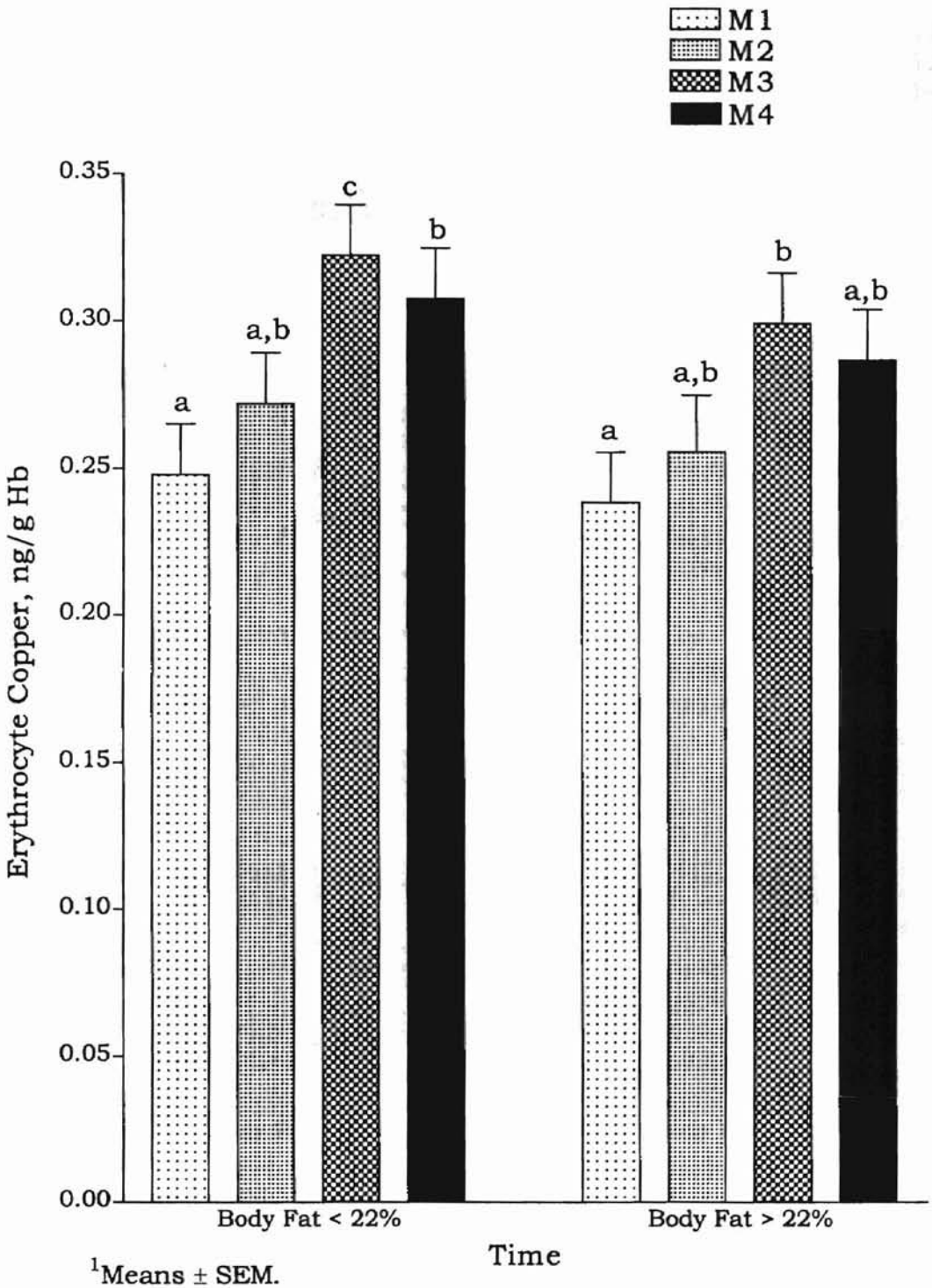
Fig 10. Difference in Plasma Copper by Body Fat Group and Over Time[1-2]



[1]Means ± SEM.

[2]Between groups, at the same measurement period, bars not sharing common numbers are significantly different ($p<0.05$).

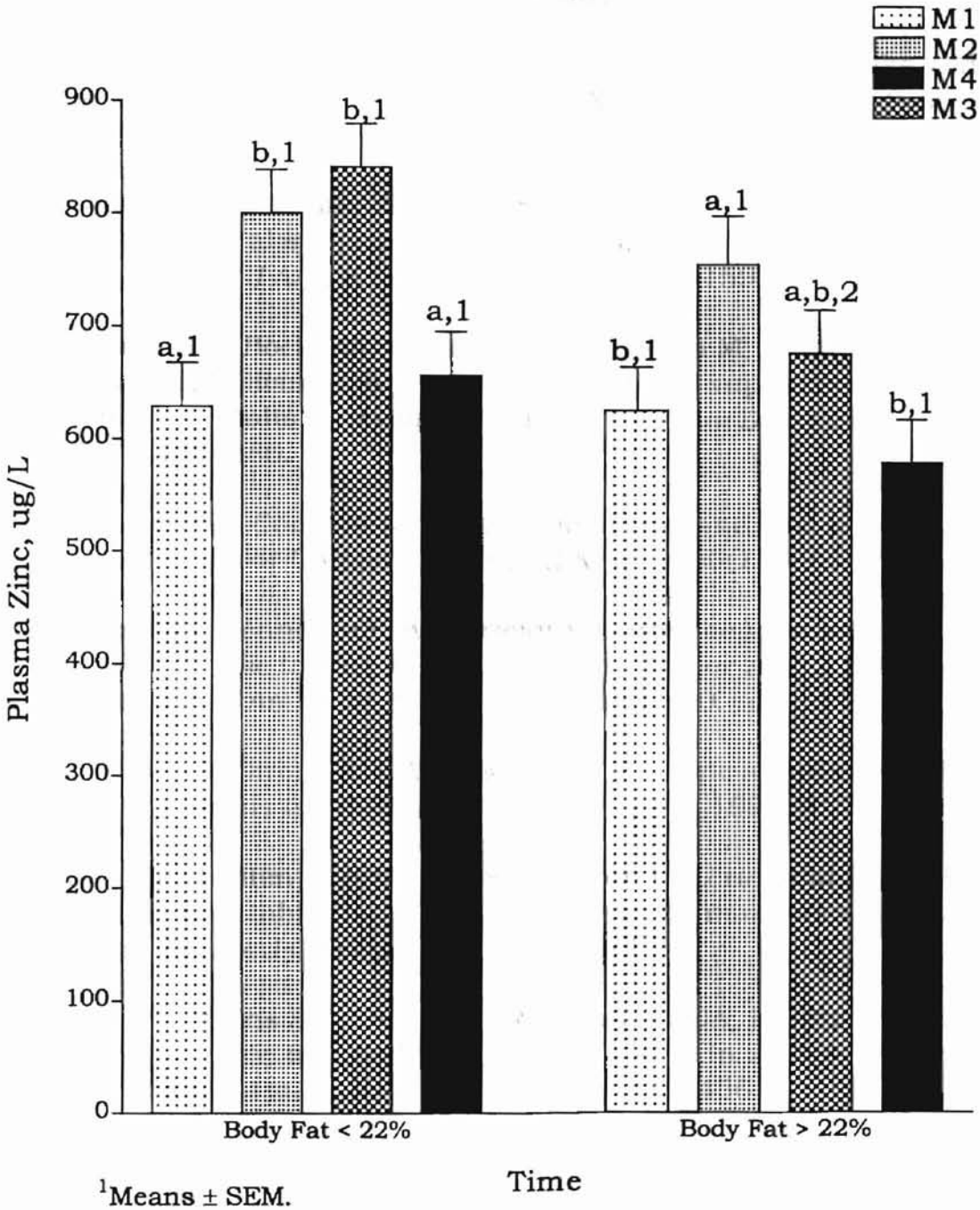
Fig 11. Difference in Erythrocyte Copper by Body Fat Group and Over Time[1-2]



[1]Means ± SEM.

[2]Within groups, bars not sharing common letters are significantly different ($p<0.05$).

Fig 12. Difference in Plasma Zinc by Body Fat Group and Over Time[1-3]



[1]Means ± SEM.

[2]Within groups, bars not sharing common letters are significantly different ($p<0.05$).

[3]Between groups, at the same measurement period, bars not sharing common numbers are significantly different ($p<0.05$).

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Chapter V

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate whether moderate zinc supplementation had a significant effect on iron, copper, and zinc status in apparently healthy premenopausal women.

The objectives of the study included the following:

1. to determine the erythrocyte and plasma concentrations of iron, copper and zinc in apparently healthy premenopausal women;
2. to determine the effect of zinc supplementation on iron, copper and zinc status indicators in apparently healthy premenopausal women;
3. to determine the relationship between dietary intake and iron, copper and zinc status in apparently healthy premenopausal women; and
4. to determine the relationship between body composition and mineral status in apparently healthy premenopausal women.

The hypotheses postulated in this study were:

H_1: There will be no significant effect of zinc supplementation on iron status as measured by plasma and erythrocyte iron concentrations and plasma ferritin in apparently healthy premenopausal women.

H_2: There will be no significant effect of zinc supplementation on copper status as measured by plasma and erythrocyte copper concentrations in apparently healthy premenopausal women.

H_3: There will be no significant difference in mineral status of individuals differing in body composition measures.

Erythrocyte and plasma iron concentrations were assessed at each measurement period in subjects. Plasma iron and zinc concentrations at baseline were within normal values while plasma copper was lower than the low end of the range. We must reject the first hypothesis (H_1) because zinc supplementation did have an effect on iron status. Plasma iron concentration increased during zinc supplementation and plasma ferritin decreased over time, although not significantly, with supplementation indicating declining iron stores. Erythrocyte iron increased with eight weeks of zinc supplementation compared to baseline. Hemoglobin did not increase significantly in whole blood during the study.

The second hypothesis (H_2) is rejected as well. Plasma copper did not change significantly over time with zinc supplementation, but erythrocyte copper increased as a response to supplementation and remained higher than baseline at post supplementation.

Dietary intakes of iron, copper and zinc did not change significantly during the study, but intakes were lower than recommended levels. It is possible that the changes in plasma and erythrocyte mineral

concentrations during supplementation also may have been affected by low trace mineral intakes.

We did find significant differences in iron, copper and zinc status when subjects were separated into body fat groups and therefore must reject the fourth hypothesis (H_4). Lean subjects had higher erythrocyte copper concentrations than did less lean subjects, but not erythrocyte iron or zinc concentrations. Lean subjects had higher plasma iron and zinc concentrations during supplementation than less lean subjects, but less lean subjects had higher plasma copper concentrations. Plasma ferritin did not significantly differ within or between lean and less lean subjects throughout the study. Hemoglobin concentration was higher in lean subjects than in less lean subjects over the duration of the study. Fricker et al (46) reported higher hemoglobin and serum ferritin in obese subjects, but this may be due to higher energy intake and not to a higher intake of iron dense foods. Our data do not support those findings as plasma ferritin did not change significantly in either group and hemoglobin was found to be higher in lean subjects. The differences could be attributed to changes in body composition. Whereas Fricker et al (46) studied obese individuals, only one subject in this study was found to meet the parameters for obesity.

In summary, moderate zinc supplementation taken with meals did have a significant effect on iron, copper, and zinc status in apparently healthy premenopausal women.

However, in order to better understand the mechanisms of trace mineral interactions, additional study is needed in males, different age groups, individuals with varying body composition, and those with higher baseline trace mineral status measures. The effect of zinc supplementation on copper status needs further investigation using copper ESOD and ceruloplasmin to measure copper nutriture in addition to plasma and erythrocyte concentrations. Data collection needs to be extended more than four weeks post-supplementation to measure the continuing effects of zinc supplements on iron, copper and zinc status measures after supplement withdrawal.

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APPENDICES

APPENDIX A

RESEARCH STUDY RECRUITMENT FLYER

HELP WANTED

Have you ever wondered what it would be like to be a participant in a research study?

Would you like to know the effects of nutrient supplements on nutritional status?

We have an *opportunity* for you if you meet the following conditions:

FEMALE

Between the ages of 18 and 65

Not currently pregnant nor planning to become pregnant in the next 6 months

Not using oral contraceptive agents nor taking estrogen replacement therapy

Do not have a chronic disease

This study is designed to determine the effect of zinc supplements on measures of zinc status including plasma, blood cells, and urinary excretion of zinc. The study involves participation for 12 weeks during which time you will participate in body composition measurements (height, weight, skinfold measurements, and bioelectric impedance), record food intakes and blood and urine collections.

The first collection period will be at the beginning of the study to provide baseline data. For eight weeks you will take a zinc supplement twice a day (15 mg each). Data will be collected at four and eight weeks of this supplement period. Four weeks following the end of supplementation the final data collection period will occur.

All that is required of you is to record food intakes for three days prior to each data collection, take the supplement daily for eight weeks, and come to the FNIA Department at four week intervals for blood collection (25 mL or about 5 teaspoons) by a licensed technician, and provide a urine specimen at each blood collection period. Weight and body composition measurements will be recorded at each data collection period. We ask that you do not attempt to lose weight during this period.

Sound like fun?!! For further information contact:

Andrea B. Arquitt, PhD, RD/LD
421 Home Economics
744-5040

APPENDIX B

APPROVAL OF INSTITUTIONAL REVIEW BOARD FOR HUMAN SUBJECTS RESEARCH

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
FOR HUMAN SUBJECTS RESEARCH

Proposal Title: Evaluation of Blood Cells, and Interleukin-2 as Measures of Zinc Status in Adult Women

Principal Investigator: Andrea B. Arquitt

Date: November 26, 1990 IRB # HE-91-007

This application has been reviewed by the IRB and

Processed as: Exempt [ ] Expedite [ ] Full Board Review [X]

Renewal or Continuation [ ]

Approval Status Recommended by Reviewer(s):

Approved [X] Deferred for Revision [ ]

Approved with Provision [ ] Disapproved [ ]

Approval status subject to review by full Institutional Review Board at next meeting, 2nd and 4th Thursday of each month.

Comments, Modifications/Conditions for Approval or Reason for Deferral or Disapproval:

Signature: Steven W Edwards Date: December 19, 1990
Chair of Institutional Review Board

APPENDIX C

INDIVIDUAL'S CONSENT TO PARTICIPATE IN RESEARCH

Individual's Consent to Participate in Research

Evaluation of Blood Cells and Interleukin-2 as Measures of Zinc Status in Adult Women

I, _____. voluntarily agree to participate in the above titled research which is sponsored by The College of Home Economics at Oklahoma State University.

I understand that:

(1) the purpose of the study is to measure the effect of zinc supplementation at the level of 30 mg/day on zinc and other trace minerals in plasma, blood cells, and urine;

(2) I will be requested to record three days of food intake four times during this study;

(3) during this study period I should attempt to avoid consumption of oysters, and the following breakfast cereals: General Mill's Total and Kellogg's Nutri-grain Raisin Bran and Just Right;

(4) I will not take any nutrient supplements other than those that are a part of this study;

(5) I will consume a 15 mg zinc supplement twice a day for eight weeks and any inadvertently omitted will be returned at the next collection date; and that supplements of this amount are readily available over the counter;

(6) I will record menstrual cycle information by date of cycle beginning and ending throughout this study;

(7) a licensed medical technician or medical technologist will draw fasting blood samples of 25 mL (about 5 teaspoons) by venipuncture prior to the study, at midpoint and end of the supplementation, and four weeks following supplementation and that slight bruising or discomfort may result from the venipuncture;

(8) urine specimens will be collected following an overnight fast at the same time as blood collections;

(9) as a reward for participation and as an incentive to complete the study, I will receive one coupon for a complimentary luncheon special at the Taylor Dining Room at each blood collection period;

(10) all records are confidential and that my name will not be associated with any reports or data records at the end of the study;

(11) participation is voluntary and that I have the right to withdraw from this study at any time by contacting the principal investigator;

(12) I will not participate if I am under age 18, am pregnant or planning to become pregnant during the study, am taking oral contraceptive agents or estrogen replacement or have a chronic health problem;

(13) I will withdraw from the project if I become pregnant during the study;

(14) this research is beneficial to the public in that many individuals take nutrient supplements without knowledge of the interactions among the nutrients; and

(15) I may contact Dr. Andrea Arquitt at (405) 744-5040 should I wish further information. I may also contact Terry Maciula, University Research Services, 001 Life Sciences East, Oklahoma State University, Stillwater, OK 74078 telephone (405) 744-9991.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date_____ Time_____(am/pm)

Signed_____

I certify that I have been personally explained all elements of this form to the subject before requesting the subject to sign it.

Signed _____
(project director or her authorized representative)

APPENDIX D

INSTRUCTIONS TO SUBJECTS

INSTRUCTIONS TO SUBJECTS

Because this study involves the use of nutrient supplements, we ask that you do not take any additional supplement during the study period (March through June). In addition, we ask that you do not consume any foods which are outstanding dietary sources of zinc. The following breakfast cereals are highly fortified and contain 15 mg of zinc per serving:

Kellogg's Nutri-Grain Raisin Bran
Kellogg's Just Right
General Mill's Total

Therefore, please do not eat any of these cereals during this study. The only other outstanding zinc source is oysters. Again, we ask that you avoid eating these during the study.

You will be provided with our own set of food models to help you identify portion sizes of foods. Please use these on each of the occasions during which you keep these records. The records will be kept on the three days preceding each blood collection. You will be reminded by phone when you should begin your food records.

Please keep menstrual cycle information on the calendar pages provided. You will return these on the day of data collection each month.

Zinc concentrations in the blood fluctuate with time of day and period in the menstrual cycle. Food intake also affects the concentration. Therefore, it is most important that you do not eat anything after 7 PM ON THE DAY BEFORE A BLOOD COLLECTION.

Total body water may be altered by alcohol consumption. Because we will be using a bioelectric impedance test to determine body composition, it is important that you do not consume any alcohol for 24 hours prior to the test. That will be one Friday a month—the day before your data collection for that month.
We really appreciate your help with this study. We hope you enjoy participating, and that the information you gain about your own hemoglobin, cholesterol and body composition values will be beneficial to you.

Thank you very much for your help.

Sincerely,

Andrea B. Arquitt, PhD, RD/LD

Investigator

Your collection dates are: _____ _____ _____ _____

APPENDIX E

HEALTH INFORMATION QUESTIONNAIRE

HEALTH INFORMATION QUESTIONNAIRE

Zinc Supplementation Study
FNIA Department
Oklahoma State University

Subject Number _____

Age _____

Race _____

Do you have or have you had any of the following diseases?

	No	Yes	When
Inherited disorder	____	____	_____
Uremia	____	____	_____
Sickle cell anemia	____	____	_____
Neoplastic disease	____	____	_____
Diabetes	____	____	_____
Liver disease	____	____	_____

Are you on any type of special diet:

Allergy ____ ____ Specify _____

Weight loss ____ ____

Weight gain ____ ____

Other ____ ____ Specify _____

Do you currently take any medications on a regular basis?

____ ____

Specify all drugs taken _____
_____
_____
_____

In the last 6 months have you taken any nutrient (dietary) supplements?

IF YES, Specify how recently _____

How regularly _____

IF YES, Specify brand and frequency of consumption:

_____
_____
_____

Do you have an Intrauterine Device (IUD) inserted at the present time?

_____ _____ Name_____

Have you recently had a serious illness or surgery?

_____ _____ Specify_____

Do you know your cholesterol "number" (concentration)?

_____ _____

IF YES, what is it? _____total _____HDL

APPENDIX F

MONTHLY DATA COLLECTION FORM

MONTHLY DATA COLLECTION FORM

Zinc Supplementation Study
FNIA Department
Oklahoma State University

Subject Number _____

Date _____

	No	Yes
1. Have you had a cold in the last month?	____	____
IF YES, when?	_____	
how long did it last?	_____	
did you have a fever?	_____	
2. Have you had the flu in the last month?	____	____
IF YES, when?	_____	
how long did it last?	_____	
did you have a fever?	_____	
3. Have you had any other illness during the last month?	____	____
IF YES, what type of illness?	_____	
how long did it last?	_____	
did you have a fever?	_____	
4. IF YES TO ANY OF THE QUESITON 1 – 3, did you continue to take your supplement during the illness?	____	____

5. What was the date of the start of your last menstrual cycle?

_____

How many days did it last?_____

VITA

Laura Kay Savage

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF ZINC SUPPLEMENTATION ON IRON, COPPER AND ZINC STATUS IN PREMENOPAUSAL WOMEN

Major Field: Nutritional Sciences

Biographical:

Personal Data: Born in London, England, March 30, 1968, the daughter of Bonnie S. Rooker and the late Alfred A. Savage.

Education: Graduated from Cypress Creek High School, Houston, Texas, in May, 1986; received Bachelor of Science Degree in Psychology from Oklahoma State University in May, 1990; Completed the Approved Preprofessional Practice Program in May, 1993; Passed the Registration Examination for Dietitians in October, 1993; Completed the Requirements for the Master of Science Degree with a major in Nutritional Sciences at Oklahoma State University in May, 2000.

Professional Experience: Research Assistant, Department of Nutritional Sciences, Oklahoma State University, January, 1990 to 1992; Teaching Assistant, Department of Nutritional Sciences, Oklahoma State University, January, 1990 to 1992; Teaching Assistant, Department of Hotel Restaurant/Nutritional Sciences, Oklahoma State University, August, 1993 to December 1993; Registered Dietitian, WIC Supervisor, Mary Mahoney Memorial Health Center, Oklahoma City, Oklahoma, March, 1994 to May, 1997: Nutrition Education Coordinator, WIC Service, Oklahoma State Department of Health, Oklahoma City, Oklahoma, June 1997 to present.