EFFECTS OF TRACE MINERAL SUPPLEMENTATION ON STATUS MEASURES OF ZINC, COPPER, AND IRON IN OLDER ADULTS

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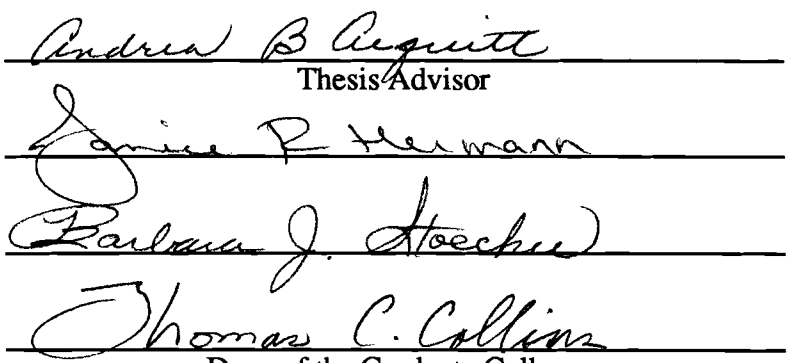

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

The elderly are at risk for impaired nutritional status due both to dietary intake and to physiological changes. Approximately five to six percent of the elderly residing in nursing homes are malnourished due to low or marginal dietary intake. The elderly may also have changes in nutritional requirements secondary to disease conditions (Kerstetter et al., 1992).

The elderly have been reported to have dietary intakes lower than the Recommended Dietary Allowances for micronutrients as well as macronutrients (Payette and Gray-Donald, 1991b). Sahyoun et al. (1988) reported that the elderly consume less than the Recommended Dietary Allowances for zinc and B-vitamins. Hematological indices (albumin, prealbumin and transferrin levels) also are reported to be lower in elderly populations than in younger populations. Lower dietary intakes of trace minerals including zinc have been found in both sexes, especially in the institutionalized elderly (Sahyoun et al., 1988).

Data from the Second National Health and Nutrition Examination Survey (NHANES II) reported that mean serum zinc values changed with age, being relatively low during childhood, increased during adolescence, peaked in young adulthood and then declined in older and elderly adults (Pilch and Senti, 1985). Turlund et al. (1986) reported a lower absorption of zinc in elderly men than in younger men which may reflect a lower requirement of absorbed zinc by the elderly. Plasma zinc is frequently reported as an indicator of zinc status (King, 1990). Plasma zinc was reported to be a useful indicator of the exchangeable zinc pool size. Decreased plasma zinc reflected a loss of

zinc from bone and liver and, thus, indicated an increased risk for development of metabolic and clinical signs of zinc deficiency (King, 1990). With increasing age, zinc status may be affected due to changes in the regulation of zinc metabolism. Wastney et al. (1992) reported that zinc taken up by red blood cells decreased with age. The response of plasma zinc to a zinc load of 100 mg per day was 115 percent in 80 year old subjects as compared to 55 percent in 20 year old subjects. In addition, the excretion of zinc in urine was over five-fold greater in the older subjects than in the younger subjects. Perhaps this excess excretion of zinc in urine is due to increased release of zinc from red blood cells.

Analysis of NHANES II data showed that almost 35 percent of the total US population took vitamin and mineral supplements regularly. In addition, supplement use was more common among older adults. Supplement use was reported to be higher among older women than older men (Koplan et al., 1986; Looker et al., 1988). However, a downward trend in supplement use has recently been reported by Bender et al. (1992) from data comparing the 1980 Food and Drug Administration study and the 1986 National Health Survey. This analysis revealed that supplement use among adults decreased significantly from 42 percent in 1980 to 38 percent in 1986. Nevertheless, other studies reported that supplement use was high among older adults (Bender et al., 1992).

Individuals self-reporting health problems have been reported to be more likely to use supplements. In a cohort study, Kim et al. (1993) reported that the elderly as a group were regular supplement users. Those persons on specialized diets were greater users than those on unrestricted diets. Nevertheless, no evidence was found for an association between supplement use and decreased mortality. However, Read et al. (1989) reported that the majority of supplement users believed that supplements decreased the susceptibility to and/or severity of health problems. Additional findings were that those who consumed nutrient supplements also consumed diets which were adequate in the nutrient(s) being supplemented (Payette and Gray-Donald, 1991b).

The excess intake of supplements is of great concern with respect to trace mineral interactions. One of the trace element interactions of significance in human nutrition is the negative effect of excess zinc on copper bioavailability (O'Dell,1989). Plasma zinc is responsive to zinc supplementation. In a 28-day zinc supplementation study that provided 30 mg per day to elderly subjects, plasma zinc concentration increased by 25 percent, but did not affect the platelet and leukocyte zinc content (Swanson et al., 1988).

Zinc supplementation has been shown to affect plasma zinc and indicators of copper status in both young and old men and women. Boukaiba et al (1993) reported that in elderly subjects serum zinc concentration increased by 20 percent after supplementing with 20 mg of zinc per day. A reduction in serum copper concentration was reported due to the negative effect of zinc supplementation. In adult females, zinc supplementation resulted in decreases in copper-zinc superoxide dismutase and serum ferritin which are indicators for copper and iron status, respectively. Use of iron with zinc lessened the effect of zinc on iron status (Yadrick et al., 1989).

The interaction between zinc and copper may be a reciprocal one, since low plasma zinc with zinc deficiency is often accompanied by high plasma copper (Turnlund, 1988). Copper deficiency was observed when a therapeutic level (150 mg/day) of supplemental zinc was used over an extended period (Prasad et al., 1978). In a case study of a male with sickle cell anemia who previously received 220 mg of zinc sulfate three times a day for seven months followed by 110 mg six times a day for 2 years, Prasad et al. (1978) reported a correction of hypocupremia, microcytosis, and increased leucocyte and neutrophil count with copper supplementation. Similar effects although to a lesser extent were observed with dietary zinc. Fischer et al. (1981) suggested that dietary zinc exerted its antagonistic effect by producing a copper binding ligand making copper unavailable for absorption. However, no effect was observed on iron status measures. Rossander-Hulten et al. (1991) reported that high dietary zinc did not interfere with iron

absorption, but manganese in the meal inhibited iron absorption suggesting a competitive inhibition by manganese on iron absorption.

Iron supplementation was also found to have a negative effect on serum zinc. Newhouse et al. (1993) reported that a therapeutic dose of 320 mg of elemental iron resulted in a downward trend in serum zinc and magnesium concentrations from the supplementation phase to the discontinuation phase. The values at 18 and 24 weeks were significantly lower than baseline concentrations.

The alteration of trace mineral status in the elderly, the interactive properties of trace minerals and the increased use of supplements supports the need for research to investigate the effects of supplementation on mineral status measures in elderly. The present study examined the zinc, copper, and chromium supplementation effects on trace minerals in plasma.

Purpose and Objectives

The purpose of this study was to determine the effect of zinc, copper, and chromium supplementation on zinc, copper, manganese and iron status in hypercholesteremic adults.

The following objectives were formulated for this study:

1. To determine trace mineral (iron, copper, zinc and manganese) concentrations in plasma in hypercholesteremic adults.
2. To determine the effect of trace mineral supplementation on the status indicators of zinc, copper, iron and manganese.
3. To determine the relationship between initial levels of zinc, copper, manganese and iron and the concentrations of these minerals in plasma during supplementation.
4. To determine the effects of withdrawal of supplements on measures of trace mineral status in hypercholesteremic adults.

Hypotheses

1. There will be no significant effect of chromium supplementation on status measures of zinc.
2. There will be no significant effect of chromium supplementation on status measures of copper.
3. There will be no significant effect of chromium supplementation on status measures of iron.
4. There will be no significant effect of copper supplementation on status measures of zinc.
5. There will be no significant effect of copper supplementation on status measures of copper.
6. There will be no significant effect of copper supplementation status measures of iron.
7. There will be no significant effect of zinc supplementation on status measures of zinc.
8. There will be no significant effect of zinc supplementation on status measures copper.
9. There will be no significant effect of zinc supplementation on status measures of iron.

Assumptions

1. It was assumed that the subjects consumed the supplements as per the directions.
2. It was assumed that the subjects fasted 12 hours before every blood collection.
3. It was assumed that the subjects did not alter their eating habits during the study period.
4. It was assumed that the subjects completely reported food intake and that these records reflected usual eating practices.

Limitations

1. Accuracy of the dietary records were limited to the ability of the subjects to estimate and record their food intake, and to the ability of researchers to interpret and code data.
2. The Food Processor Plus 5.03 used for the dietary analysis only estimated the intake of certain nutrients and does not contain values for chromium.
3. The sample group was not representative of the entire population; thus, the results are applied to this sample group only.
4. There was no control over the subjects' personal nutrient supplement intake.

CHAPTER II

REVIEW OF LITERATURE

This chapter includes reviews of research related to assessment of the nutritional status of the elderly, particularly related to certain trace elements. Supplement use among the U.S. population with emphasis on the trace mineral supplement use among the elderly is reviewed. In addition, reviews of the biochemical roles of zinc, copper, and chromium and their assessment measures are included. Trace mineral interactions of zinc/copper and zinc/iron are also included. Included in the methodological reviews are dietary and anthropometric assessments.

Supplementation

Extent of Supplement Use

Vitamin and mineral supplements are widely used in the United States. The 1987 National Health Interview Survey (NHIS), a collaborative study of the National Center for Health Statistics and the National Cancer Institute, reported that 51.1 percent of adults aged 18 to 99 years in the United States consumed a vitamin/mineral supplement in 1986, and 23.1 percent consumed supplements daily. The results of this study also suggested that there has been a change in supplementation practices since the 1970's. The most commonly used supplements in 1986 were multivitamins followed by vitamin C, calcium, and vitamins E and A (Subar & Block, 1990). In the reports from the Second National Health and Nutrition Examination Survey (NHANES II) data almost 35 percent of US population between 18 and 74 years of age took vitamin/mineral preparations

regularly. These supplementation practices were more common among older adults than younger people. The use of supplements was positively associated with older ages, high income, higher education level and white race (Koplan et al., 1986; Looker 1988).

Garry et al. (1982) conducted a 5-year longitudinal study on 304 elderly men and women over the age of 60 years living in the area of Albuquerque, NM. Fifty-five percent of the men and 61 percent of the women in this group ingested one or more vitamin or mineral supplements regularly. The supplemental intake of this sample population of elderly was higher compared to supplemental data obtained from national studies. Payette and Gray-Donald (1991a) reported that 43 percent and 62 percent of elderly Canadian men and women, respectively, consumed vitamin and/or mineral supplements during the preceding year.

Nutrient supplements were consumed for a variety of reasons. Bender et al., (1992) compared the 1980 Food and Drug Administration Vitamin and Mineral Supplementation Survey and the 1986 National Health Interview Survey. The results revealed that supplement use varied with the number of physical conditions. Use of supplements was directly proportional to the number of conditions. Nevertheless, supplement use was higher among individuals who considered their health to be excellent or very good rather than good, fair or poor (Bender et al., 1992).

In a cohort study, Kim et al., (1993) examined the relationship between the reported use of vitamin and mineral supplements and mortality. Between 1982 and 1984, they reinterviewed surviving members from the NHANES I study. At the baseline, the mean age was 50.2 years, and the subjects were followed for a period of 13 years. The use of supplements was highest among the oldest group, 65-74 years. More often, supplement usage was noted among persons on a special diet or who were alcoholics. Although subjects reported that they used supplements for health benefits, the authors concluded that supplement use was not associated with decreased mortality. In contrast, Read et al. (1989) assessed health status and food and nutrient beliefs using a

questionnaire. The results indicated that older individuals of ages 60-70 and above who were occasional and/or regular vitamin/mineral supplement users rated their health as excellent. The respondents of a supplement usage study by Park et al. (1991) also agreed that supplements reduced their susceptibility to and/or the severity of health problems.

Zinc Supplementation

In 1980, an estimated 13.5 percent of U.S. adults consumed nutrient supplements containing zinc. The mean intake from these supplements was fifty percent of the RDA. However, many individuals consumed zinc at levels approaching three times the RDA for this nutrient (Stewart et al., 1985). Zinc had been used therapeutically to correct other mineral deficiencies. Prolonged supplementation (2 years) with high levels of zinc (150-200 mg/day) in a patient with sickle cell anemia resulted in hypocupremia which was later corrected by copper supplementation (Prasad et al., 1978).

Biochemical and Physiological Roles of Zinc

Zinc plays a important role in certain physiological conditions. Maternal zinc is essential for normal growth of the fetus (Keen et al., 1993). There is a progressive decrease in plasma zinc during the gestation period which is not influenced by zinc intake (Hambidge et al., 1983). In a study in which pregnant women received multivitamin-mineral supplements containing 30 mg of zinc gluconate, plasma zinc decreased suggesting an increased requirement for zinc by the mother and by growth of fetus. They also reported an increase in polymorphonuclear zinc and a decrease in polymorphonuclear copper. Plasma zinc concentrations of the fetus were higher than maternal plasma zinc concentrations (Hambidge et al., 1983).

Depressed zinc status was reported among people with eating disorders. McClain (1992) suggested that zinc deficiency may be a sustaining factor for abnormal eating behavior. Serum and 24-hour urinary zinc values were lower than normal in these eating

disordered patients. Serum zinc was significantly depressed in individuals with bulimia and anorexia nervosa compared to healthy controls. Upon supplementation with 25 mg/day of zinc, serum zinc concentration and urinary zinc increased. In addition, hemoglobin and hematocrit concentrations decreased in supplemented groups. Dietary zinc intake was also reported to be lower for individuals with bulimia and anorexia nervosa prior to supplementation.

Zinc supplementation increased plasma zinc in patients with chronic inflammatory rheumatic disease. Supplementation with 45 mg of zinc (as zinc gluconate) led to an increase in plasma zinc from baseline concentrations. Nevertheless, there were no changes in erythrocyte or leucocyte zinc concentrations (Peretz et al., 1993).

Zinc supplementation might cause side effects. Side effects of zinc supplementation such as headaches, abdominal cramps, nausea, loss of appetite and vomiting were registered at concentrations of 50 mg of elemental zinc consumed over 12 weeks (Samman and Roberts, 1987).

Trace Mineral Status in Elderly

Zinc Status

Dietary Intake

The Recommended Dietary Allowance for zinc for males and females aged 51 and over are 15 and 12 mg/day, respectively (National Research Council, 1989). Nationwide surveys have provided information on dietary zinc intake of the people of all age groups. Dietary zinc intake for elderly has been reported to be less than two-thirds of the Recommended Dietary Allowances (Howarth, 1989; Dreosti, 1984; Greger, 1977 and Hutton and Hayes-Davis, 1983).

Several national and small studies reported a reduced intake of zinc in populations. Moser-Veillon studied the data from the Food and Drug Administration's

Total Diet Study, the U.S. Department of Agriculture's Nationwide Food Consumption Survey of individuals, and a year-long Beltsville diet study. She indicated that adult males selected diets containing 90 percent of the RDA for zinc, whereas, women selected diets containing less than 81 percent of the RDA for zinc. These data were from elderly between the ages of 60-65 years. According to the Total Diet study, women aged 60 to 65 years consumed a mean of 8.7 mg of zinc per day, and in men of the same age mean consumption was 12.9 mg per day (Pennington and Young, 1991).

Harland et al. (1980) analyzed the mineral content of adult diets based on published data from USDA's 1965 Household Food Consumption Survey. At an intake of 2,800 Kcal, zinc intake was 89 percent of the Recommended Dietary Allowance. When zinc intake was assessed based on the commodity group (market basket), the intake was 18.7 mg/day.

Zinc intake was reported to be lower in institutionalized Australian individuals between the ages of 60-99 years compared to independently living elderly (Flint et al., 1981). This study reported that institutionalized elderly consumed 7.6 ± 1.1 mg zinc whereas controls (non-institutinalized) consumed 11.0 ± 0.5 mg per day. Dietary intake of institutionalized elderly was also studied by Greger (1977). Both men and women had intakes of less than two-thirds of the RDA.

Several studies were performed to assess zinc intake and nutriture of non-institutionalized elderly. Fosmire et al. (1984) studied zinc intake in rural elderly individuals 65 years and older. The mean zinc intake of the whole sample was 9.8 ± 3.1 mg per day. The mean zinc intake of males and females was 11.6 ± 4.2 and 9.0 ± 2.2 mg per day, respectively. For the total group, the mean zinc intake was 5.87 mg/1000 kcal. The nutrient density for males and females was 5.98 and 5.81 mg per 1000 kcal. Bogden at al. (1987) studied the zinc nutriture in non-institutionalized elderly subjects aged 60-89 years. The dietary intake was found to be less than the RDA for both men and women, with a mean daily intake of 10.1 mg and 7.9 mg, respectively.

Greger and Sciscoe (1977) studied the zinc nutriture of elderly participants of an urban feeding program. From one-day dietary records they found that the mean daily dietary intake for men and women was 10.2 and 9.9 mg, respectively. In a study on elderly Canadian women (58-89 years) on intake of zinc, Gibson et al. (1985) reported that the mean intake was 7.6 mg/day.

The data from the above studies indicated a lower zinc intake in women compared to men. From the literature it can be concluded that dietary zinc intake in both institutionalized and non-institutionalized elderly is below the RDA. In addition, elderly women consumed less dietary zinc compared to men of the same age group.

Biochemical Measures of Zinc

Reduced zinc concentrations in plasma and other tissues have been reported in older adults (Pilch and Senti, 1985; Thomas et al., 1988; Wastney et al., 1992). Serum zinc concentrations, the only biochemical assessment of zinc status measured in NHANES II, changed with age and gender. Values were significantly higher in males (89.6 ± 0.53 ug/dL) than in females (84.0 ± 0.40 ug/dL). In males, the concentrations were low during childhood (80.6 ± 0.64 ug/dL), increased during adolescence (87.8 ± 0.71 ug/dL), peaked in young adulthood (93.0 ± 0.61 ug/dL), and then declined in older adults (89.1 ± 0.63 ug/dL) and in the elderly (85.6 ± 0.79 ug/dL). The authors suggested that the decrease could be due to a decreased release of zinc from erythrocytes with age (Pilch and Senti, 1985).

Several studies reported reduced plasma or serum zinc concentrations in females compared to males. Mean serum zinc was measured in elderly males with a mean age of 78 years and females with a mean age of 82 years. Younger subjects aged 22 years served as controls in this study. Mean serum zinc was significantly lower in independently living elderly males (mean 15.0 umol/L) compared to younger males (mean 17.24 umol/L). Mean serum zinc in elderly females was lower (mean 14.18 umol/L) than

younger females (15.36 umol/L), although these were not significantly different. Thus, irrespective of sex, serum zinc concentrations were lower in older compared to younger subjects. In addition, both younger and older males had higher serum zinc concentrations than women. However, this gender difference was significant only between the younger controls (Rea, 1989). Goode et al. (1991) found a similar trend in serum and plasma zinc. They found plasma zinc to be lower in both healthy (10.8 umol/L) and hospitalized elderly (10.5 umol/L) compared to a younger control group (12.7 umol/L).

Due to the reduced dietary zinc intake in the elderly, mild zinc deficiency appears to be a significant clinical problem. Lindeman et al. (1971) measured plasma zinc concentrations in males (20-84 years) and females (20-58 years). A significant decrease in plasma zinc concentration with increasing age was reported in both males and females. However, plasma zinc concentrations were found to be significantly lower in males than in females. The authors did not report the concentrations; however, they gave regression equations to calculate plasma and red cell concentrations for males and females of different age groups based on the plasma zinc concentration in micrograms per deciliter.

Bunker et al. (1984a) conducted a zinc metabolic balance study with twenty-four healthy elderly people aged 69.7 to 85.5 years. Individuals younger than the study group served as controls. Elderly males had plasma zinc concentration of 10.9 umol/L as compared to 11.0 umol/L in females. The plasma zinc concentrations of the elderly as a whole group was significantly lower (11.0 ± 1.2 umol/L) than the younger controls (13.0 ± 1.8 umol/L). These results suggested that there is a significant difference between the age groups in plasma zinc concentrations. However, no significant differences were reported in whole blood and leucocyte zinc concentrations between elderly and young adults (Bunker et al., 1984a).

Plasma zinc response to the same zinc load was reported to be higher in older than younger subjects indicating a change in zinc metabolism with age (Wastney et al., 1992). This kinetic study assessed changes in zinc metabolism with age in healthy adults aged

20-84 years after giving a single oral or intravenous bolus of ^{65}zinc. The study consisted of two consecutive 9 month periods. The first period consisted of a normal diet containing 10 mg of zinc/day; the second period consisted of a normal diet with 10 mg of zinc and an additional 100 mg zinc/day orally. Plasma zinc concentration increased due to zinc loading (100 mg/day) over the basal concentration in both older and younger subjects. This response was significantly related to age with the response being higher in older than younger subjects. Plasma zinc and urinary zinc were used in regression equations to estimate absorption. Zinc excretion was fivefold greater in older than in younger subjects. In another study by Bales et al. (1986), plasma response to the same zinc load was reported to be lower in elderly compared to younger subjects. The elderly (mean age 72.5 years) and the younger controls (mean age 24 years) were given 25 mg of zinc orally as zinc gluconate and plasma zinc was measured at 0, 1, 2, 3, and 4 hours post dose. At 2 and 3 hours, mean plasma zinc concentrations were significantly lower in elderly than in younger subjects. Total area under the response curve was significantly lower for elderly than for younger subjects suggesting a reduced uptake of zinc in elderly compared to the young. This difference in response to zinc load could be due to a small dose of 25 mg compared to 100 mg in the study conducted by Wastney et al. (1992). In addition, this dose was given one time, compared to the nine months of supplementation by Wastney et al. (1992).

Zinc utilization changes with age. A 30 day metabolic study with elderly subjects aged 56-83 years showed a negative zinc balance upon zinc supplementation with either 7.80 or 23.26 mg of zinc in addition to 6.03 mg of dietary zinc (Burke et al., 1981). Thus, a total of 29.09 mg of zinc still led to a negative balance in these elderly subjects. Zinc intake did not affect zinc retention. However, in another study, 15 mg of zinc fed in a metabolic study was sufficient to maintain balance in men aged 65 - 74 years (Turnlund et al., 1981).

Food consumption also seems to affect plasma zinc concentration. Plasma zinc concentrations decline with the intake of a meal. In a study of premenopausal women aged 37 ± 8 years, Wallock et al. (1993) studied the response of plasma zinc to meal trials. Seventeen hour fasting increased plasma zinc concentrations by 9 percent from 12.5 ± 0.44 to 13.6 ± 0.6 umol/L. Plasma zinc concentrations decreased by 11 percent from 12.7 ± 0.3 to 11.3 ± 0.2 umol/L in one meal trial compared to 18.5 percent decrease from 12.6 ± 0.4 to 10.6 ± 0.2 umol/L in three meal trial. However, erythrocyte and erythrocyte membrane zinc did not change significantly with meal feeding (Wallock et al., 1993). Couzy et al (1993) did not find any effect of diet on zinc absorption in elderly subjects aged 70 to 83 years. A stable isotope label (0.8 mg ^{70}Zn) was added to a test meal of high or low zinc bioavailability based on phytic acid content. Their results showed that aging did not lead to nutritionally relevant changes in zinc absorption nor in the effects of dietary inhibition of zinc absorption. Serum zinc concentrations did not differ between these elderly subjects and younger controls. Thus, the ability to absorb zinc seems to be preserved in healthy elderly people, at least until 80 years of age. However, a study by Turnlund et al. (1986) suggested a decreased absorption of zinc with age. They determined zinc absorption with ^{67}Zn and ^{70}Zn stable isotopes in six young men (22-30 yr) and six elderly men (65-74 yr) in a 12 week metabolic study. The purified formula diet supplemented with selected food items contained 15 mg of zinc per day was fed. Serum, urinary, and fecal zinc, and zinc balance was determined by atomic absorption spectrophotometry. Zinc absorption was significantly less in elderly at 17 percent compared to younger men at 31 percent suggesting decreased zinc absorption with age.

Zinc absorption responds to changes in dietary zinc. In a 75-day metabolic study on young men, absorption was 25 percent when 16.5 mg zinc was fed. Zinc absorption increased to 53 and 49 percent after 13 and 42 days, respectively, when 5.5 mg zinc was fed. However, total zinc absorption decreased from 4.1 to 2.78 mg/day during low zinc

period (Wada et al., 1985). Even though the percent absorption is greater with low zinc diet, the total amount absorbed is also reduced and may compromise zinc status.

Plasma and serum zinc

There is currently no specific sensitive laboratory or functional indicator for zinc status. There is little functional zinc reserve in the body. The body's zinc concentrations are maintained by both conservation and redistribution of tissue zinc. In mild zinc deficiency, conservation is manifested by reduction or cessation of growth in growing organisms and by reduced excretion in mature organisms (King, 1990).

Plasma zinc frequently has been used to assess zinc nutriture by determining circulating zinc concentrations (Solomons, 1979). Plasma zinc responds to supplemental zinc intake. Field et al. (1987) supplemented elderly individuals with 50, 100, and 150 mg of elemental zinc daily for 28 days. As a result, plasma zinc increased with the 100 and 150 mg/day doses in these individuals. Supplementation of 100 mg of zinc led to a significant increase in plasma zinc from baseline (10.31 ± 2.38 umol/l) to the end of the study (11.98 ± 2.43 umol/l). Similarly, a significant increase in plasma zinc was reported due to supplementation with 150 mg of zinc/day from baseline (11.83 ± 1.70 umol/l) to the end of the study (17.76 ± 5.15 umol/l).

Zinc nutriture is reflected through plasma and serum zinc. Experimental zinc deficiency induced in human volunteers was accompanied by a progressive decrease in circulating zinc (Baer & King, 1984; Hess et al., 1977). A 10-week confined metabolic study was performed on six healthy young male volunteers by Baer and King, (1984). Subjects were fed a semisynthetic liquid diet providing only 0.28 mg of zinc/day during a 5-week depletion period. This was followed by a repletion period during which only three of the subjects were fed zinc at levels of 6.0, 23.2, or 46.3 mg per day. A significant decrease in plasma zinc was reported from pretreatment (89 ± 17 ug/100g) to end of the depletion period (50 ± 20 ug/100g). During a one week repletion period,

plasma zinc increased by 260 percent. The increase was significant from 36 ± 21 ug/100g to 93 ± 5 ug/100 g. However, the increase after an additional week of repletion was not significant (Baer and King, 1984).

In another study zinc excretion under conditions of negligible zinc intake (0.17 mg/day) was measured in women taking a combination oral contraceptive agent (+OCA) and in women with normal menstrual cycles (-OCA). In this 35 day study, serum zinc dropped significantly by 47 percent (from 84.5 ± 11.1 ug/dL to 44.9 ± 12.7 ug/dL) in the +OCA group and by 21 percent (from 80.8 ± 14.4 to 63.5 ± 22.3 ug/dL) in the -OCA group. A significant decline in urinary zinc was also reported in both groups. Urinary zinc declined to 83 (0.06 mg/day) and 62 percent (0.15 mg/day) in both the +OCA and -OCA groups. Subjects in both groups had initial urinary zinc excretion within the normal range (140 - 780 ug/day). It was noted that the higher the initial level of zinc in urine, the greater the decline during the study. Although not significant, mean urinary zinc was lower in the +OCA group compared to the -OCA group. Whole body zinc losses as measured by urinary and fecal zinc were similar for both groups. These data suggested that accessible zinc stores are not extensive, and the depletion of storage zinc due to a low-zinc diet results in a decrease in serum zinc (Hess et al., 1977).

Decreases in serum zinc concentrations was observed in patients receiving total parenteral nutrition without trace mineral supplementation (Fleming et al., 1976). Serum zinc was monitored from 3-13 weeks in patients receiving total parenteral nutrition. Three out of eight patients showed a significant decline in serum zinc to less than 70 ug/dL for at least 2 consecutive weeks. Further decline in serum zinc was found in three patients after resumption of oral zinc.

Effect of Protein on Zinc

Dietary protein has been positively associated with zinc absorption (Sandstrom et al., 1980, 1989). Several metabolic studies were conducted to study the effect of protein

on zinc. Zinc absorption from a composite meal was studied in adult women by Sandstrom et al. (1980). Zinc absorption from a meal with bread was measured with a radionuclide technique using ^{65}zinc and whole-body counting. The bread was prepared from 100 and 72 percent extraction wheat and enriched with zinc chloride. A significant positive correlation was found between zinc absorption and protein content. Total zinc absorption from white bread containing 0.4 mg zinc was significantly lower than from wholemeal bread containing 1.3 mg zinc.

Another study was conducted to investigate the effects of increasing levels of various protein sources on zinc absorption from a legume-based meal. The basal test meal consisted of cooked white beans (35 grams of dry wt), 100 g of white bread and tomato sauce. The basal diet contained 15 g of protein and 25 umol of zinc. The protein level was increased to approximately 20 and 30 g by adding protein sources such as chicken, fish, beef, or milk products. One test meal had 44 g protein. The results indicated that the amount of zinc absorbed from the meals was positively correlated with protein and zinc content. Zinc absorbed from the basal meal with the lowest protein content was 5.9 ± 1.7 umol, and zinc absorption increased to 10.3 ± 2.0 umol from the test meal with chicken (doubled protein content and low zinc). However, zinc absorption was even higher at 15.9 ± 4.7 umol from the diet with the addition of beef (doubled protein content and high zinc). These differences in zinc absorption were significant. Thus, the authors concluded that the zinc content of the main protein source affects zinc absorption (Sandstrom et al., 1989). Similar results were observed in rats (Hunt and Johnson, 1992).

Greger and Snedeker (1980) conducted a 51 day balance study using adult males to investigate the effect of different levels of protein on zinc status. There were four treatments with high and low levels of protein and phosphorus. The low and high protein consisted of 8.1 g and 24.1 g of nitrogen respectively. The moderate and high phosphorus diets contained 1,010 mg and 2,525 mg of phosphorus. Subjects on high

protein diets lost significantly more zinc in the urine compared to subjects on low protein diets. However, phosphorus level did not affect urinary zinc. Subjects fed high protein diets had higher serum zinc concentrations, and protein was positively correlated with zinc losses. Colin et al. (1983) found a similar effect of protein on zinc absorption in females. They studied the effect of protein on fecal zinc levels in a metabolic balance study. The study consisted of four periods of 6 hours each. Subjects were fed moderate and high protein in combination with low or high zinc. Similar to the findings of Greger and Snedeker (1980), they also found significantly greater urinary losses in subjects consuming high protein diets (0.38 ± 0.12 mg/day) compared to subjects fed low protein diets (0.27 ± 0.12 mg/day). However, zinc retention was not affected by protein level. No significant differences were found in fecal zinc excretion with intake of 50 g or 100 g of protein per day (Colin et al., 1983).

Similarly, no effect of protein on fecal zinc was reported by Mahalko et al. (1983) from a metabolic balance study. Male volunteers (19-64 years) participated in this study. Subjects were fed two different diets containing 65 and 94 g of protein with similar mineral composition. Both diets contained approximately 7 mg of zinc. Similar to the other studies, subjects on high protein diet had significantly higher urinary zinc losses compared to the subjects on low protein diet (Mahalko et al., 1983).

Zinc and Serum Alkaline Phosphatase

Alkaline phosphatase is a zinc metalloenzyme found in bone, placenta, and intestine. Elevated concentrations were found in conditions involving increased deposition of calcium in bone and certain liver diseases. Low alkaline phosphatase concentrations usually are not clinically significant (Lee and Neiman, 1993; pg 244).

Increased alkaline phosphatase activity was demonstrated in response to zinc supplementation in adults and young children. Adult pregnant women receiving 15 mg of supplemental zinc (zinc sulfate) daily during pregnancy had higher alkaline phosphatase

activity compared to non-supplemented controls (Hambidge et al., 1983). In another study by Udomkesmalee et al. (1992) young children aged 6-13 years supplemented with 25 mg of zinc/day for six months also had significantly increased alkaline phosphatase activity over baseline.

Studies have shown that serum alkaline phosphatase concentrations vary with season. McKenna et al. (1985) studied vitamin D status in relation to alkaline phosphatase concentration in elderly Irish people. A higher serum alkaline phosphatase was found in vitamin D depleted groups compared to vitamin D replete group. In addition, they also found a decrease in serum alkaline phosphatase activity with seasonal rise in 25-hydroxyvitamin D levels. Thus it was hypothesized that serum alkaline phosphatse varies with seasons, the values being lower in summer compared to winter.

Copper Status

Dietary Intake

The Food and Nutrition Board recommends 1.5 to 3.0 mg of copper a day as a safe and adequate range of dietary intake for adults (National Research Council, 1989). Klevay et al. (1979) conducted a metabolic ward study on 13 men consuming a variety of typical U.S. diets. To replace fecal and urinary losses, they found 1.3 mg of copper/day were needed. However, Turnlund et al. (1981) found copper intakes at a level of 3 mg a day fed in a metabolic study were sufficient to maintain copper balance in elderly men aged 65-74 years.

Studies have shown inadequate dietary intake in both institutionalized and non-institutionalized elderly. According to the Total Diet Study, women aged 60 to 65 years had a copper intake of 0.86 mg per day as compared to 1.18 mg for men, aged 60 to 65 years (Pennington and Young, 1991). In another study conducted to assess the dietary copper intake of elderly of age range 65-69 years (Howarth, 1989), a self-completed semi-quantitative food frequency questionnaire was used to collect the dietary intake

information. The mean dietary copper intake for men was 1.9 mg, and 1.74 mg/day for women. Both the men and women consumed below two-thirds of the ESADDI for copper.

Bogden et al. (1987) reported copper intakes below the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for copper in elderly men and women aged 60-89 years recruited from a senior citizen center. Consumption of copper for men was between 1.17 mg and 1.33 mg, and for women between 0.86 mg and 1.19 mg per day. In a metabolic balance study, copper levels in self-selected diets of subjects aged 14 to 64 years were analyzed by atomic absorption spectrophotometry (Holden et al., 1979). Each subject was asked to maintain a fourteen-day food record for the items eaten as well as the linear measure, volume, and weight. Information about brand names and recipes were also recorded. Foods and beverages were collected separately without contamination. Homogenized food was used for analyses. The mean daily copper intake was below 2 mg/day. The majority (81 percent) of the subjects consumed less than two-thirds of this recommended amount. However, there was no significant difference in copper intake between men (1.2 mg/day) and women (0.9 mg/day). The mean copper:calorie ratio for both males and females was 0.50 mg per 1,000 kcal as compared to the suggested 0.85 mg per 1,000 kcal. Thus, diets supplying adequate amounts of energy do not guarantee adequate levels of copper (Holden et al., 1979). Bunker et al. (1987) also reported lower copper intakes in house-bound elderly consuming self-selected diets.

In institutionalized elderly copper intake was also reported to be below the ESADDI (Pennington and Young, 1991; Dreosti et al., 1984; Thomas et al., 1988; Howarth, 1989). Thomas and co-workers (1988) conducted dietary analyses of twenty-one patients in a geriatric rehabilitation unit with a mean age of 81.7 years. The food intake for a period of five days was reproduced exactly including the hospital meals, sweets, medicines, and any food brought by the visitors. Leftover food was collected as "rejects" and used for analysis. Food was collected in trace mineral free plastic

containers. Blood was collected between 09.00 and 10.00 hours. The meals provided 18 umol (1.14 mg) of copper if consumed completely. This amount was significantly less than the ESADDI for this age group.

Biochemical Measures of Copper Status

Serum copper and ceruloplasmin concentrations are frequently used by researchers as indicators of copper status. Age and sex influence serum copper concentration (Yuncie et al., 1974). A significant increase in serum copper was observed with increasing age in male subjects, ranging in age from 20 to 89 years. However, this effect was not observed in females of the same age range. Age had no effect on serum ceruloplasmin concentrations, but the concentration was higher in females than in males.

Bunker et al. (1984a) conducted a metabolic balance study using elderly subjects, 76 to 78 years of age, with a younger group as controls. Duplicates of self-selected diets, feces, and urine were collected over a 5-day period into trace mineral free containers. The mean copper concentrations in blood for men and women were 17.8 and 20.7 umol/L respectively. Mean serum ceruloplasmin was 330 ± 80 mg/L for the elderly subjects compared to 310 ± 55 mg/L for the control group; however these concentrations were not significantly different. Mean plasma copper concentrations of the elderly were significantly higher (19.4 ± 3.4 umol/L) compared to younger controls (16.4 ± 2.4 umol/L).

The amount of copper in the diet can affect copper absorption. August et al. (1989) studied copper absorption from natural diets containing 3 mg of copper in young and elderly men and women. Copper absorption was 60 percent in younger subjects and 53 percent in the older ones. Absorption increased to 67 percent in older individuals when a diet low in copper (0.5 mg) was given.

Serum ceruloplasmin was used as an indicator of copper status in copper-supplemented rheumatoid arthritis (RA) patients by DiSilvestro et al. (1992). Twenty-

three RA patients age range 35-53 years received medication to treat their disease. Healthy adults served as controls of this study. Subjects and controls were supplemented with 2 mg of copper for a period of four weeks. Copper supplementation resulted in a significant increase in erythrocyte copper superoxide dismutase in both patients and controls. Supplementation did not have any significant effect on serum ceruloplasmin in either group; although, arthritic patients had significantly higher serum ceruloplasmin concentrations at both pre-supplementation (41 ± 10 mg/dL) and post-supplementation (43 ± 16 mg/dL) compared to controls at pre-supplementation (29 ± 7 mg/dL) and at post-supplementation (29 ± 9 mg/dL). The hypothesis for this difference in serum ceruloplasmin concentrations was that arthritis patients have marginal copper status, and ceruloplasmin synthesis is higher due to inflammation. In addition, the dosage and duration of supplementation may have been too low and too short to have a significant effect on serum ceruloplsamin. A similar non-response of ceruloplasmin to copper supplementation was reported by Pratt and coworkers (1985) in normal adult subjects. Their study was a double-blind placebo trial with seven subjects with mean age of 42 years. Subjects either received a placebo or 10 mg of copper/day as copper gluconate for 12 weeks. Supplementation did not have any significant effect on serum, urine, or hair copper concentrations. The urinary copper was 126 ± 22 ug/100 ml before copper supplementation; and 123 ± 16 ug/100 ml after supplementation. Serum and hair copper concentrations were not provided by the authors. However, the lack of response could be explained by the fact that the sample size was too small to detect any effect due to supplementation. (Pratt et al., 1985)

Chromium Status

Dietary Intake

The current recommendation of the Food and Nutrition Board is 50 to 200 ug chromium per day (National Research Council, 1989). Studies estimating chromium

intake in the United States reported lower intakes than the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) (Offenbacher et al., 1986; Anderson and Kozlovsky, 1985; Bunker et al., 1984b).

Several metabolic studies assessed the chromium intake of adults. Anderson and Kozlovsky (1985) conducted a metabolic study using adults of (20-65 years) in Beltsville, Maryland. Duplicates of self-selected diets were collected for a period of seven days. The homogenized samples were analyzed by atomic absorption spectrophotometry. Average analyzed daily chromium intake was 33 ug and 25 ug for males and females, respectively. Lower chromium intake of females could be due to females' lower total energy intakes although the chromium density was greater in females than in males. Caloric intake of males and females was 2,300 and 1,600 Kcal, respectively.

Similar results were obtained from another metabolic study on the elderly. Offenbacher et al. (1986) conducted a metabolic study using two elderly normal male subjects aged 62 and 66 years. They were placed in a metabolic unit for 12 days (two 6-day periods). Subjects' diets were analyzed using atomic absorption spectrophotometry. The average dietary chromium intake of the two subjects was 36.9 and 36.7 ug/day, respectively, which was below the ESADDI.

Bunker and co workers (1984b) conducted a metabolic balance study on healthy elderly people ranging in age from 70 and 85 years, eating self-selected diets. Ashed duplicate diets were analyzed by atomic absorption spectrophotometry. The mean daily chromium intake for males and females was 29.8 ug/day and 20.1 ug/day, respectively. The intake was below the Estimated Safe and Adequate Daily Dietary Intake for chromium.

Biochemical Measures of Chromium Status

Reported plasma chromium concentrations of the elderly are not different from young adults. Offenbacher (1992) reviewed different research studies on chromium including, supplementation trials, monitoring studies, balance studies, and animal studies. She suggested that chromium retention decreases with age suggesting an altered chromium metabolism.

The effect of chromium supplementation on chromium status was studied by Anderson and co-workers (1985) using adult subjects (21-69 years). Subjects were given either a placebo or 200 ug of inorganic chromium (chromium chloride) daily for 3 months in a double-blind cross-over study. After three months of supplementation serum chromium significantly increased three-fold from 0.13 ± 0.02 ng/ml to 0.38 ± 0.02 ng/ml. However, serum chromium was not significantly different between males and females.

Chromium absorption from meals with chromium content found in typical U.S. diets was inversely related to dietary intake. The 7-day average intake from self-selected diets of adults was well below the minimum suggested safe and adequate intake. Anderson and Kozlovsky (1985) studied chromium metabolism in adults (25-65 years) consuming self-selected diets. Duplicates of food and beverages were collected daily for a period of seven days. An inverse relationship between chromium absorption and chromium in the diet was found. Absorption at a dietary intake of 10 ug was approximately two percent and absorption further decreased to 0.5 percent from an intake of 40 ug. However, urinary chromium excretion was not related to dietary chromium intake. Fecal chromium was not measured in this study (Anderson and Kozlovsky, 1985).

The effect of chromium supplementation on urinary chromium excretion was reported by Anderson et al. (1982). Supplementation with 200 ug of chromium for 2 months resulted in a significant increase in urinary chromium. Subjects had a mean initial

urinary chromium concentration of less than 0.3 ng/ml which increased significantly to 1.02 ng/ml after two months of supplementation. However, an additional month of supplementation did not further significantly increase urinary chromium. The non-supplemented subjects had significantly lower urinary concentration compared to supplemented subjects. A five-fold increase in supplementation corresponded to a five-fold increase in urinary chromium excretion. Thus, the authors suggest that urinary chromium excretion might be a useful indicator of intake but not status (Anderson et al., 1982).

The effect of zinc status on chromium uptake was documented in rats by Hahn and Evans (1975). When rats were given test doses of zinc and chromium, the zinc deficient rats showed a chromium uptake of five-fold compared to zinc supplemented rats suggesting that chromium and zinc share a common absorption pathway. Thus, when rats were zinc deficient, chromium was better absorbed.

Trace Mineral Interactions

This section reviews trace mineral interactions. Interactions of zinc-copper, zinc-iron, and copper-iron are discussed.

Interaction between Zinc and Copper

The negative effect of excess zinc on copper bioavailbility has been identified as one of the significant trace element interactions (O'Dell, 1989). Early animal studies reported that high dietary zinc induced copper deficiency which was reversed by copper supplementation (Magee and Matrone, 1960).

Boukaiba et al. (1993) found a significant effect of zinc supplementation on serum copper in institutinalized elderly. In a 16-week crossover study, subjects were supplemented with 153 umol of zinc for eight weeks and later with placebo for another eight weeks. After zinc supplementation, serum copper was significantly lower (16.9 ±

1.10 umol/L) compared to post placebo period (20.3 ± 0.87umol/L). The mean baseline serum copper concentration of the total group was 21.3 ± 0.64 umol/L, suggesting the significant decrease in serum copper was due to zinc supplementation.

Therapeutic doses of zinc have been reported to cause copper deficiency in different age groups. Botash et al., (1992) reported a case study of a thirteen month-old who was hospitalized with a fever of 40.8°C. She was anemic with poor weight gain and abnormal sparse hair. The mother and the other sibling had a history of Acrodermatitis Enteropathica and were treated with zinc gluconate. However, this child did not have any such history. In spite of normal baseline serum zinc levels and no clinical signs of Acrodermatitis Enteropathica, at the age of six months she was given oral zinc gluconate (16 mg elemental zinc/d) and increased (24 mg of elemental zinc/day) one month prior to hospitalization. Bone marrow findings upon admission suggested copper deficiency. The subject's plasma zinc concentration was 36.7 umol/L (normal range 9.2 to 19.9 umol/L), serum ceruloplasmin level was 20 mg/L (normal range 230 to 580 mg/L), and serum copper was undetectable (limit of detection was 1.6 umol/L). Copper therapy of 600 ug/day was initiated and continued for 4 days. The patient was discharged and treated with 600 ug copper chloride per day. Serum zinc decreased to normal values after one year of copper treatment, whereas serum ceruloplasmin and serum copper reached the normal range within two months of the therapy (Botash et al., 1992).

Several other case studies have also reported a negative influence on copper with the intake of therapeutic levels of zinc (Prasad et al., 1978; Hoogenraad et al., 1978; Hoogenraad et al., 1984; Hoffman et al., 1988, Patterson et al., 1985, and Gyroffy and Chan, 1992). Hoffman et al., (1988) described a case study of a thirty-five year old female patient with a gastric ulcer who received daily doses between 440-660 mg of zinc (as zinc sulfate) for ten months in addition to a daily multi vitamin-mineral preparation containing 80 mg of zinc sulfate. Serum zinc concentration more than doubled to 1.44 ug/dL from baseline (0.63 ug/dL) and serum copper concentration decreased to 0.15

ug/dL from baseline (0.6 ug/dL). Serum ceruloplasmin was not detectable (normal 22.9-43.1 mg/dL). This zinc therapy also increased the excretion of zinc in urine ten times normal (normal 15-60 ug/24 hrs). A 10 mg intravenous preparation of cupric chloride given over a five day period restored ceruloplasmin levels to normal concentrations. Serum ceruloplasmin levels increased to 19.5 mg/dL after two weeks and to 35 mg/dL after six months of supplementation; however, urinary zinc excretion remained high for the next three months.

Copper deficiency occurred in a fifty-seven year old man with fatigue and microcytic anemia who ingested 810 mg of elemental zinc/day for eighteen months. Serum zinc was more than 3000 ug/L (normal 550-1400 ug/L), serum ceruloplasmin was 23 mg/dL (normal 70-155 mg/dL), and urinary copper was <10 ug/24 hours (normal 15-150 ug/24 hours). After discontinuing the zinc supplements, serum copper and ceruloplasmin increased to normal concentrations; after 8 weeks, serum copper increased significantly to 134 ug/dL and ceruloplasmin increased significantly to 32 mg/dL. Anemia and hypocupremia were corrected after discontinuation of zinc (Gyorffy and Chan, 1992). In another case study of a 57 year old man Patterson et al. (1985), reported similar changes in copper status after discontinuing zinc supplements. This person also had symptoms of easy fatigability and anemia. The subject took zinc supplements of 450 mg/d for two years with 2000 ug/d of vitamin B-12 five weeks prior to admission. His diagnosis was sideroblastic anemia resulting from copper deficiency. Upon admission, zinc supplements were discontinued. By eighty-three days serum zinc, copper, and ceruloplasmin concentrations were within normal ranges. There was a decrease in serum zinc concentration to 110 ug/dL (normal 50-160 ug/dL); serum copper increased to 104 ug/dL (normal 70-90 ug/dL); and ceruloplasmin increased to 37 mg/dL (20-35 mg/dL) (Patterson et al., 1985).

Samman and Roberts (1988) found gender differences in the effect of zinc on copper status indicators in young adults. Supplementation with 660 mg of zinc sulfate

per day for six weeks increased plasma zinc in both males and females. However, reduced ferroxidase activity, the name proposed for ceruloplasmin, and erythrocyte superoxide dismutase (E-SOD) antioxidant activity was found in female subjects only. In a separate study with adult men, Fischer et al., (1984) gave 50 mg of zinc for six weeks resulting in significant increases in plasma zinc. Plasma copper and serum ferroxidase activity (ceruloplasmin) were not altered by zinc supplementation, but erythrocyte superoxide dismutase activity decreased at six weeks in zinc treated subjects but not in controls. Similar findings were reported by Yadrick et al., (1989) in a study using adult women. Zinc supplementation of 50 mg/day for 10 weeks did not affect serum ceruloplasmin concentration, but E-SOD levels declined significantly after six and ten weeks of supplementation. The levels were significantly lower compared to pretreatmant levels. Thus, conflicting results have been reported for gender differences in the effect of zinc supplementation on indicators of copper status.

Festa et al. (1985) also found a negative effect of zinc intake on copper retention in adult men. Subjects consumed diets containing 2.6 mg of copper/day in addition to 1.8, 4.0, 8.0, 18.5 or 20.7 mg of zinc per day for one or two week periods in a 63-day study. Copper retention and fecal copper were influenced by the level and duration of zinc intake. An intake of 18.5 mg of zinc/day for two consecutive weeks resulted in increased fecal copper and reduced copper retention even with a copper intake of 2.6 mg daily. This excretion was significantly higher from all the other periods and exceeded intake, leading to negative copper balance. Copper excretion was not significantly altered at other tested levels (Festa et al., 1985).

Zinc supplementation (24 mg per day) did not affect serum copper concentrations in a short term study in adult women. Taper et al., (1980), found that supplemental zinc did not affect serum zinc or copper. They gave either 8, 16 or 24 mg of zinc along with 2 mg of copper for 12-days and found neither an increase in serum zinc concentration nor a decrease in serum copper concentration. However, all the subjects were in negative

copper balance as determined by copper retention and urinary excretion of copper at the end of the study.

The effect of low dietary zinc on copper status was assessed in a 24 hour metabolic balance study in young adult females by Colin et al. (1983). Subjects were given different diets with (18.4-19.9 mg/day) zinc and low (9.5-10.1 mg/day) zinc along with either a high (nitrogen intake 15.2 g/day) or a moderate protein (nitrogen intake 7.9 g/day) diet with a constant copper intake of 2.0 mg. Dietary zinc did not affect copper retention or excretion. Plasma copper was reported to be within the normal range of 87-153 ug/100 ml. However, women consuming the high protein - low zinc diets had higher plasma copper levels at the end of the study than did moderate protein - high zinc diets, but the difference was not significant. Changes in measures of copper status were not significant for any treatment group.

The amount of dietary copper may cause changes in copper status in response to zinc supplementation. Greger et al. (1978a) found different results in two different metabolic studies. No differences in fecal copper excretion or copper retention occurred when 7.4 or 13.4 mg of zinc and 2.9 mg of copper/day were fed to 14 adolescent girls for 18 days. All the subjects were found to be in positive copper balance (Greger et al., 1978a). However, in a different metabolic study with adolescent girls when dietary copper was reduced to 1.2 mg/day, they found a significantly higher fecal copper excretion and reduced copper retention when fed 14.7 compared to 11.5 mg of zinc/day during 10-day periods (Greger at al., 1978b). The results from the above studies suggest that dietary copper intake at the lower level of ESADDI inhibited the effect of zinc supplements on copper status during short term metabolic studies.

Negative effects of zinc on copper were reported in studies with a high ratio of zinc to copper in rats. Van Campen (1966) reported that negative effects of zinc on copper could be found only with a very high zinc and copper ratio (500:1). At this high ratio of Zn:Cu, a significant depression of approximately 60 percent occurred in copper

uptake from the stomach and duodenum in rats. However, at a ratio of 50:1, copper absorption was not altered.

Negative effects of zinc on copper have been used to treat diseases like Wilson's disease (copper toxicity). In two separate case studies, patients with severe neurological symptoms due to Wilson's disease were corrected by zinc therapy. Subjects were treated with 300 mg of zinc three times a day. Plasma copper and ceruloplasmin concentration decreased and plasma zinc concentration increased during the treatment (Hoogenraad et al., 1984). In another case study of a Wilson's disease patient, Hoogenraad et al. (1978) examined the influence of oral zinc sulfate. The patient had very high urinary copper excretion of 350 ug/24 hours (normal 5-25 ug/24 hours), low serum copper 50 ug/dL (normal (80-140 ug/dL), and low serum ceruloplasmin concentration of 9 mg/dL (normal 25-43 mg/dL). The patient was supplemented with 600 mg zinc along with 1.6 mg dietary copper daily for 3 months. Urinary copper decreased to 100 ug/24 hours, serum copper to 35 ug/dL, and serum ceruloplasmin to 4.5 mg/dL. The authors suggested that zinc treatment not only prevented further tissue copper accumulation but also contributed to the gradual removal of deposited copper. However, serum copper and ceruloplasmin which were below normal ranges before treatment continued to decrease further below normal ranges.

Interaction between Zinc and Iron

Changes in iron metabolism due to excessive zinc may result from both an indirect multi-element interaction of zinc:copper:iron. A direct antagonistic interaction is found between zinc and iron (Kirchgessner et al., 1982). Inorganic zinc impairs the intestinal absorption of iron (Crofton et al., 1989). They conducted an absorption test on healthy adult males to study the effect of zinc on iron. The study consisted of two parts. The first part was the administration of two test solutions: one had 842 umol of elemental iron, and the other solution had 344 umol of elemental zinc in addition to the same

amount of iron. The second part of the study consisted of three solutions with 421 umol of elemental iron in the first solution, 421 umol of zinc along with iron (Fe/Zn 1:1) in the second solution, and 1048 umol of elemental zinc with iron (Fe/Zn 1:2.5) in the third solution. Co-administration of zinc did not affect radiolabeled iron absorption at three or six hours, total plasma iron, or iron retention. In the second part plasma iron declined significantly when the second solution was given (Fe/Zn 1:1). Plasma iron further decreased significantly when the ratio was increased to 1:2.5.

The interaction of zinc and iron in the intestine of healthy human adults was studied by Solomons and Jacob (1981). The change in plasma zinc concentration after oral zinc administration was used as the index of zinc absorption. The standard zinc dose was 110 mg as zinc sulfate dissolved in Coca-Cola and ingested either alone or with non-heme iron at doses of 25, 50, or 75 mg. These solutions represented Fe/Zn ratios of 1:1, 2:1, and 3:1. In this study a ratio of Fe/Zn of 1:1 slightly inhibited zinc absorption while Fe/Zn ratios of 2:1 and 3:1 substantially inhibited zinc uptake. However, when heme iron (heme chloride) was given at a ratio of 3:1 Fe/Zn and zinc in an inorganic form, iron did not have an effect on plasma zinc. Absorption of the organic form of zinc (54 mg provided by oysters) with or without 100 mg of ferrous iron (Fe/Zn ratio 2:1) was not affected by iron. Thus, the results revealed that the competitive interaction of zinc and iron was strongest between nonheme iron and inorganic zinc.

The effect of inorganic zinc on iron status was measured by Yadrick and co-workers (1989). Young women were either given 50 mg zinc (zinc gluconate) or 50 mg of iron (ferrous sulfate monohydrate) in addition to 50 mg of zinc for 10 weeks. In the group receiving only the zinc supplement, serum ferritin and hematocrit decreased significantly. In the zinc and iron supplemented group, serum ferritin increased significantly after 10 weeks of supplementation, but there was no effect on hemoglobin or hematocrit. Thus, the supplemental iron compensated for the loss in storage iron when zinc alone was given.

Effects of iron and folic acid on zinc bioavailability were studied by Meadows et al. (1983). They conducted a two week study with pregnant women as a study group and non-pregnant women as the control group. They found that zinc bioavailability decreased with intake of oral iron as ferrous fumerate. Zinc bioavailability as estimated by plasma zinc was evaluated before and after 24 hours of fasting following two weeks of oral iron supplementation and folic acid in controls. After 12 hours of fasting, subjects were given 220 mg of zinc sulfate (by mouth) and then oral iron and folic acid supplements (100 mg iron and 350 ug folic acid) daily for 14 days. The results indicated decreased zinc bioavailability after iron and folic acid supplementation as measured by changes in the plasma zinc curve. The shape of the plasma curves suggested that this was due to impairment of intestinal absorption of zinc. However, contrasting results were obtained when infants were studied. Serum zinc in 12 month old infants was not affected by thirty milligrams of ferrous sulfate per kilogram body weight given before breakfast every day for 3 months. However, serum ferritin and iron concentrates were significantly higher in the iron supplemented group than in the palcebo group. An increase in hemoglobin was reported in the iron group but not in the placebo group. Although there was a slight decrease in serum zinc in the iron supplemented group, the decrease was not significant (Yip et al., 1985).

Pregnant women are commonly supplemented with iron which might affect zinc status. Iron status was assessed in zinc supplemented pregnant women by Simmer et al. (1987). Healthy pregnant women were given 25 mg of oral zinc (zinc sulfate) after an overnight fast. Blood samples were collected before and 1, 2, 3, and 4 hours after the zinc dose. Hemoglobin, total iron binding capacity (TIBC), serum iron and mean corpuscular volume (MCV) were measured in the first blood sample. Subjects were then supplemented daily with 100 mg of ferrous iron and 350 ug of folate for two weeks, and zinc absorption was reassessed 24 hours after the final doses. For comparison, zinc absorption was also measured in non-pregnant healthy volunteers. After an overnight

fast, they were given 50 mg of oral zinc. Blood samples were collected and analyzed similarly to pregnant subjects. These volunteers were also supplemented with 350 ug oral folate but not iron daily for two weeks, and zinc absorption was reassessed after the final dose. In the pregnant women, zinc absorption was reduced 24 hours after iron-folate supplementation suggesting a mucosal rather a luminal effect. Also, zinc absorption was reduced by folate supplements. Therefore, the researchers concluded that routine iron and folate supplementation may have deleterious effects on zinc metabolism due to inhibition of iron and folate on zinc absorption.

Research has also shown that zinc can affect measures of iron status. Research was conducted to investigate iron-zinc interactions in humans under conditions of high zinc/iron ratios. Hambidge et al. (1983) examined measures of zinc status in pregnant women either supplemented or unsupplemented with 15 mg Zn/day. These subjects also received daily iron supplements. Negative correlations between the level of prenatal iron and plasma zinc and between iron and alkaline phosphatase activity (measure of zinc status) were reported in these subjects. Furthermore, the non-supplemented group had higher alkaline phosphatase activity than did the supplemented group (Hambidge et al., 1983).

Zinc status is affected by the extent of iron supplementation and by its discontinuation. Newhouse et al. (1993) studied the effects of iron supplementation and discontinuation on serum zinc in 111 healthy women between the ages of 18 and 40 years. Forty-five subjects were either iron deficient (hemoglobin level below 120 g/L) or iron deplete (serum ferritin below 20 ug/L) before entering the study. These subjects were supplemented with 100 mg elemental iron per day for 12 weeks. Serum ferritin increased significantly from baseline (15.9 ± 22.5 ug/L) to the end of the 12 week supplementation period (36.5 ± 32 ug/L) of 12 weeks and remained significantly higher than baseline during next 12 week discontinuation period. The treatment did not significantly affect serum zinc concentrations during the supplementation period.

However, during the discontinuation phase, there was a decrease in serum zinc concentrations so that by 18 and 24 weeks the values were significantly lower than baseline concentrations.

Due to the interaction between iron and zinc, excessive iron levels in a multi-mineral supplement may negatively affect zinc absorption. Sandstrom et al., (1985) reported a significant effect of high iron to zinc ratio on the zinc absorption in healthy men and women between 20-50 years of age. Fasted subjects were given test solutions of 40 umol oral elemental zinc (^{65}Zn) and three other solutions containing additional elemental iron of 40, 100, and 1000 umol corresponding to 1:1, 2.5:1, and 25:1 ratios of Fe:Zn respectively. The absorption of zinc was determined from measures of whole body retention after 14 days. When given alone, zinc absorption was 74 percent; zinc absorption decreased significantly to 58 percent at 1:1 Fe/Zn ratio. At a Fe:Zn ratio of 2.5:1, zinc absorption remained at 58 percent. However at a Fe/Zn ratio of 25:1, zinc absorption decreased significantly to 34 percent. At that same ratio (25:1), the inhibitory effect of high levels of iron was alleviated by adding histidine (4 mmol), a zinc ligand, to the water solution (1000 umol of iron). This histidine addition resulted in increased absorption from 34 to 47 percent. Thus, the intake of a supplement with a meal may overcome the inhibitory effect of iron. In the same study, a test meal consisting of rice and meat sauce with an inherent ratio of Fe/Zn of 1:1 resulted in zinc absorption of 25 percent. Addition of ferrous sulfate (100 or 1000 umol) increased Fe/Zn ratios to 2.5:1 and 25:1, but did not significantly change zinc absorption.

Nutritional Assessment

Nutritional assessment evaluates the nutritional status of individuals or populations. This section reviews evaluation of dietary methods and anthropometric methods.

Dietary Assessment

Dietary assessment measures the quantity of individual food consumed for a certain period of time or the pattern of food consumption. The sensitivity of dietary assessment instruments to changes in the nutrient intake or dietary behavior under study should be considered in nutrition-intervention research (Kristal et al., 1994).

Several dietary assessment techniques are used to assess food intake. Of those, commonly used methods include 24-hour recall, food frequency questionnaires, and food records. The validity of any method is influenced by the intelligence, motivation, co-operation of the subject, and also the techniques used by the investigator. In addition, rapport between the subject and the interviewer is important for collecting complete and accurate information (Beal, 1967).

A 24-hour recall is a retrospective method to evaluate food intake over the past 24 hours. The disadvantage of this method is that it may result in inaccurate reporting or deviation from usual eating pattern (Buzzard, 1994). According to Gersovitz et al. (1974), decline in short-term memory with age makes this method unreliable to be used in the studies involving elderly. In their study the elderly subjects (mean age of 72 years) reported mean intakes of each nutrient greater than the mean actual intake; however, this was significant for protein only.

A food record is a written record of the food eaten and is written at the time of consumption. Food records vary in length of time for which the information is recorded. However, food records longer than 3 days may not provide valid information on intake. Validity of the seven-day food record was investigated among non-institutionalized elderly who were participating in a congregate meal program. Records collected during the first few days provided more accurate estimates of the mean intake compared to fifth, sixth, and seventh days (Gersovitz et al., 1978). In a study using adolescents, Mullenbach et al. (1992) found that 3-day food records provided accurate estimates of dietary intake compared to 24-hour dietary recall.

Different methods of dietary assessment to obtain information on the quality of diets have been reviewed by Medlin and Skinner (1988). This review described individual assessment methods and their evolution over 50 years. The authors suggested that knowledge of the strengths and limitations of current dietary assessment methodology is essential for researchers and practitioners in order to select appropriate methodology and to interpret the findings. The review recognized that various methods yield different results. The intakes may be underestimated or overestimated consistently with the method used.

Accurate food intake estimates can be achieved by using food models and with prior notification of subjects. Posner et al. (1992) conducted validity research on two-dimensional food models used to estimate portion sizes in nutrition research. They used the 24-hour recall method to evaluate the intake. Pearson correlation coefficients between the two- and three-dimensional methods were high. This research supported the use of food models to estimate serving sizes in nutrition research.

It was reported that using graduated food models reduced time needed for interviews and also decreased the frustration of the respondents. This also reduced the individual variation in estimating the food intake and led to more accurate estimates. In addition, models helped in easy handling of the data since the answers were "definite, comparable, and consistent" (Moore et al., 1967).

Despite the method used, the accuracy of the calculated estimates depends on the degree of food description detail. In addition, quantification coding and calculation procedures that are used to convert the food intake data into nutrient intakes also affect the accuracy of the data (Buzzard, 1994).

Anthropometric Assessment

Anthropometric measurements frequently used in clinical settings and nutrition surveys include weight, height, skinfold thickness, and bioelectrical impedance to

estimate percentage of body fat and lean body tissue in the body. It was reported that anthropometric measurements in the elderly vary with age, sex, health practices, and the presence of certain disorders like diabetes and hypertension (Kubena et al., 1991). Kubena et al. (1991) studied the relationship between anthropometric measurements and health behaviors and disorders in elderly non-institutionalized Caucasians. Their results showed differences in anthropometric indexes due to age and sex.

Master and Lasser (1960) presented height and weight standards for persons aged 65-94 years. The mean height range of men was reported to be 154-185 cm with an average weight of 72 kilograms. Whereas, women were reported to have a mean height range of 147-175 cm with the average weight being 64 kilograms. In both the sexes, the average weight decreased with advancing age. In a later study, Roubenoff and Wilson (1993) reported that the stature often decreases with age.

With accurate height and weight measures, body mass index can be computed for individuals. It is calculated as weight (kg)/height $(m)^2$ (Deurenburg et al, 1988). Body mass index is correlated with body fat in young adults; however, it might lead to inaccurate estimates of body fat in older adults due to decreased height, increased body fat, and decreased lean body tissue. Burr and Phillips (1984) reported a decline in body mass index in elderly with advancing age. This could be due to a change in individual's height and weight of the individuals as they grow older since the BMI is adjusted for height and weight.

Bioelectrical impedance analysis (BIA) is a widely used method which gives reliable and valid assessment of body composition (Lukaski, 1987). The principle of this method is that the resistance of an electric current is proportional to the amount of fat free mass. Body composition changes with advancing age leading to a decrease in fat free mass, consequently increasing fat mass (Novak, 1972; Noppa et al., 1979). Novak (1972) conducted a study on adult men and women volunteers aged 18 to 85 years. The purpose of the study was to determine the relationship between fat free mass and age in

both males and females. The results indicated that fat content was double in the oldest group (65-85 years) compared to the youngest group (18-25 years). A steady increase in percentage fat was reported with a decrease in fat free mass in both the sexes. Noppa et al. (1979) also reported similar changes in body fat mass with advancing age. Bioelectrical impedance analysis is reported to be a valid method in predicting lean body mass in large heterogeneous population (Segal et al., 1988). Bioelectrical impedance analysis is a valid method of predicting fat free mass and fat mass in elderly when age-specific regression equations are used (Deurenberg et al., 1990).

CHAPTER III

METHODOLOGY

Subjects

Thirty four volunteers over 55 years of age participated in the study. The subjects were recruited by contacting local physicians, organizations and by mailing a letter to all the staff and faculty of Oklahoma State University (Appendix A). These volunteers reported that they were free from chronic illness, non-users of medication, and were maintaining current body weight. After explaining the study, an informed consent was obtained from each subject (Appendix B). The experimental protocol was approved by the Institutional Review Board of Oklahoma State University (Appendix C).

Supplement Preparation

The zinc, copper and chromium supplements were prepared in the nutritional sciences laboratory using a gelatin capsule filling machine (Quanterron, Inc., Minnesota). Zinc supplements were prepared by mixing 64.8415 grams of zinc sulfate ($ZnSO_4.7H_2O$) and 349.7674 grams of lactose in a ball mill for 8 hours. The gelatin capsules were filled with the mixture calculated to contain 15 mg of zinc per capsule. Similarly, 7.2551 grams of copper carbonate [$Cu_2(CO_3)_3$] in 708.7449 grams of lactose, and 1.2587 gms of chromium chloride ($CrCl_3.6H_2O$) in 427.1798 gms of lactose were mixed to make the capsules to contain 1.5 mg of copper or 100 ug of chromium in each. Number two clear gelatin capsules (Item # 16014, Apothecary Products, Inc., Burnsville, MN) were used to make the supplements. Sample capsules were wet and dry ashed

using a modification of the Hill et al.(1986) method and tested for mineral concentration using the atomic absorption spectrophotometer (Model 5100 PC; Perkin-Elmer Corp., Norwalk, CT). The average analyzed chromium, copper and zinc contents of the supplements were 120.66 ug, 1.692 mg and 15.18 mg, respectively.

Experimental Design

This study was a randomized design with time as a repeated measure. The subjects were randomly assigned to one of four supplement groups (placebo, chromium, copper, or zinc) such that the proportion of males and females in each group was similar.

Subjects consumed the supplements or placebo for eight weeks beginning immediately after baseline data collection. Subjects were instructed to consume one capsule in the morning and one in the evening with meals. Subjects received one month's supply of supplements and were asked to return the container at the following collection period for determination of actual supplement consumption. In addition to taking the supplement, subjects were advised to maintain usual diets and exercise patterns during the course of the study.

Data were collected four times during the study: baseline, at four and eight weeks of supplementation and four weeks post supplementation. Twelve hour fasting blood samples and routine anthropometric measurements were collected at each data collection period. Prior to each measurement period, subjects recorded a 3-day food intake.

Procedures

Health Information Questionnaire

Prior to the baseline measurement, each subject completed a health profile questionnaire. Information pertaining to the subject's past medical history, type of special diet, current medication, dietary supplements used in the past six months, recent

serious illnesses or surgery, serum cholesterol concentrations, exercise, and family history of elevated cholesterol or heart disease was collected (Appendix D).

Monthly Data Collection Form

Each subject completed a monthly data collection form at each of the four measurement periods. The monthly data collection form was designed to detect recent illnesses which might affect biochemical measures. In addition, maintenance of exercise pattern was determined at this time (Appendix E).

Dietary Intake Data

Subjects were given verbal and written instructions by a Registered Dietitian for recording food intake. A set of non-biasing food models were provided to each subject during the study. The food models were prepared in the laboratory using a modification of the method of Moore et al., (1967). Colored nylon net with a measured volume of dried beans were used. Different colored nylon was used to code for different amounts. A red nylon net circle of 10 and 1/8 inches in diameter was used for the one cup measurement. Yellow circular nylon net seven and a half inches in diameter was used for a half cup measure, and a blue nylon net circle six and a half inches in diameter was used for a one-third cup measure. A double layer of nylon net was sewn together on the edges and were filled with the corresponding volume of dried pinto beans. The thread was pulled together to close the bag and was knotted. A three-dimensional wooden block with the dimensions of length: 3 1/8 inches, breadth: 2 1/8 inches, and height: 0.75 inches was used to represent 3 oz of meat.

Three-day dietary records were kept by the subjects before the supplementation, at four weeks, eight weeks and four weeks post supplementation. The three-day dietary records included only the week days (Appendix F). The Food Processor Plus, version 5.03 (ESHA Research, Salem, OR) was used to analyze the reported intakes. This

program does not have values for chromium. Missing data are indicated by blanks. Additional foods were included in the database from food label information, restaurant menus and home recipes. Information about the supplements was included as a data file.

Anthropometric Data

Subject's standing height was measured at the baseline and at the 8 weeks supplementation periods. With the subject standing erect against the wall with barefoot heels together, a scaled rule attached to the wall was read to the nearest 1/4 inch. Subjects' body weight was measured at four measurement periods using a calibrated body weight scale (Continental Co., Chicago). Accuracy was read to the nearest one quarter pound increment.

Knee Height

Often stature decreases with age (Roubenoff and Wilson, 1993). Knee height measurement is used to estimate stature of adults 60 to 90 years. It can also be used with other anthropometric measures to predict weight. It is a reliable estimate of stature when used to adjust body composition measurements included in bioelectric impedance.

Knee height was measured during the second data collection period using a Ross Knee Height Caliper (1989) (Ross Laboratories, Columbus, OH). Measurements were done in duplicate and averaged to ensure accuracy of measurements within 0.5 cm. Subjects were reclined in a supine position with the left knee and left ankle bent at 90 degree angles to take the measurement. The caliper was held with a locking mechanism and read to the nearest 0.1 cm through the viewing window. Equations to estimate knee height were provided by Ross Laboratories. The equations are specific for age and gender. Stature for men is calculated from knee height by the following equation:

[2.03 x knee height (cm)]-[0.04 x age (yr)] + 64.19.

Stature for women is calculated from knee height by the following equation:

[1.83 x knee height (cm)]-[0.24 x age (yr)] + 84.8.

Bioelectrical Impedance

Bioelectrical impedance is dependant on body water. Hydration can be affected by intake of alcohol, diuretics or caffeine, recent exercise or food intake, and abnormal body temperature. Bioelectrical impedance was used as a noninvasive and simple method to measure body composition. Bioelectrical impedance measures battery-charged electrical conductivity of fat-free tissue using whole body reactance and resistance to estimate lean body mass and total body fat (Biodynamics Model 310, Body Composition Analyzer, Chattanooga Corporation, Chattanooga, TN).

Subjects were measured during the first and third data collection period by a trained research assistant. The test was performed after a 12-hour fast, and the subjects were assumed to be adequately hydrated. All the jewelry was removed before the measurement. Each subjects was instructed to lay on a horizontal surface in a supine position. The subject was positioned with hands, palms down, at least six inches apart from the body. The feet were also positioned six inches apart. The right arm and leg were exposed for the placing the electrodes. Each subject was instructed to place the hand in the dorsiflexion position and an electrode was placed near the subject's wrist at the point where the wrist creased. Each subject was told to relax the wrist, and the second electrode was placed on the same hand near the knuckles. Similarly, one electrode was placed on the subject's right foot near ankle at the point where it creased. The subject was asked to relax the foot, and the second electrode was placed on the same foot near the outer toes. The subject was told to relax and to remain motionless for the duration of the test lasting about 10 seconds. The test was repeated using a new set of electrodes, and the two values were averaged for analysis. The test was not performed on the subjects with a pacemaker. The test was performed according to the procedure

described in the User Guide. Subjects' height and weight along with age and gender were programmed into the bioelectric impedance analyzer.

Blood Collection

At each data collection period between 7:30 and 9:30 am thirty milliliters fasting blood was collected from the subjects. The blood was collected by a phlebotomist using a 21 gauge stainless steel butterfly needle and luer-monovette syringes (Starstedt, W.Germany). Twenty milliliters of blood was collected in 10 ml neutral tubes which had been treated with 100 ul of 300 g/L sodium citrate as the anticoagulant. In addition, ten milliliters of blood were collected in serum luer-monovette tubes. The plasma tubes were placed on the rotator for mixing until separation, and serum tubes were allowed to clot in an ice chest. The plasma was separated within 30 minutes of blood collection. The serum tubes were allowed to clot on ice for two hours before separation.

Plasma and Serum Separation

After transferring one ml of whole blood to a microcentifuge tube for total hemoglobin analysis, plasma was separated by centrifugation at 3000 rpm for 25 minutes at four degrees centigrade. Two and a half milliliters of plasma were transferred using a plastic transfer pipette into a mineral free Falcon tube (Becton Dickinson Labware, New Jersey)for mineral analysis. Plasma was separated and stored in different microcentrifuge tubes for analysis of cholesterol, glucose, apolipoprotein A-1, and apolipoprotein B. The remaining plasma was stored in a second Falcon tube and frozen.

The serum tube was allowed to stand on ice for 2 hours and then centrifuged at 3000 rpm for 55 minutes at 4 degrees centigrade. The clear top layer of the serum was carefully separated with a plastic transfer pipette and stored in siliconized tubes and frozen for the analysis of estradiol, insulin, vitamin D, and osteocalcin. Serum was also stored in a clear microcentrifuge tubes for ferritin analyses. Fresh serum was transferred into a

microcentrifuge tube for immediate analysis of alkaline phosphatase and then frozen for subsequent albumin analysis. The remaining serum was stored in a Falcon tube and frozen.

Preparation of Solutions

Glass distilled deionized water was used to prepare all the solutions. The water was pretreated in Barnstead filters prior to glass distillation. The same batch of water was used to make all of the solutions. Glass distilled water was frozen in acid washed plastic bottles to use for lysing red cells so that all cells would be lysed with the same batch of distilled water. Fresh bottles were thawed for each data collection period.

Phosphate buffered saline (PBS) was prepared by mixing 7.1 g of sodium chloride, 1.15 g of sodium phosphate-dibasic salt ($Na_2 HPO_4$), potassium phosphate-monobasic ($K H_2PO_4$), and 0.2 g of potassium chloride in distilled water made up to one liter. The solution was prepared and the pH was adjusted to 7.2. Six liters of PBS were prepared at a time, mixed and portioned for using throughout the study.

The anticoagulant was prepared using trisodium citrate and normal saline. Fifteen grams of trisodium citrate were added to saline and diluted to a final volume of 50 ml. This solution was mixed thoroughly to prepare 30 percent trisodium citrate. Saline was prepared by mixing 0.45 g of sodium chloride in 50 ml of distilled water.

Washing of Red Cells

After removing the plasma, the buffy coat was removed carefully by a transfer pipette. To the remaining red cells, 5 ml of cold phosphate buffer saline (PBS) was added to suspend erythrocytes. The suspended cells were transferred to a mineral free polyethylene tube and another 5 ml of PBS was added to the plasma syringe and gently rinsed to resuspend remaining cells. The tubes with red cells and a total of 10 ml of PBS solution were closed with parafilm, mixed, and then centrifuged at 3000 rpm for 25

minutes at four degrees centigrade. The clear PBS solution was carefully drawn off by a transfer pipette and discarded. Erythrocytes were resuspended as before. The cells were washed a total of three times. After the final centrifugation, one ml of red cells was removed and added to a Falcon tube containing 2 ml of distilled water and vortexed thoroughly to lyse the red cells (RBC). A lysed RBC sample was removed to a microcentrifuge tube for red cell hemoglobin analysis. The remaining lysed cells were frozen for mineral analyses. The remaining packed red cells were frozen in capped and parafilmed falcon tubes and stored.

Biochemical Analyses

Modifications from the manufaturer's methods used in this study are included in Appendix G. Otherwise, the methods used were as described in the published procedures.

Total Hemoglobin

Total hemoglobin was measured by the cyanmethemoglobin method for quantitative, colorimetric determination in whole blood at 540 nm using the visible lamp (procedure # 525, Sigma Diagnostics, St.Louis, MO). In this technique total hemoglobin at alkaline pH is rapidly converted to the cyano derivative which is determined by its absorbance. The color intensity at 540 nm is proportional to total hemoglobin concentration.

Four cyanmethemoglobin standards (0, 6, 12, and 18 g/dl) were used, and were prepared in advance and stored refrigerated in plastic bottles covered with an aluminium foil to protect from light. The standards were stable for 6 months. The same standards were used for all the samples. Whole blood samples were vortexed thoroughly before analysis. The procedure was modified by decreasing the amount of blood used for the

analyses (10 uL), and the amount of Drabkin's solution used (2.5 mL) in order to reduce hazardous waste.

Serum Albumin

Albumin was measured by the bromocresol purple method specific for human serum albumin. This is a quantitative, colorimetric determination in serum at 600 nm (procedure # 626, Sigma Diagnostics, St.Louis, MO). Human serum albumin reacts with bromocresol purple (BCP) to form a stable blue-purple color complex with maximum absorption at 600 nm. To five standards, blank and frozen sample duplicates, 1.0 ml of albumin reagent (BCP) was added. To the standard, test and blank, 0.01 ml of albumin standard (Sigma catalog # 540-10), 0.01 ml serum and 0.01 ml 0.85 % saline was added respectively. The contents were vortexed. The absorbance of standard and test was read against a blank as reference at 600 nm. The intensity of the color is proportional to the serum albumin concentration.

Alkaline Phosphatase

Alkaline phosphatase activity in serum was determined by quantitative, kinetic determination using an alkaline phosphatase (ALP) reagent at 405 nm (procedure # 245, Sigma Diagnostics, St.Louis, MO). Serum ALP hydrolyzes p-nitrophenol phosphate to p-nitrophenol and inorganic phosphate. The hydrolysis occurs at alkaline pH. The p-nitrophenol formed shows an absorbance maximum at 405 nm and is directly proportional to ALP activity of the sample.

The reagents were prepared the day before the analysis. Distilled water was used as a reference and samples were read in duplicates. Twenty microliters of fresh sample was used for each analysis.

Serum Ferritin

Serum ferritin was measured by a double antibody radioimmumoassay method (Diagnostic Products Corporation, Los Angeles, CA) which quantitatively measures serum ferritin. Frozen samples (100 uL/test) were brought to room temperature. All the samples and standards were run in duplicate. Non-specific binding (NSB) tubes were run in quadruplicate. In this procedure, ^{125}I-labeled ferritin competes with ferritin in the sample for sites of ferritin-specific antibody. After incubation for a fixed time, separation of bound ferritin from free is achieved. The supernatent solution was decanted and the precipitant was retained and counted for one minute using a gamma counter (Packard Cobra II, Auto Gamma, Packard Instrument Co., Meriden, CT). The counts are inversely related to the amount of ferritin present in the sample.

Plasma Minerals

Plasma minerals (zinc, copper, iron, and manganese) were analyzed by atomic atomic spectrophotometry (AAS). Plasma zinc and iron were read using an air-acetylene flame, and copper and manganese were read in the graphite furnace.

Plasma was wet and dry ashed by a modification of the Hill et al. (1986) method and tested for mineral using conentrations using an atomic absorption spectrophotometer, Model 5100 PC (Perkin-Elmer Corp., Norwalk, CT). The detailed procedure for ashing (wet and dry) and dilutions are included in Appendix H.

Statistical Analyses

Data were analyzed using the Statistical Statistical Analysis System (SAS) program available at Oklahoma State University Computer Center. The General Linear Model (GLM) was used to determine significant differences for unbalanced groups. Comparision of treatment groups to placebo was performed by Dunnett's test. Significance level was set at 0.05.

CHAPTER IV

RESULTS AND DISCUSSION

Sample Description

The sample for this study consisted of thirty volunteers over the age of 55 years. The volunteers were independently living elderly adults in the community of Stillwater, Oklahoma. The original sample consisted of thirty-four subjects. Four of these subjects despite their participation in the study were not included in the analyses. Data from male subjects of the chromium group were not analyzed. Two of these male subjects had high initial serum ferritin concentrations of 752 and 438 ng/ml. The third subject did not the fit the specified age range. In addition, data of one male from the placebo group was also dropped because of an elevated initial serum ferritin concentration of 377 ng/ml. The data of the only male left in the chromium group were not included in the analyses.

Subject Description by Gender

The sample consisted of thirteen males and seventeen females. Table 1 includes the baseline anthropometric measures by supplement groups. One female each in the placebo group and in the chromium group, and one male in the zinc group missed the second measurement (M2). Two females in the copper group missed the fourth measurement (M4).

Table 1. Means of anthropometric measures at baseline by gender[a,b]

Variable[c]	Placebo	Chromium	Copper	Zinc
Males				
n	4	-	4	4
Age, yr	$56 \pm 4^{*}$	-	$62 \pm 4^{*}$	63 ± 4
Ht, m	$1.81 \pm 0.02^{*}$	-	$1.78 \pm 0.02^{*}$	$1.76 \pm 0.02^{*}$
Wt, kg	$80.9 \pm 6.8^{*}$	-	80.4 ± 6.8	83.1 ± 6.8
BMI, wt/ht^2	24.7 ± 2.1	-	25.5 ± 2.1	24.7 ± 2.1
% body fat	$22.4 \pm 2.6^{*}$	-	$24.4 \pm 2.6^{*}$	22.3 ± 2.1
Females				
n	3	6	4	4
Age, yr	$72 \pm 4^{**}$	58 ± 3	$74 \pm 4^{**}$	64 ± 4
Ht, m	$1.62 \pm 0.03^{**}$	1.59 ± 02	$1.58 \pm 0.02^{**}$	$1.60 \pm 0.02^{**}$
Wt, kg	$70.5 \pm 7.9^{**}$	66.7 ± 5.6	61.2 ± 6.8	57.4 ± 6.8
BMI, wt/ht^2	26.3 ± 2.5	26.2 ± 1.7	24.6 ± 2.1	22.3 ± 2.1
% body fat	$35.5 \pm 3.1^{**}$	37.8 ± 2.4	$39.9 \pm 3.1^{**}$	33.2 ± 2.6

a. LS Means $\pm$ SEM.
b. Variables by sex in columns with different superscripts (*) are statistically significant ($p \leq 0.05$).
c. Ht = Height, Wt = Weight, BMI = Body Mass Index.

Anthropometric Measurements at Baseline

By Gender. Mean age, height, body weight, body mass index, and percent body fat of the subjects according to gender are presented in Table 1. The mean age of females was significantly higher than males in both placebo and copper groups. However, there were no significant differences in age between males and females in zinc group. Males of all the four groups had significantly greater height compared to females. In the placebo group males were significantly heavier (80.9 ± 6.8 kg) than females (70.5 ± 7.9 kg). Weight did not differ significantly between males and females in copper and zinc groups (Table 1). There was no significant difference between males and females for the body mass index between groups. However, females in the placebo and the copper groups had significantly higher percentage body fat compared to males.

By Supplement Group. No significant differences were found among males of different supplement groups for age, height, weight, body mass index, and percentage body fat. Similarly, there were no significant differences in age, height, weight, body mass index, and percentage body fat among females of different groups. Thus, the sample was homogeneous by gender in supplement groups in relation to anthropometric measurements at baseline.

Biochemical Measurements at Baseline

By Gender

Baseline means of hemoglobin, serum ferritin, albumin, alkaline phosphatase, and plasma minerals are reported in
Table 2. The mean initial values for hemoglobin, serum ferritin, albumin, alkaline phosphatase, plasma zinc, and plasma iron were within normal ranges. However, subjects had lower than normal concentrations of plasma copper concentrations.

Males in the copper group had significantly higher hemoglobin concentration (16.5 ± 0.4 mg/dL) at baseline compared to females (13.4 ± 0.4 mg/dL) (Table 2). This

Table 2. Means of biochemical measurements at baseline by gender [1,2,3]

Variable[4]	Placebo	Chromium	Copper	Zinc	Normal values[5]
Males					
n	4	-	4	4	
Hemoglobin, g/dL	15 ± 0.9	-	16 ± 0.9*	15 ± 0.9	14.0 - 18.0
Serum Ferritin, ng/mL	100 ± 27	-	152 ± 27*	158 ± 27*	18 - 300
Albumin, g/dL	4.9 ± 0.3	-	5.0 ± 0.3	4.8 ± 0.3	4.0 - 6.0
Alk Phos, U/L	62 ± 6	-	60 ± 6	61 ± 6	30 - 120
Plasma zinc, umol/L	13.62 ± 0.8 a	-	11.93 ± 0.6b	11.01 ± 0.6 b	11.5 - 18.5
Plasma iron, umol/L	15.76 ± 3.4	-	21.85 ± 2.9	17.55 ± 2.9	14 - 32
Plasma copper, umol/L	6.52 ± 0.91*	-	7.59 ± 0.91	7.38 ± 0.08	11.0 - 22 0
Females					
n	3	6	4	4	
Hemoglobin, g/dL	15 ± 1.0	14 ± 0.7	13 ± 0.9**	15 ± 0.9	11.5 - 15.5
Ferritin, ng/mL	97 ± 31 a	57 ± 22 b	35 ± 27 b**	55 ± 27b**	18 - 300
Albumin, g/dL	4.7 ± 0.3	4.5 ± 0.2	4.7 ± 0.2	4.5 ± 0.3	4.0 - 6.0
Alk Phos, U/L	65 ± 7	75 ± 5	68 ± 6	67 ± 6	30 - 120
Plasma zinc, umol/L	12.69 ± 0.6	11.47 ± 0.4	11.02 ± 0.6	11.02 ± 0.4	11.5 - 18.5
Plasma iron, umol/L	19.34 ± 3.4	15.76 ± 2.3	21.67 ± 3.4	17.01 ± 2.9	11 - 29
Plasma copper, umol/L	9.58 ± 0.91**	8.89 ± 0.64	7.57 ± 1.12	8.47 ± 0.79	11.0 - 22.0

1. LS Means ± SEM.
2. Rows with different alphabetic superscripts are statistically significant ($p \leq 0.05$).
3. Variables by sex in columns with different superscripts (*) are statistically signifiant ($p < 0.05$).
4. Young DS: Implementation of SI units for clinical laboratory data. Ann Int Med 1987;106:114-29.
5. Alk Phos = Alkaline Phosphatase.

was not found in the other groups. Significant differences for serum ferritin concentrations were found between males and females in copper and zinc groups. Males had significantly higher values than females (Table 2). However, there was no significant difference in the placebo group between males and females for serum ferritin concentrations.

No significant differences were found in serum albumin and alkaline phosphatase concentrations between males and females of all the groups. Similarly, no differences were found in plasma zinc, plasma copper, and plasma iron in any of the groups at baseline.

By supplement group

There were no significant differences between the supplement groups in hemoglobin, serum ferritin, albumin, alkaline phosphatase, plasma copper, and plasma iron concentrations in males. However, the placebo group had significantly higher initial plasma zinc concentrations in males (12.2 ± 0.8 umol/L) compared to the copper (11.9 ± 0.6 umol/L) and zinc groups (11.0 ± 0.5 umol/L) (Table 2).

No significant differences were found in females for hemoglobin, albumin, alkaline phosphatase, and plasma minerals between the groups. However, females of the placebo group had significant higher initial serum ferritin concentrations compared to females of chromium, copper, and zinc groups (Table 2).

Dietary Intake at Baseline

Whole Sample

For the whole sample, 96.7 percent reported protein intakes above the two-thirds of the Recommended Dietary Allowances (RDA). The frequency distributions for intakes of selected vitamins and minerals are presented in Figures 1 and 2. Only 56.6 percent of subjects met two-thirds or more of the RDA for zinc, and 20 percent of the subjects had

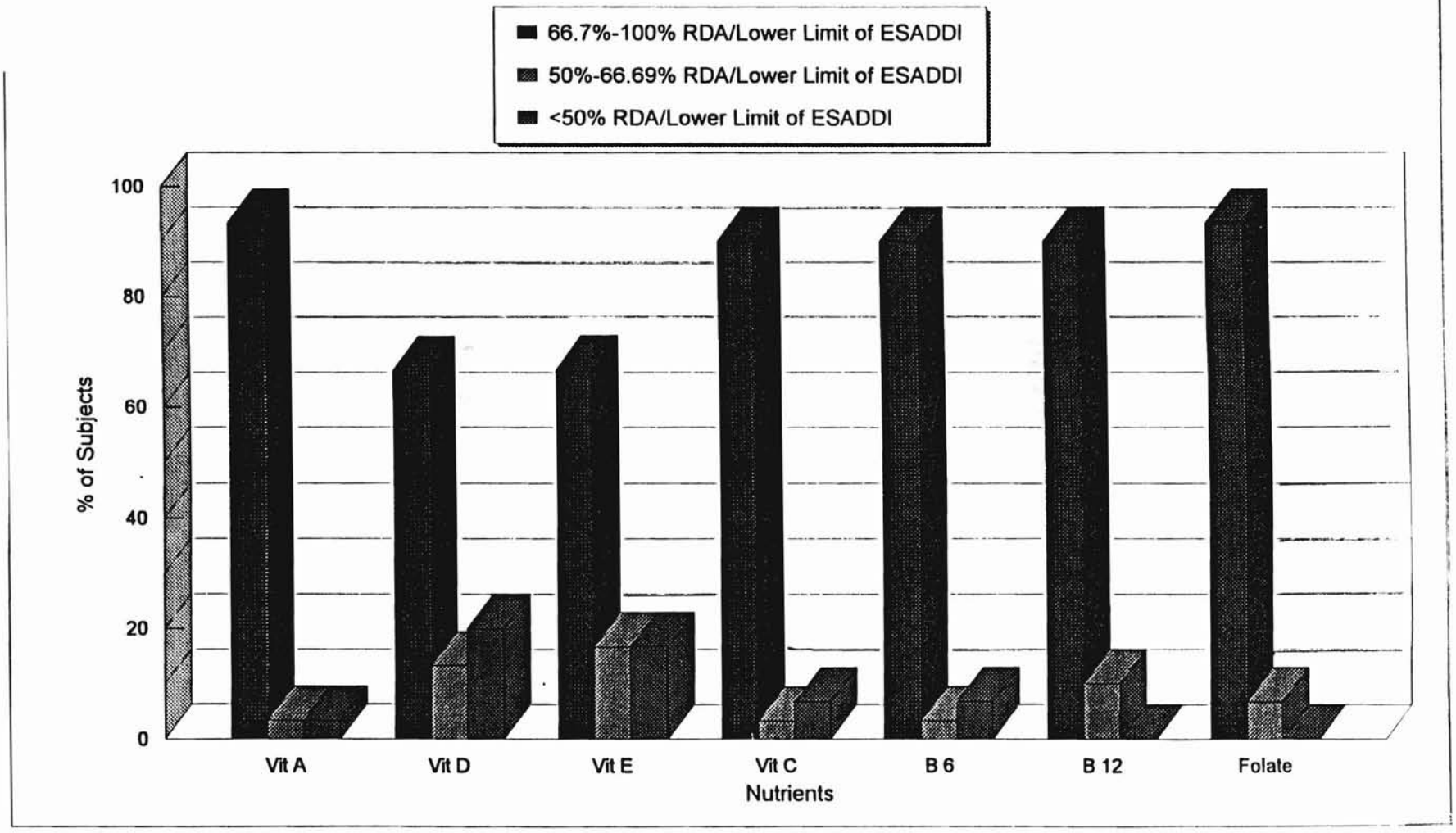

Fig 1. Frequency distribution of vitamin intake at baseline.

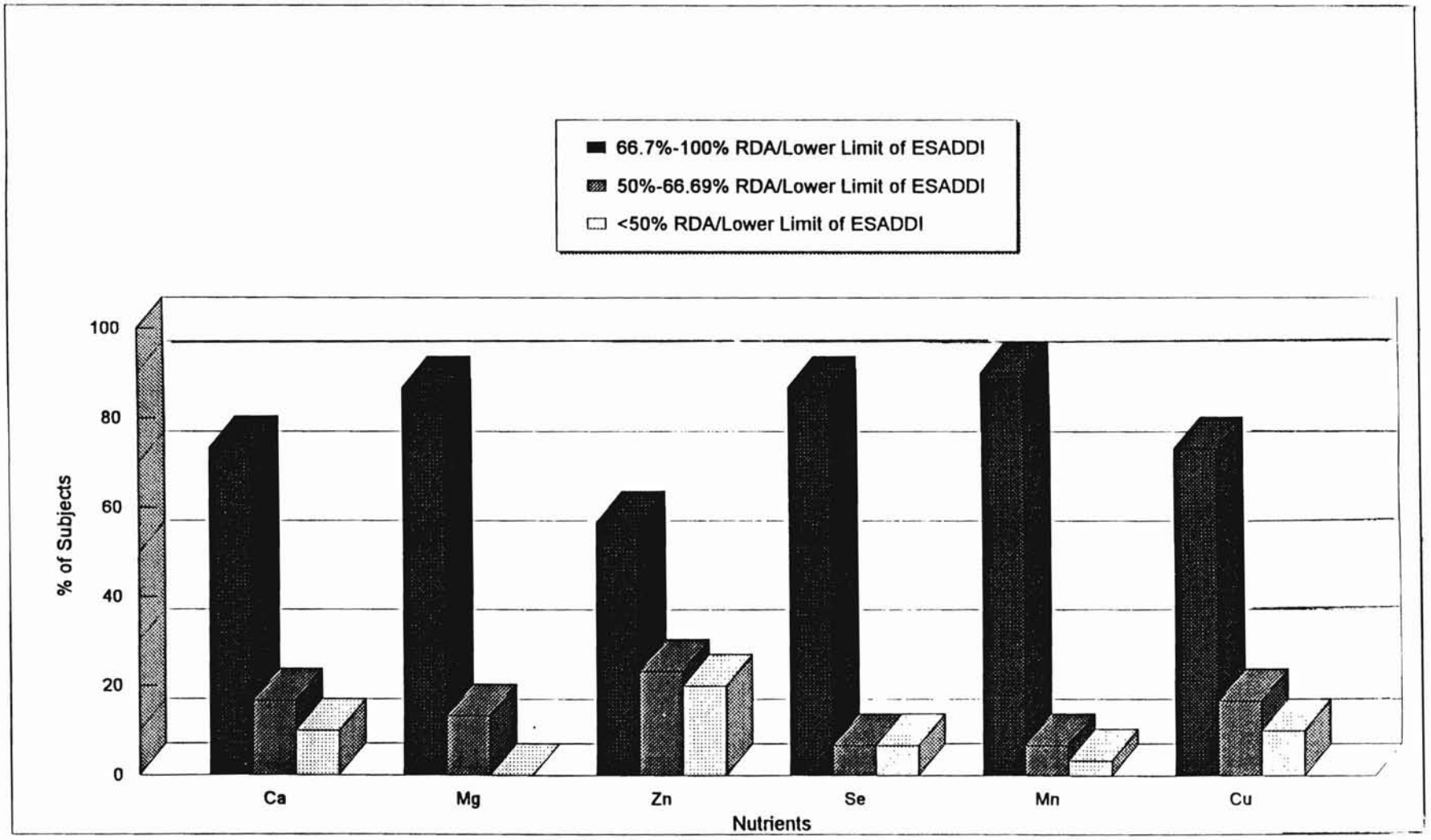

Fig. 2. Frequency distribution of mineral intake at baseline.

zinc intakes of less than 50 percent of the RDA. Mean intakes of less than two-thirds of the recommended intake for zinc were previously reported by other researchers in elderly subjects (Howarth, 1989; Hutton and Hayes-Davis, 1983). Hutton and Hayes-Davis (1983) studied elderly individuals living in government housing complexes. The mean total intake of the subjects was 7 mg which was less than 50 percent of the recommended intake for this age group. This could be due to the fact that their mean food energy was less than recommended; however, their protein intakes were equal to or exceeded the RDA. In our study, almost all the subjects had two-thirds or more of the recommended intakes for protein; however, zinc intakes of less than two-thirds of RDA in 43.4 percent of our subjects could be due to the quality of foods they selected. National studies reported a mean intake of eighty-nine percent of RDA for zinc in adult males (Harland et al., 1981). Their study was based on the data collected during 1974 and 1975, and the subjects consumed diets containing a mean energy level of 2,800 kcal. These findings showed that zinc intake was dependent upon energy level of the diet. The lower intakes of zinc in males in our copper and zinc groups compared to the placebo group could be due to corresponding calorie intakes in these groups which were significantly lower than in the placebo group.

While seventy-three percent of the subjects consumed above two-thirds of the lower limit of the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for copper, only 20 percent of the subjects met 100 percent of the lower limit of the recommended intake for copper. Furthermore, 10 percent reported consumption of less than 50 percent of the recommended intake for this nutrient (Figure 2). National studies have reported intakes less than the recommended levels for copper in both males and females aged 60-65 years. In the Total Diet Study, intakes of men and women were 1.18 and 0.86 mg per day respectively. These intakes were compared to the recommended intakes of 1989 (Pennington and Young, 1991). However, our subjects reported consumption of greater amounts than reported by Pennington and Young (1991). Males

and females of our study consumed an average of 1.36 (90 percent of the lower limit) and 1.17 mg (78 percent of the lower limit) per day, respectively. In our study all the subjects consumed two-thirds or more of the recommended intakes for iron. Pennington and Young (1991) also reported an increased intake of iron in elderly. Their study showed that elderly males and females consumed iron exceeding their RDA at 151 and 106 percent respectively.

By Supplement Group and Gender

Placebo. The placebo group consumed adequate amounts of nutrients at baseline. Mean intakes of both males and females met two-thirds or more of the recommended intakes for all the nutrients.

Chromium. Eighty-three percent of the females in the chromium group met two-thirds or more of the recommended intakes for protein and folic acid. All the females of this group met two-thirds of the recommended intakes for iron and manganese. Thirty-three percent of the females in the chromium group consumed less than 50 percent of the recommended intakes for copper, and zinc. Only one female in the chromium group met 100 percent of the lower limit of ESADDI for copper and 100 percent of the RDA for zinc.

Copper. In the copper supplemented group, all the males met two-thirds or more of the recommended intake for protein, riboflavin, magnesium, iron, and manganese. However, 25 percent of males in this group reported consumption of less than 50 percent of the recommended intakes for zinc, and none of them met 100 percent of the RDA for zinc. The females of the same group met two-thirds or more of the recommended intakes for protein, vitamins A and C, and all B vitamins. Seventy-five percent of the females met two-thirds or more of the recommended intakes for zinc, and copper. Similar to the chromium group, only one female met 100 percent of the recommended lower limit of ESADDI for copper and 100 percent of the RDA for zinc.

Zinc. In the zinc group, all of the males met two-thirds or more of the recommended intakes for protein, vitamins A and C, and all B vitamins. However, only 25 percent of the males met two-thirds or more of the recommendation for zinc. Seventy-five percent of them met the two-thirds or more of the recommended intake for manganese and copper. None of the males in the zinc group met 100 percent of the RDA for zinc and only one male in this group met 100 percent of the lower limit of the ESADDI for copper. All the females in the zinc group met two-thirds of the recommended intakes for protein, vitamin A, thiamin, riboflavin, niacin, folate, iron, and copper. Seventy-five percent of the females met the two-thirds or more recommended intakes for vitamin C, B_6, B_{12}, magnesium, and manganese. However, only 25 percent of the females met this recommendation for zinc. None of the females in the zinc group met 100 percent of lower limit of ESADDI for copper or 100 percent RDA for zinc.

Overall, the reported nutrient intakes of the sample were good except for copper and zinc. A majority of the subjects did not meet 100 percent of the RDA for zinc or 100 percent of the lower limit of the ESADDI for copper. Few subjects consumed less than 50 percent of the recommended intakes for zinc and copper. However, these subjects met 100 percent of the recommended intake for iron.

Differences in Nutrient Intakes Between Groups by Gender

Males. The mean nutrient intakes and percent RDA/lower limit of ESADDI at baseline for males is reported in Table 3. The mean calorie and protein intake of the placebo group was significantly higher than that of the copper group. The placebo group reported consumption of significantly greater amounts of riboflavin and niacin compared to the copper and zinc supplemented groups . Thiamin intake of the placebo group was significantly greater compared to copper and zinc groups. In addition, copper group consumed greater amounts than zinc group. Zinc, manganese, and magnesium intakes of the placebo group were significantly greater than reported in the copper and zinc

Table 3. Mean nutrient intake of males at baseline [1,2,3]

Nutrient	Placebo	Copper	Zinc
n	4	4	4
Energy, Kcal	**2214 ± 497[a]**	**1514 ± 86[b]**	**1647 ± 363[a,b]**
Protein, g	**105.2 ± 28[a]**	**63.4 ± 10[b]**	**78.4 ± 10[a,b]**
	(168 ± 57)	(98 ± 10)	(119 ± 23)
Vit A, RE	**1536 ± 648**	**795 ± 397**	**877 ± 120**
	(154 ± 65)	(79 ± 40)	(88 ± 12)
Thiamin, mg	**2.24 ± 0.38[a]**	**1.42 ± 0.37[b]**	**1.38 ± 0.14[b, c]**
	(187 ± 32)	(118 ± 31)	(115 ± 12)
Riboflavin, mg	**2.20 ± 0.36[a]**	**1.40 ± 0.26[b]**	**1.86 ± 0.17[b]**
	(157 ± 25)	(100 ± 18)	(133 ± 12)
Niacin, mg	**31.05 ± 6.4[a]**	**18.3 ± 2.4[b]**	**23.5 ± 1.5[b]**
	(207 ± 42)	(122 ± 16)	(157 ± 10)
B 6, mg	**2.88 ± 0.56[a]**	**1.32 ± 0.53[b]**	**1.89 ± 0.26[b]**
	(144 ± 29)	(66 ± 27)	(95 ± 13)
B 12, ug	**8.54 ± 2.9[a]**	**4.1 ± 2.20[b]**	**3.81 ± 1.72[b]**
	(427 ± 145)	(205 ± 110)	(191 ± 86)
Folate, ug	**306 ± 18**	**226 ± 93**	**277 ± 61**
	(153 ± 9)	(113 ± 46)	(138 ± 30)
Vit C, mg	**185 ± 83[a]**	**78 ± 62[b]**	**129 ± 52[a,b]**
	(309 ± 138)	(129 ± 104)	(215 ± 87)
Vit D, ug	**8.59 ± 6.72**	**5.77 ± 3.85**	**4.98 ± 4.24**
	(172 ± 134)	(115 ± 77)	(100 ± 85)
Calcium, g	**949 ± 345[a]**	**548 ± 207[b]**	**941 ± 142[a]**
	(119 ± 43)	(79 ± 19)	(118 ± 18)
Copper, mg	**1.77 ± 0.35[a]**	**0.98 ± 0.23[b]**	**1.33 ± 0.38[a,b]**
	(118 ± 24)	(65 ± 15)	(89 ± 25)
Iron, mg	**17.5 ± 4.2**	**13.2 ± 2.7**	**13.8 ± 4.2**
	(175 ± 42)	(132 ± 27)	(138 ± 45)
Mg, mg	**423 ± 84[a]**	**244 ± 24[b]**	**312 ± 59[b]**
	(121 ± 24)	(70 ± 7)	(89 ± 17)
Mn, mg	**4.77 ± 2.29[a]**	**2.21 ± 0.52[b]**	**2.28 ± 1.16[b]**
	(239 ± 115)	(111 ± 26)	(114 ± 58)
Zinc, mg	**15.0 ± 2.2[a]**	**8.95 ± 2.32[b]**	**9.55 ± 3.01[b]**
	(100 ± 15)	(60 ± 15)	(64 ± 20)

1. LS Means ± SEM.
2. Figures in parenthesis are percent RDA/lower limit of ESADDI.
3. Values in a row with different superscripts are significantly different ($p \leq 0.05$).

supplemented groups as were the intakes of B_6 and B_{12}. The placebo group reported greater intakes of calcium and copper than the copper group but not greater than the zinc group. However, there were no significant differences in the nutrient intakes among males of the different groups for vitamin A, D, folate and iron. The intake of iron in the placebo group was higher compared to copper and zinc groups, but this difference was not statistically significant.

Females. The mean nutrient intakes of females of all the groups at baseline are presented in Table 4. For most nutrients no significant differences were found among females of the different groups (Table 4). The placebo group consumed significantly greater amounts of thiamin, niacin, and magnesium compared to the zinc supplemented group. Riboflavin intake was significantly higher in the placebo compared to chromium and zinc groups but not different from the copper group. However, intake of riboflavin of the copper group was significantly greater than the zinc group. Intake of iron was also found to be significantly greater in the placebo group compared the zinc group. There were no significant differences in the intake of copper or zinc between the groups (Table 4).

Changes Over Time by Gender

Placebo. In males at eight weeks of supplementation (M3) protein intake was significantly less than baseline consumption in placebo group. A significant decrease in intakes of copper and zinc was also found from baseline to four weeks post supplementation (M4) in this group. However, females in the placebo group reported a significant increase in copper intake from baseline to four weeks of supplementation (M2).

Chromium. Females in the chromium group reported consistent intakes throughout the study. There were no significant changes in the nutrient intakes over time.

Table 4. Mean nutrient intake of females at baseline [1,2,3]

Nutrient	Placebo	Chromium	Copper	Zinc
n	3	6	4	4
Energy, Kcal	**1906 ± 455**	**1527 ± 713**	**1540 ± 273**	**1389 ± 196**
Protein, g	**74.7 ± 17.7**	**66.4 ± 30.0**	**56.5 ± 4.8**	**54.5 ±12.5**
	(139 ± 40)	(122 ± 30)	(117 ± 12)	(120 ± 30)
Vit A, RE	**1577 ± 205**	**1266 ± 609**	**1305 ± 656**	**1121 ± 620**
	(197 ± 26)	(153 ± 76)	(163 ± 82)	(140 ± 78)
Thiamin, mg	**1.55 ± 0.49[a]**	**1.25 ± 0.41[a,b]**	**1.46 ± 0.30[a,b]**	**0.99 ± 0.17[b]**
	(155 ± 49)	(125 ± 41)	(146 ± 30)	(99 ± 17)
Riboflavin, mg	**2.16 ± 0.54[a]**	**1.52 ± 0.43[c]**	**1.89 ± 0.16[c]**	**1.17 ± 0.13[b]**
	(180 ± 45)	(126 ± 36)	(157 ±13)	(98 ± 11)
Niacin, mg	**2.51 ± 4.9[a]**	**18.8 ± 7.5[a,b]**	**18.8 ± 2.8[a,b]**	**15.5 ± 0.60[b]**
	(193 ± 37)	(144 ± 58)	(145 ± 22)	(119 ± 34)
B6, mg	**2.27 ± 0.43**	**1.85 ± 0.62**	**1.79 ± 0.80**	**1.55 ± 0.60**
	(142 ± 27)	(116 + 39)	(112 ± 5)	(97 ± 37)
B12, ug	**4.01 ± 0.40**	**3.78 ± 3.30**	**4.22 ± 1.76**	**3.00 ± 2.17**
	(201 ± 20)	(189 ± 165)	(211 ± 88)	(150 ± 108)
Folate, ug	**315 ± 106**	**252 ± 120**	**286 ± 100**	**237 ± 64**
	(175 ± 59)	(140 ± 67)	(159 ± 56)	(132 + 36)
Vit C, mg	**148 ± 89**	**87 ± 51**	**152 ± 59**	**99 ± 60**
	(246 ± 149)	(144 ± 86)	(254 ± 98)	(164 ± 101)
Vit D, ug	**4.55 ± 0.96**	**10.09 ± 14.7**	**5.05 ± 1.80**	**3.43 ± 2.70**
	(91 ± 19)	(202 ± 295)	(101 ± 36)	(69 ± 54)
Calcium, mg	**893 ± 318**	**578 ± 249**	**848 ± 243**	**584 ± 169**
	(112 ± 40)	(88 ± 26)	(106 ± 30)	(73 ± 21)
Copper, mg	**1.31 ± 0.22**	**1.05 ± 0.36**	**1.26 ± 0.39**	**1.06 ± 0.07**
	(88 ± 14)	(70 ± 24)	(84 ± 26)	(71 ± 5)
Iron, mg	**17.3 ± 4.4[a]**	**11.6 ± 6.5[a,b]**	**14.6 ± 3.7[a,b]**	**9.94 ± 0.56[b]**
	(173 ± 44)	(116 ± 65)	(146 ± 37)	(99 ± 6)
Magnesium, mg	**338 ± 105[a]**	**276 ± 96[a,b]**	**319 ± 35[a,b]**	**225 ± 43[b]**
	(120 ± 37)	(99 ± 34)	(114 ± 12)	(80 ± 15)
Manganese, mg	**3.35 ± 0.96**	**2.82 ± 1.25**	**3.52 ± 1.89**	**2.24 ± 0.83**
	(167 ± 48)	(141 ± 63)	(176 ± 94)	(112 ± 42)
Zinc, mg	**9.69 ± 2.10**	**7.46 ± 3.29**	**9.94 ± 3.73**	**6.38 ± 1.61**
	(81 ± 17)	(62 ±27)	(83 ± 31)	(53 ± 13)

1. LS means ± SEM.
2. Figures in parenthesis are percent RDA/lower limit of ESADDI.
3. Values in a row with different superscripts are significantly significant ($p \leq 0.05$).

Copper. Males in the copper group reported significant increase in folate and zinc intake from baseline to M2. Females in copper group did not report any significant changes in nutrient intakes except for a significant decrease in calcium intake from M1 to M4.

Zinc. Males in the zinc supplemented group reported intakes of vitamin C and calcium significantly lower at M4 compared to baseline. The intake of vitamin C at M4 was reduced to half the amount consumed at baseline in this group. Females in the zinc group consumed significantly greater amounts of iron and zinc at eight weeks of supplementation (M3) compared to baseline. The intake of vitamin C doubled from baseline to four weeks of supplementation (M2) in this group of females.

Thus, dietary changes over time of particular importance in this group were zinc, copper, and iron. There were decreases in the intake of copper and zinc in males of the placebo group, whereas copper consumption increased in females of the placebo group. There were increases in zinc consumption in males in the copper group unlike males in the placebo group. Increases in iron intake was reported by females in the zinc group.

Nutrient Density

Nutrient densities (amount of nutrient/1000 kcal) were calculated and compared for all measurement periods. No significant differences were found in the nutrient densities between the supplement groups at baseline. There were no significant differences in nutrient densities between males and females except for protein. The nutrient density for protein was significantly higher in males (44.9 g/1000 Kcal) compared to females (39.2 g/1000 Kcal). Thus, the quality of the diets were similar for both the sexes in all the groups at baseline.

Changes Over Time by Gender

Placebo. No significant changes occurred in nutrient densities in males of the placebo group except for thiamin, vitamin C, and zinc. The nutrient densities for these nutrients were significantly lower at four weeks supplementation (M2) compared to baseline. The nutrient density for vitamin C and zinc decreased from M2 to M3 and M4, but the decreases at M3 and M4 were not significant from M2. Nutrient densities did not change significantly overtime in females of the placebo group except vitamin A, riboflavin, B_{12}, and copper. There was a significant increase in the nutrient densities of these nutrients from baseline to M2. However, nutrient density for copper decreased significantly from M2 to M3 and the values were similar to baseline at M4. The nutrient density for zinc decreased significantly ($p=0.02$) from baseline (7.04 mg/1000 kcal) to four weeks of supplementation (5.97 mg/1000 kcal) in males in the placebo group. Females in the placebo group consumed diets with significantly higher nutrient density for copper at four weeks of supplementation (1.36 mg/ 1000 kcal) compared to baseline (0.70 mg/1000 kcal) ($p = 0.0017$). These intakes were greater compared to the findings of Holden et al. (1979). They reported a mean nutrient density of copper in adult men and women from a metabolic balance study to be 0.50 mg per 1000 kcal.

Chromium. There were no significant changes over time in the nutrient densities for females except for fiber in the chromium group. A significant decrease was reported from baseline to M4 in females in the nutrient density for fiber.

Copper. Significant differences were reported in nutrient densities for males in the copper group only for vitamin A, B_6, and folate. Nutrient densities for these increased significantly from baseline to four weeks of supplementation. There were no significant differences in the nutrient densities in females in copper group except for calcium which was significantly lower at M2 and M4 compared to baseline.

Zinc. No significant differences were observed in the nutrient densities in males and females in zinc group except for thiamin and vitamin C. The nutrient density for

thiamin at M4 was significantly greater compared to baseline in females, and the nutrient density for vitamin C was significantly greater at M2 compared to baseline in females.

Changes Over Time in Anthropometric Measurements

A significant increase in body mass index was found in females in copper group from baseline to eight weeks of supplementation ($p=0.01$). A significant decrease was found in body mass index in males in zinc group from baseline to eight weeks of supplementation ($p=0.02$). At four weeks of supplementation, males in the copper group were significantly lighter (mean 75 kg) compared to baseline (mean 80 kg) ($p=0.0015$). A significant decrease in percent body fat was found in males in copper group from baseline (24 percent) to eight weeks of supplementation (20 percent).

Effects of Trace Mineral Supplementation on Status Measures of Zinc, Copper, and Iron

Anthropometric and biochemical measures in males and females of each of the supplement groups were compared separately with males and females of the placebo group. The comparisons were made for each measurement period using Dunnett's equation. Significant differences were found for males or females of supplemented groups compared to the placebo group for the biochemical measures for hemoglobin, serum ferritin, plasma copper, and plasma zinc. No significant differences by gender were found in anthropometric measures of the supplement group compared to placebo. Plasma manganese concentrations were not included in the analyses. Our results were greater than reported values. The anticoagulant used was tested and found to be contaminated with manganese, thus the values were not used.

The treatment groups are compared with the placebo group (Table 5). Dunnett's was used separately for males and females to compare each treatment group with the placebo at every measurement period. The level of significance was set at 0.05.

Table 5. Significant differences within gender between the placebo and supplement groups using Dunnett's[1,2]

	Placebo			
	Baseline (M1)	Four Weeks (M2)	Eight Weeks (M3)	Four Weeks Post (M4)
Males				
Copper	Serum Ferritin ↑	Serum Ferritin ↑	-	Serum Ferritin ↑
Zinc	Serum Ferritin ↑	-	-	-
Females				
Chromium	Serum Ferritin ↓ Total Hemoglobin ↓	-	Serum Ferritin ↓ Plasma Copper ↑	-
Copper	Serum Ferritin ↓ Total Hemoglobin ↓	Serum Ferritin ↓ Plasma Copper ↑	Serum Ferritin ↓	Serum Ferritin ↓
Zinc	Serum Ferritin ↓	-	-	-

1. $P < 0.05$.
2. The direction of arrows represent the values of treatment groups as compared to the placebo group.

Chromium

The effects of chromium supplementation on status indicators of zinc, copper, and iron are presented in Table 6. Within the chromium group, serum alkaline phosphatase decreased significantly (p=0.0006) in females from baseline (74.8 U/L) to four weeks post supplementation (M4) (56.0 U/L). The values decreased steadily from baseline through M4, but only by M4 did the difference from baseline, M2, and M3 reach significance. However, this change did not occur in females of the placebo group. While the values in the chromium group for alkaline phosphatase were not significantly different from the individual measurement period values in the placebo group, the decrease overtime in the chromium group alone was significant. No significant changes were found in albumin and plasma zinc in females due to chromium supplementation. In women in the placebo group, there also were no changes in albumin or plasma zinc. Our subjects had initial plasma zinc values closer to the lower limit of the normal range. Hahn and Evans (1975) found a significant effect of zinc supplementation on chromium uptake. Zinc deficient rats had a significantly higher uptake of chromium compared to zinc replete rats. It shows that chromium and zinc compete with each other, and zinc absorption is affected in a depressed zinc status.

Plasma copper increased significantly in females from baseline (8.89 umol/L) to M3 (11.02 umol/L). A significant decrease was found from eight weeks of supplementation (M3) to four weeks of post supplementation (M4) (Table 6). However, no significant changes were found for plasma copper in females of the placebo group. The changes in plasma copper from baseline to eight weeks of supplementation in the chromium group were also significantly different from the placebo group (Dunnett's, Table 5).

Chromium supplementation led to a significant increase in hemoglobin in females from baseline to M4 (p=0.03) which was not found in females of the placebo group. In addition, a significant increase in plasma iron was found from M2 to M4 in the chromium

Table 6. Effects of chromium supplementation on status measures of zinc, copper, and iron[1,2,3,4]

Nutrient	Placebo				Chromium			
	Baseline (M 1)	Four Weeks (M 2)	Eight Weeks (M 3)	Four Weeks Post (M 4)	Baseline (M 1)	Four Weeks (M 2)	Eight Weeks (M 3)	Four Weeks Post (M 4)
Males (n)	4	4	4	4				
Albumin, g/dL	4.89 ± 0.16	4.75 ± 0.16	4.65 ± 0.16	5.12 ± 0.16	-	-	-	-
Alk Phos, U/L	61.6 ± 4.7	51.9 ± 4.7	57.3 ± 4.7	53.9 ± 4.7	-	-	-	-
Plasma Zn, umol/L	12.2 ± 0.8	11.6 ± 0.5	10.9 ± 0.5	11.3 ± 0.5	-	-	-	-
Plasma Cu,umol/L	6.20 ± 0.80*	6.07 ± 0.80*	5.95 ± 0.66*	6.29 ± 0.66*	-	-	-	-
Total Hb, g/dL	15.2 ± 0.4[a]	16.0 ± 0.4[a,b]	16.6 ± 0.4[b,*]	16.6 ± 0.4[b]	-	-	-	-
Serum Ferr,ng/mL	100 ± 9	89 ± 9	94 ± 9	88 ± 9	-	-	-	-
Plasma Fe,umol/L	13.5 ± 2.2*	15.9 ± 2.2	18.2 ± 1.8	18.9 ± 1.8	-	-	-	-
Females (n)	3	2	3	3	6	5	6	6
Albumin, g/dL	4.66 ± 0.19	5.02 ± 0.24	4.58 ± 0.19	4.94 ± 0.19	4.50 + 0.13	4.46 + 0.15	4.18 + 0.13	4.31 + 0.12
Alk Phos, U/L	65.2 ± 5.4	57.4 ± 7.0	59.4 ± 5.4	55.6 ± 5.4	74.8 + 3.8[a]	66.7 + 4.3[a]	67.1 + 3.8[a]	56.0 + 3.6[b]
Plasma Zn umol/L	12.8 ± 0.68	12.7 ± 0.8	12.3 ± 0.6	12.8 ± 0.6	11.4 + 0.4	11.4 + 0.5	11.5 + 0.4	11.2 + 0.4
Plasma Cu, umol/L	9.58 ± 0.76**	8.97 ± 0.98**	8.58 ± 0.76**	9.03 ± 0.76**	8.89 + 0.5[a]	10.31 + 0.6[a,b]	11.02 + 0.5[b]	9.51 + 0.6[a]
Total Hb, g/dL	15.5 ± 0.5	15.5 ± 0.6	14.9 ± 0.5**	15.7 ± 0.5	14.0 + 0.4[a]	14.5 + 0.4[a,b]	14.5 + 0.4[a,b]	15.1 + 0.3[b]
Serum Ferr, ng/mL	97 ± 10	78 ± 13	86 ± 10	84 ± 10	57 + 7	55 + 8	50 + 7	55 + 7
Plasma Fe, umol/L	19.3 ± 2.1**	18.5 ± 2.7	20.7 ± 2.1	18.9 ± 2.1	15.8 + 1.5[a,b]	14.5 + 1.7[a]	15.1 + 1.5[a,b]	18.2 + 1.4[b]

1. LS Means ± SEM. Alk Phos = Alkaline Phosphatase, Total Hb = Total Hemoglobin, Serum Ferr = Serum Ferritin.
2. Chromium group is compared against Placebo group within gender and measurement period (Dunnett's; $p \leq 0.05$). Differences in variables within each row with different symbols (#) are significantly different ($p \leq 0.05$).
3. Rows within supplement groups different alphabetic superscripts are significantly different ($p \leq 0.05$).
4. Variables by sex in columns with different superscripts (*) are significantly different ($p \leq 0.05$).

group (Table 6). This change did not occur in the females of the placebo group, but the placebo group was not significantly different from the chromium group at any of the measurement periods. While serum ferritin decreased from M1 to M3, these changes were not significant. However, from the Dunnett's equation, a significant difference was found between females of chromium and placebo groups in serum ferritin at eight weeks of supplementation (Table 6); the mean serum ferritin of females in the placebo group was significantly higher than that of the chromium group (Table 5).

The increases in plasma iron and total hemoglobin with a corresponding non-significant decrease in serum ferritin suggests mobilization of iron from stores due to the effect of chromium supplementation. This mobilization could be due to increased plasma copper, because copper helps in synthesis of hemoglobin. Iron is also needed for the synthesis.

Copper

The effects of copper supplementation on status indicators of zinc, copper, and iron are presented in Table 7. Copper supplementation did not have any significant effect on albumin or plasma zinc concentrations in either males or females. However, significant decreases were found in serum alkaline phosphatase concentrations in both males and females (Table 7). At four weeks post supplementation, males had significantly ($p=0.01$) lower concentrations of serum alkaline phosphatase (43.9 U/L) compared to baseline (60.4 U/L). Similarly, females showed a significant decrease from baseline (68.3 U/L) to eight weeks (52.9 U/L) and four weeks post supplementation (43.4 U/L). However, neither males nor females of the placebo group showed any changes in alkaline phosphatase nor were the values in the copper supplementation group significantly different from the placebo group for males or females (Dunnett's, Table 5).

No significant changes were found in either males or females over time for plasma copper. In females in the copper supplementation group, total hemoglobin and plasma

Table 7. Effects of copper supplementation on status measures of zinc, copper, and iron [1,2,3,4]

Nutrient	Placebo				Copper			
	Baseline (M 1)	Four Weeks (M 2)	Eight Weeks (M 3)	Four Weeks Post (M 4)	Baseline (M 1)	Four Weeks (M 2)	Eight Weeks (M 3)	Four Weeks Post (M 4)
Males (n)	4	4	4	4	4	4	4	4
Albumin, g/dL	4.89 ± 0.16	4.75 ± 0.16	4.65 ± 0.16	5.12 ± 0.16	4.98 ± 0.16	4.72 ± 0.16	4.98 ± 0.16	5.06 ± 0.16
Alk Phos, U/L	61.6 ± 4.7	51.9 ± 4.7	57.3 ± 4.7	53.9 ± 4.7	60.4 ± 4.7^{a}	51.8 ± 4.7^{a,b}	50.3 ± 4.7^{a,b}	43.9 ± 4.7^{b}
Plasma Zn, umol/L	12.2 ± 0.8$^{\#}$	11.6 ± 0.5	10.9 ± 0.5	11.3 ± 0.5	11.9 ± 0.6	12.7 ± 0.6	11.6 ± 0.6	11.0 ± 0.7
Plasma Cu, umol/L	6.20 ± 0.80*	6.07 ± 0.80*	5.95 ± 0.66*	6.29 ± 0.66*	7.65 ± 0.79	7.58 ± 0.66	7.16 ± 0.66	8.44 ± 0.66
Total Hb, g/dL	15.2 ± 0.4^{a}	16.0 ± 0.4^{a,b}	16.6 ± 0.4^{b,*}	16.6 ± 0.4^{b}	16.5 ± 0.4*	15.8 ± 0.4*	16.1 ± 0.4*	16.6 ± 0.4*
Serum Ferr, ng/mL	100 ± 9	89 ± 9	94 ± 9	88 ± 9	152 ± 9*	147 ± 9*	141 ± 9*	144 ± 9*
Plasma Fe, umol/L	13.5 ± 2.2*	15.9 ± 2.2	18.2 ± 1.8	18.9 ± 1.8	21.8 ± 1.8^{a}	15.5 ± 1.8^{b}	18.1 ± 1.8^{a,b}	17.8 ± 1.8^{a,b}
Females (n)	3	2	3	3	4	4	4	2
Albumin, g/dL	4.66 ± 0.19	5.02 ± 0.24	4.58 ± 0.19	4.94 ± 0.19	4.69 ± 0.16	4.68 ± 0.16	4.40 ± 0.16	5.15 ± 0.25
Alk Phos, U/L	65.2 ± 5.4	57.4 ± 7.0	59.4 ± 5.4	55.6 ± 5.4	68.3 ± 4.7^{a}	59.5 ± 4.7^{a,b}	52.9 ± 4.7^{b}	43.4 ± 7.2^{b}
Plasma Zn, umol/L	12.8 ± 0.68	12.7 ± 0.8	12.3 ± 0.6	12.8 ± 0.6	11.0 ± 0.6	11.3 ± 0.6	10.7 ± 0.6	11.9 ± 1.2
Plasma Cu, umol/L	9.58 ± 0.76**	8.97 ± 0.98**	8.58 ± 0.76**	9.03 ± 0.76**	8.67 ± 1.00	9.00 ± 0.76	8.50 ± 0.76	8.28 ± 1.47
Total Hb, g/dL	15.5 ± 0.5	15.5 ± 0.6	14.9 ± 0.5**	15.7 ± 0.5	13.4 ± 0.4^{a**}	14.0 ± 0.4^{a,b**}	13.3 ± 0.4^{a,b**}	15.0 ± 0.6^{b,**}
Serum Ferr, ng/mL	97 ± 10	78 ± 13	86 ± 10	84 ± 10	35 ± 9**	30 ± 9**	26 ± 9**	28 ± 9**
Plasma Fe, umol/L	19.3 ± 2.1**	18.5 ± 2.7	20.7 ± 2.1	18.9 ± 2.1	21.6 ± 0.4^{a}	15.2 ± 2.1^{b}	17.7 ± 2.1^{a,b}	24.2 ± 0.4^{a,b}

1. LS MEans ± SEM. Alk Phos = Alkaline Phosphatase, Total Hemoglobin = Total hemoglobin, Serum Ferr = Serum Ferritin.
2. Copper group is compared against placebo group within gender and measurement period (Dunnett's; $p \leq 0.05$)). Differences in variables within each row with different symbols (#) are significantly different ($p \leq 0.05$).
3. Rows within supplement groups with different alphabetic superscripts within each group are significantly different ($p \leq 0.05$).
4. Variables by sex in columns with different superscripts (*) are significantly different ($p < 0.05$).

iron increased significantly from baseline to four weeks post supplementation (Table 7). No significant effects of copper supplementation were found for serum ferritin concentrations in either males or females. However, in both males and females plasma iron decreased significantly baseline to four weeks of supplementation suggesting an initial effect of higher copper intakes. However, plasma iron partially recovered toward initial concentrations by four weeks post-supplementation period (males) or surpassed baseline concentrations (females) (Table 7). These changes were not evident in either males or females of the placebo group.

Dunnett's equation showed that there was a significant difference in values for serum ferritin concentrations at baseline, four weeks and four weeks of post supplementation in males (Table 5). Females in the copper group had significantly different serum ferritin concentrations at all measurement periods compared to females of the placebo group. Using Dunnett's, plasma copper of the females in copper group at four weeks of supplementation was significantly different from plasma copper of females in the placebo group (Table 5). Thus, when plasma copper concentration of the copper group was compared to the placebo group, four weeks of copper supplementation affected plasma copper in females although not significantly different from M1 within the group. Pratt et al. (1985) reported that supplementation with 10 mg of copper for 12 weeks did not affect serum copper concentrations in adults. However, their subjects were younger (mean age of 46 years) compared to our subjects; their sample was not divided according to sex; and the mean initial plasma copper concentration was 126 ± 22 ug/dL (19.8 umol) which was greater than the baseline means of our study. Bunker et al. (1984a) also reported higher plasma copper for elderly males (12.7 umol/L) and elderly females (14.0 umol/L) than found in this study. Their mean dietary copper intake was 1.28 ± 0.55 mg/day while in this study mean dietary copper was 1.26 ± 0.39 mg/day.

Zinc

As expected, zinc supplementation led to a significant increase in plasma zinc in both males and females. In males, plasma zinc concentrations at four and eight weeks of supplementation were significantly higher compared to baseline (Table 8). In females the decrease at M4 was significant as well as the increases at M2 and M3. A similar response to zinc supplementation was reported by Field et al. (1987). They found that with 100 or 150 mg of zinc/day for four weeks led to a significant increase in plasma zinc from baseline (11.10 ± 1.85 umol/L) to the end of the supplementation period (14.02 ± 1.55 umol/L). Their controls did not show any significant change over time. They did not find this response with 50 mg of supplementation. In our study 30 mg of supplementation for four weeks led to a significant increase in plasma zinc. No significant changes in plasma zinc was observed in our placebo group from baseline through four weeks post-supplementation.

The response of our subjects to zinc supplementation could be due to age. It was hypothesized by Wastney et al. (1992) that the response to zinc loads in the elderly is greater than is found in younger individuals. The mean basal plasma zinc of their subjects was 0.86 ± 0.10 mg/L. The baseline values for our subjects (11.0 umol/L) were at the lower end of the normal range. Their study identified the changes that occur in zinc regulation with aging. Our subjects' response to supplemental zinc was similar to that found by Wastney et al. (1991). In these studies zinc doses which ranged from 30 to 100 mg/d resulted in significant increases in plasma zinc. Samman and Roberts (1987) also reported a significant increase in plasma zinc in both adult men and women from initial concentrations when supplemented with 150 mg of zinc for six weeks. Plasma zinc increased from 15.1 ± 2.5 umol/L to 20.6 ± 4.6 umol/L in males and from 14.8 ± 2.5 to 23.2 ± 6.3 umol/L in females. In our study, males showed a significant increase in plasma from 11.0 ± 0.5 umol/L at baseline to 12.9 ± 0.6 umol/L at four weeks of supplementation; a slight increase was found from four to eight weeks of supplementation

Table 8. Effects of zinc supplementation on status measures of zinc, copper, and iron[1,2,3,4]

	Placebo				Zinc			
Nutrient	Baseline (M 1)	Four Weeks (M 2)	Eight Weeks (M 3)	Four Weeks Post (M 4)	Baseline (M 1)	Four Weeks (M 2)	Eight Weeks (M 3)	Four Weeks Post (M 4)
Males (n)	4	4	4	4	4	3	4	4
Albumin, g/dL	4.89 ± 0.16	4.75 ± 0.16	4.65 ± 0.16	5.12 ± 0.16	4.78 ± 0.16^{a,c}	5.65 ± 0.20^{b*}	4.61 ± 0.16^{c}	5.27 ± 0.16^{b}
Alk Phos, U/L	61.6 ± 4.7	51.9 ± 4.7	57.3 ± 4.7	53.9 ± 4.7	61.3 ± 4.7^{a}	46.5 ± 5.6^{b}	54.3 ± 4.7^{a,b}	43.1 ± 4.7^{b*}
Plasma Zn, umol/L	12.2 ± 0.8	11.6 ± 0.5	10.9 ± 0.5	11.3 ± 0.5	11.0 ± 0.5^{a}	12.9 ± 0.6^{b}	13.0 ± 0.5^{b}	11.7 ± 0.5^{a,b}
Plasma Cu, umol/L	6.20 ± 0.80*	6.07 ± 0.80*	5.95 ± 0.66*	6.29 ± 0.66*	7.38 ± 0.66	6.46 ± 0.79*	7.97 ± 0.65	7.09 ± 0.65
Total Hb, g/dL	15.2 ± 0.4^{a}	16.0 ± 0.4^{a,b}	16.6 ± 0.4^{b*}	16.6 ± 0.4^{b}	15.4 ± 0.4	16.6 ± 0.5*	15.7 ± 0.4	16.1 ± 0.4
Serum Ferr, ng/mL	100 ± 9	89 ± 9	94 ± 9	88 ± 9	158 ± 9^{a*}	115 ± 11^{b*}	104 ± 9^{b*}	114 ± 9^{b*}
Plasma Fe, umol/L	13.5 ± 2.2*	15.9 ± 2.2	18.2 ± 1.8	18.9 ± 1.8	17.6 ± 1.8	17.0 ± 2.2	19.0 ± 1.8	19.0 ± 1.8
Females (n)	3	2	3	3	4	4	4	4
Albumin, g/dL	4.66 ± 0.19	5.02 ± 0.24	4.58 ± 0.19	4.94 ± 0.19	4.54 ± 0.16	4.93 ± 0.16**	4.53 ± 0.16	4.90 ± 0.16
Alk Phos, U/L	65.2 ± 5.4	57.4 ± 7.0	59.4 ± 5.4	55.6 ± 5.4	67.0 ± 4.7^{a}	50.8 ± 4.7^{b}	62.0 ± 4.7^{a,b}	59.3 ± 4.7^{a,b**}
Plasma Zn, umol/L	12.8 ± 0.68	12.7 ± 0.8	12.3 ± 0.6	12.8 ± 0.6	11.0 ± 0.5^{a}	14.3 ± 0.5^{b}	13.5 ± 0.6^{b}	11.8 ± 0.5^{a}
Plasma Cu, umol/L	9.58 ± 0.76**	8.97 ± 0.98**	8.58 ± 0.76**	9.03 ± 0.76**	8.46 ± 0.65	8.95 ± 0.65**	8.68 ± 0.65	7.90 ± 0.65
Total Hb, g/dL	15.5 ± 0.5	15.5 ± 0.6	14.9 ± 0.5**	15.7 ± 0.5	15.0 ± 0.4	15.2 ± 0.4**	15.2 ± 0.4	15.5 ± 0.4
Serum Ferr, ng/mL	97 ± 10	78 ± 13	86 ± 10	84 ± 10	55 ± 9**	69 ± 9**	55 ± 9**	52 ± 9**
Plasma Fe, umol/L	19.3 ± 2.1**	18.5 ± 2.7	20.7 ± 2.1	18.9 ± 2.1	17.1 ± 1.8	21.1 ± 1.8	20.8 ± 1.8	18.4 ± 1.8

1. LS Means ± SEM. Alkal Phos = Alkaline Phosphatase, Total Hb = Total hemoglobin, Serum Ferr = Serum Ferritin.
2. Zinc group is compared against placebo group within gender and measurement periods (Dunnett's; $p \leq 0.05$). Differences in variables within each row with different symbols (#) are significantly different ($p \leq 0.05$).
3. Rows within supplement groups with different alphabetic superscripts are significantly different ($p \leq 0.01$).
4. Variable by sex in columns with different superscripts (*) are significantly different ($p \leq 0.01$).

which was not significantly different from the four week supplementation value but was different than initial plasma zinc. Females of our study also showed a significant increase in plasma zinc concentrations due to zinc supplementation from 11.0 ± 0.5 umol/L at baseline to 14.3 ± 0.5 umol/L at four weeks of supplementation. However, the increase in our subjects was lower compared to reported findings of Samman and Roberts (1987). This difference in response could also be due to the amount of dose. Freeland-Graves et al. (1982) reported a dose dependent increase in plasma zinc. Women supplemented with 100 mg zinc/day for eight weeks showed a greater increase in plasma zinc compared to women supplemented with 15 or 50 mg of zinc/day. They reported a significant increase in plasma zinc from initial values in subjects receiving 15, 30, or 100 mg of zinc daily to four weeks of supplementation. They also found that the plasma zinc in those subjects declined from four weeks and reached initial values at eight weeks of supplementation. We found a decrease in plasma zinc from eight weeks to four weeks post supplementation period which was a discontinuation phase rather than a supplementation phase as reported by Freeland-Graves et al. (1982). A significant decline was found among females in this study during the discontinuation phase but the decline in males was not significant. A significant quadratic effect on plasma zinc due to zinc supplementation was found in the zinc group (Table 8) which was not found in the placebo group. In a quadratic effect, the body responds to supplementation with increased absorption leading to an increase and then decreases after reaching the peak or by withdrawal of the supplement by excretion. This phenomenon is called homeostasis.

Significant changes in serum zinc in adult women supplemented with zinc were reported by Yadrick et al. (1989) although they reported no post-supplementation measures. Serum zinc increased significantly from baseline (12.9 ± 0.6 umol/L) to six (15.5 ± 0.7 umol/L) and ten weeks of supplementation (16.2 ± 1.1 umol/L). However, the dose was higher compared to the dose in our study and the initial concentrations were higher. Fischer et al. (1981) also reported a similar rise in plasma zinc from baseline to

two weeks of supplementation; no further changes were reported and the values plateaued at four and six weeks of supplementation. The authors did not provide any values at baseline or during supplementation.

Both males and females had significantly lower serum alkaline phosphatase concentrations at four weeks of supplementation compared to baseline (Table 8). Hambidge and coworkers (1983) also reported a lower mean alkaline phosphatase activity in pregnant women receiving 15 mg daily compared to controls who did not receive any supplements. The subjects of the placebo group in our study also did not show any significant changes in serum alkaline phophatase. Significant differences in albumin with zinc supplementation were found only in males. Albumin concentrations were significantly higher at four weeks of supplementation and at four weeks post supplementation than at baseline. However, there was a significant decrease in albumin from four to eight weeks of supplementation, and again a significant increase from M3 to M4 (Table 8). These changes were not found in males of the placebo group. A contrasting effect of zinc supplementation on albumin was reported by Hambidge et al. (1983). Mean serum albumin of the pregnant women receiving 15 mg of zinc daily decreased significantly from 3.8 ± 0.3 g/dL at 3 months to 3.2 ± 0.4 g/dL at 10 months. In this study the blood was measured every month until delivery. The difference in mean serum albumin could be due to the physiological demands of pregnancy on these subjects; also, the duration of supplementation was longer compared to our study.

Zinc supplementation did not have any significant effects on plasma copper in either males or females. Samman and Roberts (1988) also did not find any significant effect of zinc on copper. They supplemented adults with 150 mg of zinc and reported no significant changes. Their dose was higher compared to our study, and the duration was only six weeks compared to eight weeks in our study. Yadrick et al. (1989) did not find any significant effect with 50 mg of zinc supplementation on ceruloplasmin. However, they found a significant decrease in Cu-Zn erythrocyte superoxide dismutase from

pretreatment level to 10 weeks of supplementation. This suggests that superoxide dismutase is a more sensitive indicator of copper status which is affected at lower zinc doses than in plasma copper. The researchers who reported a significant decrease in plasma or serum copper used supplementation levels of over 100 mg/day of zinc unlike our study.

No significant effects of zinc supplementation were found for hemoglobin and plasma iron in either males or females in our study. However, a small non-significant increase in hemoglobin was found in females from baseline compared to M2, M3, and M4. Similar findings were reported by Yadrick et al. (1989) from a supplementation study on premenopausal adult women. However, in our study, serum ferritin decreased significantly in males from baseline to four, eight, and four weeks of post supplementation. This effect was not found in females (Table 7). Yadrick et al. (1989) reported a significant decrease in serum ferritin in premenopausal adult females from baseline (36.6 ± 7.4 ug/L) to ten weeks of supplementation (28.2 ± 5.6 ug/L). They supplemented adult women with 50 mg of zinc/day for a period of ten weeks. They also found that pretreatment levels of serum zinc were associated with the change in serum ferritin during supplementation. A greater decrease in serum ferritin was related to increased pretreatment levels of serum zinc. Initial plasma zinc concentrations of females subjects of our study was 11.0 ± 0.5 umol/L (normal: 11.5 - 18.5 umol/L) as compared to the subjects in the Yadrick study which was 12.9 ± 0.6 umol/L.

Differences Between Males and Females in Biochemical Measures at Different Measurement Periods

Placebo

At all the measurements, males in placebo group had significantly lower concentrations of plasma copper compared to females. They also had significantly higher total hemoglobin compared to females at eight weeks of supplementation. No significant

differences were found between males and females in this group for serum albumin, alkaline phosphatase, plasma zinc, and plasma iron. Though not significant, females had higher plasma iron concentrations at M1, M2, and M3 compared to males (Tables 6-8).

Copper

There were no significant differences between males and females of the copper supplemented group for albumin, alkaline phosphatase, and plasma minerals. However, total hemoglobin and serum ferritin concentrations were significantly higher in males compared to females at all measurement periods. Plasma copper was not significantly different between males and females (Table 7). However, Bunker et al. (1984a) reported a difference in plasma copper in different sexes in the elderly. From a metabolic balance study on elderly subjects consuming self-selected diets, the mean plasma copper levels for males and females were 12.7 and 14.0 umol/L. Our subjects had lower plasma copper concentrations, but, like Bunker and co-workers study, mean female plasma copper concentration was greater than that of males except at four weeks post-supplementation.

Zinc

At all the four measurement periods, males had significantly higher serum ferritin concentrations compared to females. Plasma copper in females was found to be significantly higher than males at four weeks of supplementation only. At four weeks of supplementation serum albumin and hemoglobin concentrations were significantly higher in males compared to females. Alkaline phosphatase was significantly higher in females compared to males at four weeks post supplementation. However, there were no significant differences in alkaline phosphatase between males and females at other measurement periods. No significant differences were found between males and females in plasma iron and plasma zinc concentrations at any measurement periods (Table 8).

Regardless of supplement group females had significantly higher concentrations of plasma copper and plasma iron compared to males. Males had significantly higher concentrations of serum ferritin and albumin than females.

CHAPTER V

SUMMARY AND CONCLUSIONS

Thirty volunteers over the age 55 years completed this trace mineral supplementation study conducted in summer of 1993. The purpose of the study was to investigate the effects of chromium, copper, and zinc supplementation on status indicators of zinc, copper, and iron. The study was approved by the Institutional Review Board of Oklahoma State University. An informed consent was obtained from the subjects prior to beginning of the study. Information about subjects' health, use of medications, and use of nutrient supplements was collected at the beginning of the study. Subjects were trained in keeping three-day food records by a Registered Dietitian.

The subjects were randomly assigned to different treatment groups. For these analyses the chromium group consisted of only females, while the other groups had both males and females. The chromium group (females = 6) was supplemented with 241 ug/day of chromium as chromium chloride; the copper group (males = 4; females = 4) was supplemented with 3.8 mg/day of copper as copper carbonate, and zinc group (males = 4; females = 3) was supplemented with 30.36 mg/day of zinc as zinc sulfate; and the placebo group (males = 4; females = 3) consumed 0.50 mg of lactose daily. The supplements were provided in divided doses taken in the morning and evening with meals. The subjects were supplemented for eight weeks. Fasting blood was collected at baseline (M1), four weeks of supplementation (M2), eight weeks of supplementation (M3), and four weeks post supplementation (M4). Three-day food records were kept by the subjects for all the measurement periods. Subjects were weighed at all the

measurement periods; however, height was obtained only at baseline and eight weeks of supplementation.

Plasma minerals were analyzed using atomic absorption spectrophotometry. Plasma zinc and iron were analyzed by flame with deuterium background correction; whereas plasma copper and manganese were analyzed on the graphite furnace with zeeman background correction. In addition to plasma mineral concentrations, serum albumin, alkaline phosphatase, hemoglobin, and serum ferritin were analyzed as status measures of zinc, copper, and iron. The SAS General Linear Model (GLM) procedure was used to test the effects of supplementation over time and interactions between measurement periods and supplements. Dunnett's test was calculated to compare males and females of each of the supplement groups separately with males and females of the placebo group for both anthropometric and biochemical measures. Dietary intakes and nutrient densities were calculated at all four measurement periods. Changes over time in nutrient intakes were assessed using the GLM procedure.

When the subjects were divided by gender, no significant differences were found between the supplement groups for anthopometric and biochemical measures except hemoglobin and serum ferritin at baseline.

A significant decrease was observed in alkaline phosphatase in females in the chromium group from baseline to four weeks of supplementation. Chromium supplementation did not affect albumin or plasma zinc in females. A significant increase in plasma copper was found from baseline to four weeks of supplementation and also during the discontinuation phase in females in the chromium group. Hemoglobin decreased significantly in females in the chromium group from baseline to four weeks post supplementation. At M4, plasma iron was significantly lower than at baseline in the chromium group.

Serum alkaline phosphatase concentrations were significantly lower at four weeks post supplementation than at baseline in both males and females in the copper group. A

significant increase in hemoglobin was found in females in the copper group from baseline to four weeks of supplementation. Both males and females showed significant decreases in plasma iron from baseline to four weeks of supplementation.

Zinc supplementation did not significantly affect plasma copper in either males or females. However, zinc supplementation led to a significant increase in plasma zinc after four weeks of supplementation in both males and females. In females in this group, plasma zinc decreased significantly during the post supplementattion period. A significant decrease from baseline values was found for serum alkaline phosphatase in both males and females at four weeks of supplementation. A significant increase in albumin after four weeks of supplementation was only found among males in the zinc group. Serum ferritin levels were significantly lower at M2, M3, and M4 compared to baseline in males in the zinc group.

A significant decrease for serum alkaline phosphatase was found in all the groups during the supplementation period. It was hypothesized that alkalaline phosphatase activity was affected due to bone metabolism. A decrease in the serum alkaline phosphatase could be related to increased bone metabolism. Since we collected our data during summer, it can be expected that the subjects were more exposed to sunlight which in turn may have increased bone deposition, thus, reducing serum alkaline phosphatase concentrations.

Conclusions

Testing of Hypotheses:

The placebo group was found to be different from the supplement groups in nutrient intakes as well as biochemical measurements. The males in the placebo group had significantly greater concentrations of plasma zinc compared to males in the copper and zinc groups at baseline. In addition, males in the placebo group consumed significantly higher amounts of zinc and copper compared to males in the copper and zinc

groups. Similarly, females in the placebo group had significantly higher concentrations of serum ferritin compared to the supplemental groups at baseline. Hence, the acceptance or rejection of hypotheses was based on the changes that occurred within the supplement groups only. The effects of supplementation rather than the differences between the placebo group and the supplement groups as calculated from Dunnett's equation were considered.

Breakdown of the groups according to gender resulted in very small sample numbers within each subgroup. This caused problems in statistically analyzing the data. Changes over time may be important biologically but may also not be apparent statistically.

Hypothesis one stated that there will be no significant effect of chromium supplementation on status measures of zinc. We accepted this null hypothesis because alkaline phosphatase decreased with supplementation and in the post supplementation period. However, no changes were found in plasma zinc, an indicator of zinc status in this study due to supplementation (Chapter IV, Table 6).

Hypothesis two stated that there will be no significant effect of chromium supplementation on status measures of copper. This hypothesis was rejected for females because there was a significant increase in plasma copper in females at eight weeks of supplementation compared to baseline. Plasma copper decreased significantly from M3 to M4 in the chromium group (chapter IV, Table 6). Also, plasma copper levels in the chromium group at M3 were significantly different from those of the placebo group (Dunnett's).

Hypothesis three stated that chromium supplementation will not have any significant effect on status measures of iron. We rejected this hypothesis for females, because a significant increase in plasma iron was found from baseline to four weeks post supplementation. A significant increase in hemoglobin from baseline to four weeks post supplementation was also found in the chromium group (chapter IV, Table 6). There

were no significant differences in serum ferritin levels at different measurement periods. There were also no significant differences between placebo and chromium groups for serum ferritin values at any measurement period.

Hypothesis four stated that there will be no significant effect of copper supplementation on status measures of zinc. No significant changes were found in either males or females in plasma zinc concentrations in the copper group. Thus, this hypothesis was accepted for either males and females.

Hypothesis five stated that there will be no significant effect of copper supplementation on status measures of copper. We accepted this hypothesis for both males and females since no significant changes over time were found in either males or females due to copper supplementation. However, from the Dunnett's equation it was found that at four weeks of supplementation, plasma copper in females of the copper group were significantly different from females in the placebo group. These discrepancies in the significant changes in plasma copper in females of the copper group could be explained with the fact that number of subjects were few, and concentrations were variable.

Hypothesis six stated that there will be no significant effect of copper supplementation on status measures of iron. No significant effects were found for serum ferritin concentrations in either males or females due to copper supplementation. A significant decrease in plasma iron was found in both males and females from baseline to four weeks of copper supplementation. In addition, only in females in the copper group hemoglobin concentration at M4 was significantly greater than at baseline (chapter IV, Table 7). Thus, the hypothesis was rejected for both males and females.

Hypothesis seven stated that there will be no significant effects of zinc supplementation on status measures of zinc. We found a significant increase in plasma zinc concentration in both males and females at four and a nearly significant effect ($p = 0.0508$) at eight weeks of supplementation (chapter IV, Table 8). In females in the zinc

supplemented group, a significant decrease was found from eight weeks of supplementation to four weeks post supplementation. Thus, a significant quadratic effect of zinc supplementation on plasma zinc was found. This hypothesis was rejected for both males and females.

Hypothesis eight stated that zinc supplementation will not significantly affect status measures of copper. This hypothesis was accepted for both males and females because no significant changes in plasma copper were found in either males or females over time due to zinc supplementation.

Hypothesis nine states that there will be no significant effects of zinc supplementation on status measures of iron. A significant decrease in serum ferritin concentrations was found in males, but not in females, from baseline to four, eight, and four weeks post supplementation (chapter IV, Table 8). Thus, we accepted this null hypothesis for females and rejected it for males.

RECOMMENDATIONS

Based on the results and experience gained in the study, recommendations for further research were developed by the author.

1. Include a larger sample size to minimize the variability in means of baseline biochemical and anthopometric measures.
2. Conduct the research separately for males and females of different age groups, because the Recommended Dietary Allowances are different for males and females.
3. Supplement the subjects with increased doses to have a greater effect on status indicators. This will ensure that the intake of the minerals is over the recommended levels. Higher than the RDA levels are frequently found in supplements available over-the-counter; the results then will be more applicable to the elderly populations consuming the nutrient supplements especially iron.

4. Increase the duration of supplementation giving subjects adequate time to adapt to higher levels of supplementation.
5. Compare older subjects with younger subjects to allow a better evaluation of the effects of supplementation in older people as compared to younger people.
6. Use a nutrient analysis program which contains chromium values or locate chromium values for key foods and calculate chromium values for recorded dietaries. This helps in assessing the dietary chromium intake of the subjects which has a bearing on the effects of chromium supplementation.
7. Train the subjects in recording consistent and accurate food intakes. This reduces the mistakes in recording the food intakes and leads to more accurate analysis of foods for nutrients.
8. Test the anticoagulant as well as all the solutions used for mineral contamination before using to decrease the potential for contamination.

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APPENDIX A

LETTER OF RECRUITING ANNOUNCEMENT

DO YOU HAVE HIGH BLOOD CHOLESTEROL?

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 blood cholesterol, bone metabolism and nutritional status?

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

Over the age of 55

Not using estrogen replacement therapy or drugs to control blood cholesterol

Do not have a chronic disease

Blood cholesterol levels greater than 240 mg/dl

This study is designed to determine the effect of minerals on blood lipids, bone metabolism and measures of trace mineral status. The study involves participation for 12 weeks during which time you will participate in body composition measurements (height, weight, skin-fold measurements, and bioelectric impedance), record food intakes and blood collections.

The first collection period will be at the beginning of the study to provide baseline data. For eight weeks you will take a supplement twice a day. The supplement will contain either lactose, 15 mg zinc, 1.5 mg copper, 100 ug chromium or combinations of these concentrations of copper/zinc or copper/chromium. Data will be collected after four and eight weeks of supplementation , and then four weeks after the end of supplementation.

All that is required of you is to record food intakes for three days prior to each data collection, take the supplement twice daily for eight weeks, and come to the Department of Nutritional Sciences at OSU at four week intervals for blood collection (30 ml or about 6 teaspoons). Weight and body composition measurements will be recorded at each data collection period. We ask that you do not attempt to lose weight or change your usual eating and exercising habits during this period.

Volunteers completing the study will receive a complementary lunch at Taylors Dinning Room.

This study has been approved by the Oklahoma State University Internal Review Board for the protection of human subjects.

Sound like fun?!! If you're interested or for further information please contact or tear off and send this response to:

Andrea B. Arquitt, PhD, RD/LD
Janice R. Hermann, PhD, RD/LD
Department of Nutritional Sciences
425 HES
College of Human Environmental Sciences
Oklahoma State University
Stillwater, OK 74078
tel. no. 744-5040

I am interested in more information on the blood lipid, bone mineral metabolism and trace mineral status study.

Name _____

Telephone number _____

APPENDIX B

INDIVIDUAL'S CONSENT FORM TO PARTICIPATE IN THE RESEARCH

Individual's Consent to Participate in Research

Effect of Supplementation with Chromium, Copper and Zinc on Plasma Lipids, Bone Metabolism and Indicators of Trace Mineral Status in Adults

I, _____, voluntarily agree to participate in the above titled research which is sponsored by Agriculture Experiment Station, College of Human Environmental Sciences at Oklahoma State University.

I understand that:

(1) the purpose of the study is to measure the effect of mineral supplementation on plasma lipids, bone metabolism and status of other trace minerals in adults;

(2) I will receive supplements containing either a placebo or ONE of the following minerals or mineral combinations which are equal to or less than those available in over-the-counter mineral supplements:
   (a) 15 mg zinc
   (b) 1.5 mg copper
   (c) 100 ug chromium
   (d) 15 mg zinc plus 1.5 mg copper
   (e) 1.5 mg copper plus 100 ug chromium;

(3) I will take one supplement with each morning and evening meal;

(4) I understand that these supplements may cause slight nausea if taken on an empty stomach and that is the reason for the above statement; however, if I have any adverse reactions I will contact one of the principal investigators;

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

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

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

(8) a phlebotomist will draw fasting blood samples of 30 ml (about 6 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;

(9) I understand that this blood will only be used for analyses which involve lipid status, mineral status and bone mineral metabolism and that after these analyses are performed the remaining blood will be incinerated and that no perpetual cell lines will be maintained;

(10) as a reward for participation and as an incentive to complete the study, I will receive one coupon for a complimentary luncheon at the Taylor Dining Room at the end of the study;

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

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

(13) I will withdraw from the project if I need to begin taking medication for my health during this 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 or Dr. Janice Hermann at (405) 744-5040 should I wish further information. I may also contact the office of University Research Services, 001 Life Sciences East, Oklahoma State University, Stillwater, OK 74078 telephone (405) 744-5700.

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 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 C

APPROVAL FROM OKLAHOMA STATE UNIVERSITY INSTITUTIONAL REVIEW BOARD FOR HUMAN SUBJECTS RESEARCH

[redacted]
[redacted]
[redacted]

Proposal Title: EFFECTS OF SUPPLEMENTATION WITH CHROMIUM, COPPER AND ZINC ON PLASMA LIPIDS, BONE METABOLISM AND INDICATORS OF TRACE MINERAL STATUS IN ADULTS

Principal Investigator: ANDREA B. ARQUITT/JANICE R. HERMANN

Date: 10-22-92 IRB # HES-93-011

This application has been reviewed by the IRB and

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

Renewal or Continuation [ ]

Approval Status Recommended by Reviewer(s):

Approved [xx] 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:

PROVISIONS RECEIVED

Signature: Marcia K. Tilley Date: 11-13-92

Chair of Institutional Review Board

APPENDIX D

HEALTH INFORMATION QUESTIONNAIRE

HEALTH INFORMATION QUESTIONNAIRE

Mineral Supplementation Study
Nutritional Sciences Department
Oklahoma State University

Subject Number _____ **Date of Birth** _____ **Race** _____ **Gender** _____

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

	No	Yes	When	Specify
Allergies	_____	_____	_____	_____
Inherited disorder	_____	_____	_____	_____
Uremia	_____	_____	_____	_____
Sickle cell anemia	_____	_____	_____	_____
Cancer	_____	_____	_____	_____
Diabetes	_____	_____	_____	_____
Heart disease	_____	_____	_____	_____
Liver disease	_____	_____	_____	_____
G I disorder	_____	_____	_____	_____
Blood Clotting Disorder	_____	_____	_____	_____

Are you on any type of special diet: Specify _____

Allergy	_____	_____	
Weight loss	_____	_____	
Weight gain	_____	_____	
Low fat, low cholesterol	_____	_____	
Diabetic diet	_____	_____	(kcal level_____)
Low sodium	_____	_____	
Other	_____	_____	Specify _____

Do you currently take any medications on a regular basis? _____

Specify all drugs taken _____

_____

_____

_____

APPENDIX E

MONTHLY DATA COLLECTION FORM

MONTHLY DATA COLLECTION FORM

Mineral Supplementation Study
Nutritional Sciences Department
Oklahoma State University

Subject Number _____

Date _____

1. Have you had a cold in the last month? Yes _____ No _____

   IF YES, when? _____

   how long did it last? _____

   did you have a fever? _____

2. Have you had the flu in the last month? Yes _____ No _____

   IF YES, when _____

   how long did it last? _____

   did you have a fever? _____

3. Have you had any other illness last month? Yes _____ No _____

   IF YES, what type of illness? _____

   how long did it last? _____

   did you have a fever? _____

4. IF YES TO ANY OF QUESTIONS 1 - 3, did you continue to take your supplement during the illness ? Yes _____ No _____

5. Did your exercise pattern change last month? Yes _____ No _____

   IF YES TO QUESTION 5, what way did your exercise pattern change:_____

   Type of exercise? _____

   IF YES, how often did you exercise?_____

   IF YES, how long do you presently exercise?_____

APPENDIX F

DIETARY RECORD FORM

DIET RECORD

Subject Number _____ Date _____

Time	Amount	Coding	Description/Brand Name

APPENDIX G

MODIFICATIONS TO BIOCHEMICAL PROCEDURES

Modifications of the Biochemical Procedures

Total Hemoglobin: The volumes in the procedure were reduced to one half. We used 10 ul of whole blood or lysed erythrocytes and 2.5 ml of Drabkin's solution. Thawed frozen whole blood or lysed erythrocytes were used. No other modifications to Sigma procedure # 525 of 1984 were made.

Albumin: Thawed serum was used to measure albumin. Sigma procedure # 625 was used.

Alkaline Phosphatase: Fresh samples were used to measure alkaline phosphatase on the same day as phlebotomy. Samples were incubated at room temperature for 45 minutes.

Serum Ferritin: No modifications were made to the procedure supplied by Diagnostic Products Corporation.

APPENDIX H

PROCEDURE FOR ASHING PLASMA

Appendix H

Ashing of Plasma

Frozen plasma samples were thawed for ashing. Two milliliters of plasma were pipetted into acid washed 13 X 100 mm Borosilicate glass tubes. The sample tubes were set at 85^{o}C to avoid popping of the samples. Temperature was raised to 95^{o}C after one ahour and the temperature was again raised to 105^{o}C after another hour. To protect the tubes from environmental contamination, the heating block was placed insida a polyethylene tank in a fume hood with the lid offset for venting.and allowed to ash for 24 hours until the samples and the blanks were completly dry. Each set of tubes with the corresponding blanks were placed upright in an inverted acid washed beaker set into an acid washed petridish lid for dry ashing.

The dry ashing oven was programmed to raise the temperature to 0.5^{o}/min upto 375^{o} and maintain the temperature for 1440 minutes (24 hours). The dry ashed samples were again wet ashed by adding to all the tubes 100 ul of glass distilled water, 100 ul of double distilled nitric acid, and 100 ul of high purity hidrogen peroxide. These additions were repeated two more times. The tubes were checked for bubling every ten minutes before each addition. More hydrogen peroxide (100-200 ul) was added until the bubbling stopped to ensure thorough ashing. The temperature was gradually increased from 85^{o}C to 105^{o}C as explained before. After the samples ashed to dryness (about 24 hours), they were dry ashed as before. The samples were wet and dry ashed for a total of four times to assure complete ashing. The tubes were closed with plastic caps and stored for further dilution.

Dilution of the Ashed Samples

To the ashed plasma, four ml of 0.5% nitric acid was added and vortexed thoroughly. Plasma manganese was read directly on furnace side of atomic aborption

spectrophotometer (AAS) with a final dilution of two fold. Plasma zinc and iron were read on the flame side of the AAS using the dilution factor of four. Plasma copper was read on the furnace side of the AAS using a 41-dilution.

VITA

Sowjanya Sudabattula

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF TRACE MINERAL SUPPLEMENTATION ON STATUS MEASURES OF ZINC, COPPER, AND IRON IN OLDER ADULTS

Major Field: Nutritional Sciences - Human Nutrition

Biographical:

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