THE EFFECTS OF SUPPLEMENTATION WITH ZINC OR ZINC AND IRON ON ZINC, IRON AND COPPER STATUS

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CHAPTER I

INTRODUCTION

Biological trace element research has been an active area of endeavor for the past few decades. However, development of methods to diagnose nutritional status with regard to trace elements has not kept pace with other research emphases (Mertz, 1985). Knowledge of the importance of trace minerals to health without adequate status assessment tools has opened the door to greater use of mineral supplements on the part of the general public.

According to a study by the Gallup organization (1982), 37 percent of the general adult population (approximately 60 million adults) used nutrient supplements in 1982, 85 percent of these on a regular basis. Usage was higher among women than men (42 percent of women compared to 31 percent of men surveyed). Of adult multivitamin users, 58 percent took a product containing minerals and/or iron. Nineteen percent of supplement users took a mineral supplement.

Self-supplementation is often practiced without the guidance of a qualified health professional. Supplements may be used for a variety of reasons and with little knowledge of functions or recommended intakes of nutrients

(Schutz et al., 1982). Iron and zinc are easily obtained over-the-counter in amounts equal to five to six times the Recommended Dietary Allowance (Physicians' Desk Reference for Non-Prescription Drugs, 1982).

Mertz (1984, 1985) warns of the risks associated with self-supplementation with trace element preparations. He states

> . . . increased intakes of one trace element, long before producing clinical signs and symptoms of toxicity, will interact with others and lead to subtle metabolic changes that can be detrimental to health.

Supplementation with zinc at levels greatly in excess of requirement has long been known to interfere with copper metabolism in animals (Magee and Matrone, 1960). Recently, alterations in copper status measurements have been demonstrated in children (Ghavami-Maibodi et al., 1983) and adult men (Fischer et al., 1984; Hackman and Keen, 1984) receiving 1.5 to 7 times the Recommended Dietary Allowance for zinc.

A competitive interaction has also been demonstrated between iron and zinc in humans. Iron inhibited zinc absorption when both were given in an inorganic form, without food, in ratios ranging from 50 mg iron/25 mg zinc to 51 mg iron/6 mg zinc (Valberg et al., 1984; Solomons and Jacob, 1981). Thus, supplementation with zinc or iron at levels currently available to the public may pose a threat to the trace mineral status of those routinely practicing supplementation.

Purpose and Objectives

The purpose of this study was to determine the effects of zinc and iron supplements on zinc, iron and copper status of adult females. The following objectives were formulated:

1. To determine the effects of zinc supplements on zinc, iron and copper status.

2. To determine whether use of an iron supplement in combination with zinc alters the effects of zinc supplements on zinc, iron and copper status.

3. To determine the relationship between initial zinc, iron and copper status and the effects of zinc or zinc and iron supplements on changes in zinc, iron and copper status.

Hypotheses

1. Zinc, iron and copper status measurements will not be affected by supplementation with zinc.

2. Following treatment, subjects consuming zinc and iron supplements will not differ in zinc, iron or copper status from those consuming only zinc supplements.

3. There will be no relationship between status measurements for zinc, iron and copper prior to supplementation and changes in status measurements following supplementation.

Assumptions and Limitations

1. The researcher assumes that subjects consumed supplements as directed.

2. Accuracy of dietary records was limited by the subjects' ability to estimate and record actual food intake.

3. The nutrient analysis computer program used underestimates intake of certain nutrients, and does not contain values for copper.

4. Application of the findings is limited to the sample group under study, since it was not a representative population sample.

CHAPTER II

REVIEW OF LITERATURE

Nutrient Supplementation

Extent of Supplement Usage

Continued growth in the nutrient supplement industry suggests that supplement usage in the United States is increasing. Manufacturers' shipments of vitamins, minerals and hematinics amounted to $2,279.9 million in 1984, compared to $1,301.9 million in 1977 (Pharmaceutical Preparations except Biologicals, 1984). Industry projections suggest that the overall market for vitamins will grow at a rate of six to eight percent per year over the next decade (Prog. Grocer, 1982). While multivitamins have the largest portion of the market, multivitamins with iron and those with minerals and iron follow (Drug and Cosmetic Industry, 1983). One market research firm projects a six percent share of the market for mineral supplements, accounting for $100 million in sales.

Usage of nutrient supplements was documented in 48% of U.S. households in a 1976 FDA survey (Consumer Nutrition Knowledge Survey, 1976). Later surveys of selected populations suggest a trend of increasing supplement consumption (see Table I).

Consumption of specific mineral supplements is not uniformly reported. Where reported, percent of sample using iron, zinc or multivitamins with minerals is included in Table I.

Several investigators report a greater level of supplement usage among females than males, regardless of age (Stewart et al., 1985; Gray et al., 1983; Schutz et al., 1982).

Clinical Use of Zinc Supplements

Zinc supplements have been used to treat a variety of conditions, including wound healing, sickle cell anemia, rheumatoid arthritis, retarded growth, and probable zinc deficiency. Supplemental treatment, for both therapeutic and experimental purposes, has involved use of a wide range of zinc levels, from levels at or near the Recommended Dietary Allowance (Walravens et al., 1983; Freeland-Graves et al., 1982) to those in excess of 10 times the R.D.A. (Prasad et al., 1978). Over-the-counter zinc supplements are available in dosages as high as 100 mg zinc in a single tablet (Physicians' Desk Reference for Nonprescription Drugs, 1982). Zinc sulfate salt and gluconate chelate are commonly available. Solomons et al. (1984) reported little difference in the bioavailability of these two compounds.

TABLE I

RECENT SURVEYS OF SUPPLEMENT USAGE

Study, Date	Sample	n	Percent of Sample Using			
			Any Sup.	MV+Min.	Fe	Zn
Stewart et al, 85	U.S. adults	2751	40	25	22	14
Worthington-Roberts and Breskin, 84	dietitians	665	60	21	7	
Griffin and Innes, 83	Windsor, Ontario households	1756	59	10		
Gray et al, 83	elderly	51	72		33	35
Read and Thomas, 83	vegetarians	49	85		6	10
Schutz et al, 82	adults	2451	67	26	7	8
Garry et al, 82	elderly	270	60		33	27
Harrill and Bowski, 81	elderly	285	75	43	12	14
English and Carl, 81	adults	60	67	22	2	
Willett et al, 81	nurses	1742	38			
Bootman and Wertheimer, 80	college students	147	51		2	
Adams et al, 80	children	657	24		8	
Cook and Payne, 79	children	60	50	23		
Rhee and Stubbs, 76	urban residents	600	66	3		

Metabolic effects of pharmacologic doses of zinc in man are largely unknown. In a metabolic balance study in which men were given 120-160 mg zinc/day over a period of several weeks, plasma zinc levels increased gradually until they approximately doubled (Spencer et al., 1977). Average zinc balance increased by approximately two mg per day. In another study by the same authors on a single subject, zinc retention was 23.1 mg/day with a high protein diet (90 g/day), while only 1.4 mg/day on a low protein diet (27 g/day). Zinc intakes ranged between 146-156.8 mg/day (Spencer et al., 1982). In neither of the above studies were experimental conditions detailed.

Sandstead (1978) expressed concern over the effects of high levels of zinc on copper status. It has long been known that high levels of zinc can interfere with copper metabolism in animals (Magee and Matrone, 1960). Prasad et al. (1978) reported the occurrence of hypocupremia and anemia in a sickle cell anemia patient who had received zinc supplements of 150-200 mg/day over a period of two years. Treatment with copper produced a return of plasma ceruloplasmin to normal levels.

Fischer et al. (1984) administered 50 mg zinc/day to male subjects for six weeks. 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.

Children receiving zinc supplements over a period of a year had decreased levels of copper in serum and hair with 100 mg zinc/day, but not 50 mg/week (Ghavami-Maibodi et al., 1983). Other workers reported declines in serum copper in runners receiving 22.5 mg zinc/day for four weeks, but not in non-runners (Hackman and Keen, 1984). No changes in whole blood or hair levels of copper occurred in women receiving 100 mg zinc/day for 60 days (Freeland-Graves et al., 1982) or in pregnant women consuming 45 mg zinc/day (Jameson, 1982).

Gastrointestinal side effects such as abdominal cramps, nausea, vomiting, bloating, constipation and diarrhea were commonly experienced with zinc supplementation at levels of 45-150 mg/day (Simkin, 1976; Greaves and Skillen, 1970; Jameson, 1982). In controlled double-blind studies, symptoms experienced by subjects receiving zinc were not unlike those of subjects receiving lactose placebo under identical conditions (Simkin, 1976; Hallbook and Lanner, 1972). Prasad (1978) suggested that zinc acetate is better tolerated than zinc sulfate. Supplements have been administered with meals (Freeland-Graves et al., 1982; Pecoud et al., 1975) and with a large volume of water in a zinc tolerance test (Freeland-Graves et al., 1980) to minimize gastrointestinal upset.

Few other metabolic effects of zinc supplements have been studied. Walravens et al. (1983) and Ghavami-Maibodi

et al. (1983) reported increases in height velocity or in height in children receiving zinc supplementation. Hooper et al. (1980) studied effects of zinc supplements on high density lipoprotein cholesterol (HDLC) levels in men, reporting an increase in HDLC after four and seven weeks of supplementation, with a return to normal levels when supplements were discontinued. Freeland-Graves et al. (1982) noted a transient increase in HDLC levels in women with 100 mg zinc/day.

Solomons et al. (1983a) studied the effects of zinc supplementation at 100 mg/day on absorption of an oral zinc load of 25 mg in an uncontrolled trial. Absorption, measured by the rise in plasma zinc, declined from initial levels following 28 days of supplementation, but at 56 days did not differ from initial levels. The authors concluded that short term zinc supplementation produced a reduction in intestinal uptake of pharmacologic doses of zinc.

Assessment of Changes in Mineral Status

Literature was reviewed to identify biochemical measures for zinc, iron and copper that are responsive to changes in mineral intake.

Zinc

Zinc has been measured in plasma/serum, erythrocytes, leukocytes, saliva, urine and hair, as well as in other body tissues and fluids. No single parameter has been

established at this time as accurately reflecting changes in total body zinc nutriture. Solomons (1979) recommended the use of multiple indices in assessment of zinc status.

Serum/Plasma Zinc. Circulating levels of zinc in plasma or serum has been the parameter of zinc status most frequently measured (Solomons, 1979). While not in itself an adequate single measure of zinc status, plasma/serum zinc levels have been shown to be responsive to changes in zinc intake. Experimental zinc depletion in humans was accompanied by a progressive fall in plasma zinc levels (Hess et al., 1977; Baer and King, 1984). Repletion of depleted subjects with 23.2 or 46.3 mg zinc/day resulted in a prompt increase in plasma zinc (Baer and King, 1984). Increases in plasma zinc occurred in women supplemented with zinc at 50 mg/day after 2-8 weeks supplementation (Buchanan et al., 1980; Freeland-Graves et al., 1982). However, levels of zinc in plasma and serum have been found to vary with oral contraceptive use (Prasad et al., 1975), infection (Wannemacher et al., 1975) and circulating levels of binding proteins (Solomons, 1979).

Saliva Zinc. Salivary zinc has been suggested as a means of evaluating zinc status. Freeland-Graves and coworkers reported decreases in the zinc content of the sediment of whole mixed saliva, but not in whole mixed saliva itself, in young women fed a low-zinc diet (1981) or a lacto-ovo-vegetarian diet (1980) for 22 days. Older women

consuming zinc supplements of 15-50 mg/day had increased salivary sediment zinc after 8 weeks (Buchanan et al., 1980), but no increases occurred in young women supplemented at levels of up to 100 mg zinc/day. Greger and Sickles (1979) reported lower levels of zinc in saliva supernatant, but not in whole saliva samples, when adolescent females were fed 11.5 mg zinc/day compared to 14.7 mg zinc/day during a 30-day metabolic study. No changes in stimulated parotid saliva zinc occurred in subjects consuming a 15 mg zinc supplement each day for 5 weeks (Lane et al., 1982). Composition of saliva can be affected by a number of variables, including source of saliva, flow rate, nature of flow stimulus, duration of stimulus, and time of collection (Dawes, 1970). Flow rate had a significant effect on the zinc concentration in parotid saliva, but no differences in zinc concentration were noted in samples collected over four consecutive days or between two consecutive months (Warren et al., 1981). Parotid saliva flow rate increased in subjects following supplementation with 15 mg zinc/day for five weeks (Lane et al., 1982).

Other Measurements of Zinc Status. Erythrocyte zinc decreased in experimental zinc deficiency after 12 weeks, and increased with zinc repletion (Prasad, 1978), but did not change in young men in a zinc depletion period of up to nine weeks (Baer and King, 1984). Prasad (1982) suggested that erythrocyte zinc does not reflect recent changes of body zinc stores, due to slow turnover. Neutrophil zinc

(Prasad, 1982) or leukocyte zinc (Hinks et al., 1982) may be more useful for this purpose.

Lowered activity of alkaline phosphatase, a zinc enzyme, has been reported in human zinc deficiency due to acrodermatitis enteropathica (Nelder and Hambidge, 1975) and unsupplemented total parenteral nutrition (Kay et al., 1976). No consistent changes in alkaline phosphatase activity occurred during experimental zinc depletion for up to five weeks in young women (Hess et al., 1977) or during depletion of up to nine weeks and repletion for two or more weeks in young men (Baer and King, 1978).

Iron

Iron loss in a population of menstruating women is approximately 1.4 mg/day (Leverton and Marsh, 1942; Finch, 1959). Development of iron deficiency when balance is negative occurs as a slow progression through several stages before development of frank anemia (Cook and Finch, 1979). The earliest stage is storage iron depletion, when reserves are lost, but there is not yet a decrease in iron supply to the developing red cell. Changes in other iron parameters do not occur until iron stores have been depleted.

Body iron in excess of that required for hemoglobin synthesis is stored in tissues as ferritin. In normal subjects, serum ferritin has been demonstrated to be a valid measure of tissue iron stores, and thus is the most sensitive measurement of iron depletion in initial stages

(Walters et al., 1973; Cook et al., 1974). Absorption of iron varies with iron stores. Female subjects with a mean serum ferritin level of 37 ng/ml, corresponding to 300 mg storage iron, absorbed less than 10 percent of an iron test dose, whereas those with serum ferritin averaging 8 ng/ml (65 mg storage iron) absorbed more than 40 percent (Cook et al., 1974).

Serum ferritin has been used to monitor changes in iron stores. A decrease in serum ferritin levels was observed in men experiencing repeated phlebotomy (Jacobs et al., 1972; Birgegard et al., 1978). Increases in serum ferritin occurred in patients treated with blood transfusion for primary refractory anemia (Jacobs et al., 1972) or homozygous beta-thalassemia (Letsky et al., 1974). Oral iron supplementation resulted in elevations in serum ferritin in non-anemic pregnant women (Thane-Toe and Thein-Than, 1982) and in male blood donors (Birgegard et al., 1978).

Copper

Copper-Zinc Superoxide Dismutase Activity. Superoxide dismutase (SOD) has been suggested as one of the most sensitive indicators of copper status (Fischer et al., 1984; Bettger et al., 1978, 1979; Klevay, 1984). Decreases in erythrocyte superoxide dismutase activity occurred with a copper-deficient diet in man (Reiser et al., 1985; Klevay, 1984), rats (Bettger et al., 1978), chicks (Bettger et al.,

1979) and swine (Williams et al., 1975). Suckling mice made copper-deficient due to the low copper diet of their dams had decreased SOD activity in liver and other tissues compared to copper-sufficient controls (Prohaska, 1983). Reiser et al. (1985) measured erythrocyte SOD activity in men fed a low copper diet with 20 percent of calories as starch or as fructose in a crossover design. Ingestion of fructose resulted in decreased SOD activity compared to starch. Repletion with three mg copper/day for three weeks increased SOD activity in subjects previously fed fructose but not starch.

Zinc deficiency had no effect on SOD activity in swine (Bettger et al., 1978) or chicks (Bettger et al., 1979), but supplementation with zinc at levels of approximately four times the requirement or recommended allowance produced decreased SOD activity in liver of rats (L'Abbe and Fischer, 1984b) and in red blood cells of men (Fischer et al., 1984) compared to controls.

Ceruloplasmin. The copper in ceruloplasmin accounts for 60-99 percent of total plasma copper, depending on the species (Evans, 1973). Ceruloplasmin levels decreased as a result of copper-deficient diet in rats (L'Abbe and Fischer, 1984a; Bettger et al., 1978), chicks (Bettger et al., 1979), roosters (Planas and Frieden, 1973) and suckling mice (Prohaska, 1983).

Roosters supplemented with 8000 mg zinc/kg, but not 2000 mg/kg, showed decreased levels of ferroxidase activity

(ceruloplasmin level) compared to controls (Planas and Frieden, 1973). Decreased ferroxidase occurred in rats supplemented with 240 mg zinc/kg, but not with lower levels of zinc in the diet (L'Abbe and Fischer, 1984b). No change in ferroxidase occurred in men supplemented with 50 mg zinc/day for 6 weeks (Fischer et al., 1984).

L'Abbe and Fischer (1984b) suggested a nonlinear relationship between the level of supplemental zinc and ferroxidase activity based on zinc supplementation in rats. Rats supplemented with zinc ranging from 15-240 mg zinc/kg diet demonstrated what authors termed an "all or none" response of ferroxidase to supplementation. Based on data gathered, L'Abbe and Fischer predicted an ED 50 level of 125.4 mg supplemental zinc: that level of zinc supplement at which half of the animals would be expected to have a low ceruloplasmin activity after six weeks of diet.

Interactions among Zinc, Iron and Copper

Effects of Zinc Supplementation on Tissue Levels of Iron and Copper

Decreased liver iron and copper levels were reported in rats fed supplemental zinc ranging from 400-1000 mg/kg (Magee and Matrone, 1960; Cox and Harris, 1960; Lee and Matrone, 1969). Addition of iron to the zinc supplemented diet (Zn/Fe=5) restored liver iron to control levels, but had no effect on liver copper (Cox and Harris, 1960). Liver copper equaled or exceeded control levels when dietary

copper at 14-25 mg/kg was given with supplemental zinc (Cox and Harris, 1960; Magee and Matrone, 1960). Addition of supplemental copper resulted in a further depression of liver iron concentration, however.

To identify further the effects of high levels of supplemental zinc on liver iron, Settlemire and Matrone (1967) examined the effect of dietary zinc (7500 mg/kg) on liver ferritin. Ninety-four percent of the decrease in liver iron was associated with the ferritin fraction. Both the amount of ferritin and the percentage of iron content of liver ferritin were reduced. An increase in liver zinc concentration was also associated with the ferritin fraction.

Some of the effects noted above may have been the result of inadequate levels of dietary copper rather than excess zinc (O'Neil-Cutting et al., 1981). Subsequent studies have addressed this problem by employing a factorial design to test effects of various combinations of zinc, iron and copper intake in rats and other species.

Whanger and Weswig (1970) reported a linear decrease in liver copper in rats with the addition of up to 2000 mg zinc/kg diet, while dietary copper was held constant at 6 mg/kg. Dietary zinc at 2000 mg/kg depressed the amount of copper in the soluble fraction, but not that in liver microsomes, nuclei and debris, or mitochondria.

Lonnerdal et al. (1985) studied rats fed 2, 25 and 500 mg iron/kg and 5, 100 and 1000 mg zinc/kg for 10 days in a

3x3 factorial design, Lowest liver and mucosal iron concentrations were found in the low iron high zinc group, and highest values in the high iron low zinc group. Hemoglobin and hematocrit values were not significantly affected by level of dietary zinc.

Reinstein et al. (1984a) used weanling rats to study the interaction between copper and zinc at levels equivalent to those consumed by the U.S. population, using a 2x5 factorial design. Copper was fed at 0.5 and 10 mg/kg, and zinc at 1, 4.5, 10, 100 and 1000 mg/kg for 42 days. As dietary zinc level increased, copper concentration decreased in heart, spleen, muscle and testes in rats fed the copper-deficient diet. A significant effect of the copper-zinc interaction on copper concentration of heart, muscle, spleen and kidney was reported. There was no effect of dietary copper or the copper-zinc interaction on tissue zinc concentrations. In the copper-deficient groups, iron concentration was higher in liver and lower in muscle than in copper-sufficient groups, regardless of zinc intake.

Johnson and Greger (1985) studied the effects of varying dietary levels of tin and zinc on mineral metabolism of rats. Diet contained approximately 35 mg iron/kg and 5.2 mg copper/kg. Dietary zinc intake ranged from 14.7 to 51.8 mg/kg. No effects of dietary zinc on tissue levels of iron or copper were noted.

Fischer et al. (1981) fed rats six levels of zinc ranging from 7.5 to 240 mg/kg. Basal diet contained 6 mg

copper/kg. Liver and serum copper levels were lower in animals fed 120 or 240 mg zinc/kg compared to those with lower dietary zinc.

Reinstein et al. (1984b) studied zinc-copper interactions in the pregnant rat. A factorial design was used with diets containing 1, 10, 100 or 1000 mg zinc/kg and 0.5, 5, 10 or 100 mg copper/kg. A significant effect of dietary zinc and the zinc-copper interaction was reported on maternal kidney copper concentration, but not for copper in other maternal tissues. Whole fetus copper and fetal liver copper were also affected by dietary zinc.

Dietary zinc affected iron concentration in maternal plasma, kidney and liver. Plasma iron was lower in groups fed 10-1000 mg zinc/kg than in those fed 1 or 4.5 mg zinc/kg. Liver iron was lower in the group fed 1000 mg zinc/kg than in that fed 1 mg zinc/kg. There was also a significant effect of dietary zinc on the iron concentration of the whole fetus, fetal liver and fetal brain. Iron concentration decreased as dietary zinc increased.

Hamilton et al. (1979, 1981) reported results of experiments examining zinc-iron-copper interactions in young Japanese quail. Using a factorial design, supplemental zinc was fed at levels ranging from 0 to 2000 mg/kg, copper at 0 to 5 mg/kg, and iron at 0 to 200 mg/kg. Basal diet contained 20 or 25 mg zinc/kg (requirement = 20), 1, 1.5 or 3.6 mg copper/kg, and 100 mg iron/kg. Supplementation with zinc only at 250 mg/kg or greater resulted in decreased

liver iron and copper. Liver iron was lower and liver copper higher with 3.6 mg copper/kg than with 1 mg/kg, for five out of seven zinc supplementation levels. Hemoglobin and hematocrit levels were also reduced with ≥250 mg zinc/kg.

Supplementation with iron (100 mg/kg) and copper (1.5 mg/kg) along with 500 mg zinc/kg resulted in liver iron and copper levels which did not differ from controls given no zinc supplement. Supplemental iron (Zn/Fe=5) protected against lowered liver iron levels, as did supplemental copper (1.5 mg/kg) for liver copper.

Iron-zinc interrelationships were examined in growing chicks in 14 day growth trials (Bafundo et al., 1984). Two levels of supplemental iron (0 or 500 mg/kg) were added to diets containing either 0 or 2000 mg zinc/kg. Basal diets were more than adequate in iron (200 mg/kg), but only marginally adequate in zinc (33 mg/kg). Zinc supplementation increased zinc concentration and decreased iron concentration in liver and intestinal tissue. Administration of supplemental iron along with zinc resulted in higher levels of zinc in liver and intestine than with zinc alone.

In a separate growth assay, these researchers administered supplemental iron and zinc to growing chicks in a ratio of 40/1 iron to zinc. They found that neither growth performance nor tissue zinc levels were adversely affected by the excess iron.

Iron-Zinc Interactions in Humans

Significant effects of zinc supplements on iron parameters in humans have not been reported. In a study of the effects of zinc administered to runners and non-runners at 22.5 mg/day for four weeks, serum iron was not affected in either group (Hackman and Keen, 1984). There were no effects on hematocrit levels in women consuming 100 mg/day for 60 days (Freeland-Graves et al., 1982) or in arthritis patients consuming 150 mg/day (Simkin, 1976).

In all animal species in which zinc supplementation has been studied, decreases in liver iron have been reported. No human studies have reported measurement of serum ferritin, which has been demonstrated to provide a valid measure of storage iron (Cook et al., 1974; Lipschitz et al., 1974).

Researchers investigating iron-zinc interactions in humans have dealt more extensively with conditions in which iron is present in excess relative to zinc than with high zinc/iron ratios. Hambidge et al. (1983) examined measures of zinc status in pregnant women either supplemented or unsupplemented with 15 mg zinc/day. Subjects also routinely took prenatal vitamins and iron supplements daily. Researchers reported a negative correlation between plasma zinc and the level of prenatal iron supplementation throughout the third trimester, and between serum alkaline phosphatase and level of supplemental iron during the first

and second trimesters in those subjects who were not supplemented with zinc.

Solomons et al. (1983b, 1981) have studied the effects of iron on zinc absorption in humans. Ferrous sulfate, but not heme iron, inhibited absorption of zinc sulfate as measured by the rise in plasma zinc after an oral dose containing iron/zinc in a ratio of 2/1 or 3/1. A significant decrement in plasma zinc occurred at one, two and three hours postdose.

These researchers also examined the relationship between iron status and iron-zinc interactions using similar methodology. Plasma iron, when used as a covariate for rise in plasma zinc, showed a significant positive correlation with uptake of plasma zinc from the iron-zinc mixture. Ingestion of 130 mg iron as ferrous sulfate by preschool children for four consecutive days prior to a zinc load did not affect plasma zinc uptake when zinc was administered alone or in combination with iron, compared to uptake without previous administration of therapeutic iron. The authors suggested that when the body's requirement for iron is higher, its enhanced passage into or through mucosa reciprocally reduces zinc entry.

Valberg et al. (1984) studied the effects of iron and copper on zinc absorption as measured by body retention of [65-Zn] zinc chloride. Capacity to absorb iron, as measured by serum ferritin, was not related to zinc absorption. While copper had no effect on absorption of a test dose of

65-Zn chloride, both inorganic iron (ferric chloride, Fe/Zn = 10) and heme iron (Fe/Zn = 5) decreased zinc absorption compared to control.

Copper-Zinc Interactions in Humans

Effects of zinc supplements on copper status measurements such as ceruloplasmin and erythrocyte superoxide dismutase have been reviewed in a previous section. Interactions between these minerals in humans have also been studied using the balance technique.

Greger et al. (1978a) found no difference in fecal copper excretion or copper retention when 7.4 or 13.4 mg zinc and 2.9 mg copper/day were fed to 14 adolescent girls for 18 days. All subjects were in positive copper balance. In another study involving 11 adolescent girls fed 1.2 mg copper daily, Greger et al. (1978b) reported increased fecal copper excretion and reduced copper retention when subjects were fed 14.7 mg zinc compared to 11.5 mg zinc/day for 10 day periods. All subjects were in apparent positive copper balance, however.

Burke et al. (1981) fed 11 elderly adults 2.33 mg copper and 7.8 or 23.3 mg zinc/day for 24 day periods. Mean fecal copper excretion was higher and apparent copper retention lower at 23.3 mg zinc compared to the lower level. Two subjects out of six fed the high zinc diet were in negative copper balance.

Taper et al. (1980) fed adult females 2 mg copper and 6, 8 or 24 mg zinc/day for 12 day periods. There was no difference among groups in fecal copper excretion, but all subjects were in negative copper balance.

Young women consuming 2.0 mg copper, low (9.5 or 10.1 mg) or high (18.4 or 19.9 mg) zinc, and low or high levels of nitrogen for 18 day periods were in slightly positive copper balance (Colin et al., 1983). The researchers suggested that mean balance would have been slightly negative if allowances had been made for whole-body surface losses.

Festa et al. (1985) studied the effects of varying levels of zinc intake on copper balance in men fed 2.6 mg copper/day. Subjects consumed 1.8, 4.0, 6.0, 8.0, 18.5, or 20.7 mg zinc/day, in one or two week periods, for a total of 63 days. Plasma copper was not altered outside the normal range in any subject, though it tended to decrease during the latter half of the study. Fecal copper increased and apparent copper retention decreased in subjects following an intake of 18.5 mg zinc for two weeks, after a period of lower zinc intake.

Iron-Zinc-Copper Interactions in Absorption

Interactions among zinc, iron and copper at the absorptive level have been studied in rats and mice using a variety of techniques.

Absorption of zinc from an intragastric test dose of 65-zinc chloride was increased in mice with a high capacity to absorb iron induced by a low iron diet, compared to iron-replete controls (Hamilton et al., 1978). Intestinal uptake and transfer of zinc, measured using perfusion of labeled zinc through open-ended duodenal loops, was greater in iron-deficient than iron-loaded mice. Presence of iron in the perfusate (at ratios of Fe/Zn of 5/1 and 10/1) inhibited intestinal uptake and transfer of zinc in both iron-deficient and iron-replete mice. A lower ratio of 2.5/1 Fe/Zn inhibited uptake and transfer in iron-deficient but not replete mice. Zinc in the perfusate at a ratio of 8/1 Zn/Fe, but not at lower ratios, inhibited iron uptake and transfer in iron-deficient mice.

In a later experiment using the same technique, zinc (Zn/Fe 10/1) inhibited iron uptake but not absorption (transfer to carcass) in iron-replete mice, and in those rendered iron-deficient by diet or bleeding (Flanagan et al., 1980). In both studies, zinc absorption was increased over absorption in iron-replete mice with dietary iron deficiency but not iron deficiency induced by bleeding. Similar effects of bleeding and dietary iron deficiency on zinc absorption in rats were noted by Pollack et al. (1965), but these authors reported no difference in copper absorption in iron-deficient rats compared to controls.

O'Neil-Cutting et al. (1981) studied the effect of supplemental zinc at 7500 mg/kg on iron absorption in rats.

Animals were fed the high zinc diet for a period of five weeks, then fed diet tagged with 59Fe. There was no difference in 59Fe retention at five days in zinc-supplemented rats compared to controls.

Fischer et al. (1981) measured copper absorption in rats fed varying levels of zinc using everted duodenal segments tied into sacs. Animals were fed 6 mg copper/kg and 7.5, 15, 30, 60, 120, or 240 mg zinc/kg for five weeks. Duodenal segments were incubated for 30 minutes in nutrient medium containing 6 mg copper/l, and copper transfer/mg mucosal cell protein was measured. There was a greater transfer of copper by those animals fed low levels of zinc compared to high levels. Rats fed 30-240 mg zinc/kg had more copper taken up in mucosal cells compared to those fed 7.5 mg zinc/kg. Rats fed 60-240 mg zinc/kg had greater mucosal cell copper uptake than those fed 15 mg zinc/kg. Mucosal cell supernatant was fractionated using a Sephacryl S-200 column. Copper was found to be associated with a protein of molecular weight similar to metallothionein.

Oestreicher and Cousins (1985) studied absorptive interactions of copper and zinc using the isolated, vascularly perfused rat intestinal system in a series of three experiments. In the first experiment, rats were fed 1, 6 or 36 mg copper/kg and 5, 30 or 180 mg zinc/kg in a 3 x 3 design. Following a seven-day period of intake, intestines were perfused with a medium containing 6 mg copper and 30 mg zinc per liter. There was no effect of

previous dietary intake of copper or zinc on the transfer of either mineral to the vascular perfusate during perfusion, nor was retention of copper or zinc in the mucosal cell affected by dietary levels of either mineral. Fractionation of the soluble fraction of the mucosal cells following perfusion revealed that with the higher zinc diets, there was a greater amount of cytosolic copper bound to metallothionein than to high molecular weight proteins, compared to the low zinc diet.

In the second experiment, rats were fed 30 mg zinc/kg and 6 mg copper/kg. Concentration of zinc in perfusate was 5, 30 or 180 mg/l, and of copper, 1, 6 or 36 mg/l. Low copper and medium or high zinc in perfusate resulted in decreased copper absorption compared to that with higher copper levels. This combination in the perfusate also resulted in increased zinc absorption compared to the low copper, low zinc combination.

In the third experiment, total intestinal metallothionein levels were measured in rats fed as described in experiment one. Rats fed low or high copper and low zinc had decreased metallothionein compared to the zinc and copper adequate (medium level of each) group. Those receiving high zinc had more intestinal metallothionein than medium or low zinc groups. The authors concluded that dietary zinc influences intestinal metallothionein content more than dietary copper.

Further, they suggested that over the range of dietary intake included in this study, neither metal significantly altered the absorption of the other, and that wide variations in intraluminal concentration seem to be necessary to influence absorption.

CHAPTER III

METHODS AND PROCEDURES

Subjects

Eighteen female volunteers, 25 to 40 years of age, participated in the study. The experimental protocol was approved by the Oklahoma State University Institutional Review Board and informed consent was obtained from each subject (Appendix A). Subjects were in good health, with no recent use of mineral supplements or drugs, including oral contraceptives.

Procedures

Experimental Design

Prior to the beginning of the experiment, serum ferritin levels and erythrocyte superoxide dismutase activity were measured for all subjects. Subjects were then ranked according to serum ferritin level and erythrocyte superoxide dismutase activity, and randomly divided into two groups, such that the groups did not differ for these two parameters of iron and copper status. Each group was then randomly assigned to a treatment.

Subjects consumed a mineral supplement each day for a 10 week period. Group Z took 50 mg of zinc as the gluconate and group F-Z 50 mg of zinc (gluconate) and 50 mg of iron as ferrous sulfate monohydrate daily.

Supplements were prepared in the lab by filling gelatin capsules with pulverized mineral tablets, so that supplements for the two treatments appeared identical. Subjects were thus blinded to treatment condition. Each day's allotment for a single subject was divided into two capsules. Subjects were instructed to consume one in the morning and the other in the evening, with a vitamin C source. Subjects received a 10 day supply of supplements at a time, and were instructed to bring the empty container from the previous time period with them to receive the next supply. This provided an opportunity to check on the actual supplement consumption.

Subjects were instructed to consume their normal diets during the course of the study. Blood and saliva samples were collected and three-day dietary records kept by the participants before inititation of supplementation, and after six and 10 weeks of supplementation.

Dietary Intake Data

Three-day food records were kept by subjects prior to supplementation, and after six and 10 weeks of supplementation (Appendix B). Subjects were trained to estimate intakes using food models and other tools.

Nutrient intakes were computed from food records using the Healthaide microcomputer program, which uses USDA Handbook 8 as a data base. Additional foods were added to this program by the investigator.

Clinical Data

A questionnaire was used to obtain data on menstrual cycle, exercise patterns, alcohol use, smoking, drug and nutrient supplement use and medical history for each subject (Appendix B).

Blood and Saliva Collection

Fasting samples of venous blood were collected from each subject between 8:00 and 10:00 A.M. using stainless steel needles and vacutainer tubes suitable for trace mineral analyses.

Plasma and serum were obtained following centrifugation at 3000 rpm for 20 minutes in a clinical centrifuge and frozen for subsequent analysis of zinc, ceruloplasmin and ferritin. Hemoglobin and hematocrit levels were measured using whole blood, and red cells were separated and prepared for analysis of superoxide dismutase activity.

Unstimulated fasting samples of saliva were collected between 7:00 and 10:00 A.M. Prior to collection each subject thoroughly cleansed her tongue, teeth and oral cavity with a toothbrush and deionized, distilled water and rinsed three times with deionized water (Appendix B).

Saliva samples were frozen for later analysis of salivary sediment zinc.

All glassware and plasticware used for storage or mineral analysis of samples was acid washed and rinsed thoroughly with deionized, distilled water.

Biochemical Analyses

Salivary Sediment Zinc

Salivary sediment zinc (SSZn) was determined according to the method of Freeland-Graves et al., 1981. Frozen saliva samples ranging in volume from 6 to 15 ml were thawed and centrifuged for 10 minutes at 14,800 x g at 4 degrees C to obtain salivary sediment. The supernatant solution was discarded and the sediment transferred to porcelain crucibles. The sediment was dried at 105 degrees C for 24 hours, ashed in a muffle furnace at 500 degrees C for 24 hours, dissolved in 7N nitric acid, and diluted with deionized water as indicated in Table II. Duplicate sample blanks were included with each analysis.

Sample solutions and blanks were compared with aqueous zinc standards containing four percent 7N nitric acid. Certified atomic absorption standard zinc reference solution of 1002 micrograms/ml was used to prepare standard solutions ranging from 0.1-0.4mg zinc/l. Samples were analyzed for zinc using a Perkin-Elmer atomic absorption spectrophotometer, model 5000. EPA zinc standards were used for quality control.

TABLE II

DILUTION OF SALIVARY SEDIMENT ASH

Salivary Sediment Dry Weight (mg)	Volume of 7N Nitric Acid Added (ml)	Final Dilution Volume (ml)
≤10mg	0.2	5
>10mg≤20mg	0.4	10
>20mg	1.0	25

Zinc in salivary sediment was calculated according to the following formula:

Zinc concentration(mg/l) X 1 liter/1000 ml X dilution volume(ml) X 1/dry weight of sample(g) = mcg zinc/g salivary sediment (dry weight).

Serum Zinc

Zinc in serum (BZ) was analyzed according to methods described by Smith et al. (1979). Frozen serum samples were allowed to come to room temperature, then mixed by gently inverting storage vials six times. A 1.0 ml serum sample was diluted with 4.0 ml deionized water in a plastic sample bottle and mixed by inversion.

Sample solutions and blanks were compared with zinc standards prepared in glycerol/water (5/95 by volume). Certified atomic absorption standard zinc reference solution

containing 1002 micrograms/ml was used to prepare zinc standards ranging from 0.1 mg-0.4 mg/l. Serum samples were analyzed using a Perkin-Elmer atomic absorption spectrophotometer, model 5000. EPA zinc standards were used for quality control.

Hemoglobin and Hematocrit

Whole blood hemoglobin and hematocrit levels were determined by the cyanmethemoglobin method and by microhematocrit centrifugation, respectively.

Serum Ferritin

Serum ferritin (Sfer) was measured using the nEIA-Ferritin enzyme immunoassay. Frozen serum samples were brought to room temperature. Standards, controls, and 50 microliter serum samples, in triplicate, were placed in tubes coated with antibodies to human ferritin, and incubated on a clinical rotator for two hours. Contents were aspirated, and 500 microliters of a ferritin-specific antibody conjugated to alkaline phosphatase added to each tube. Tubes were incubated as before. The incubate was discarded and tubes washed three times with distilled, deionized water. Water remaining in the tubes was aspirated after a three to five minute standing period. One milliliter of p-nitrophenyl-phosphate was added, and tubes were incubated for 1/2 hour at 37 degrees C. Following incubation, 300 microliters of 0.5M NaOH was added to each

tube and tubes were shaken, stopping the reaction of alkaline phosphatase with substrate. This reaction produces a colored end-product, p-nitrophenol. Product is directly proportional to the amount of ferritin bound to the solid phase antibody. Absorbance of samples and standards was measured at 405nm using distilled water as a blank.

Serum Ceruloplasmin

Ceruloplasmin (Cp) was determined on five microliter samples of serum using the radial immunodiffusion technique of Mancini et al. (1965). NOR-Partigen Ceruloplasmin kits for the assay were obtained from Behring Diagnostics. Standards and serum samples were applied to cylindrical wells cut in gel matrix containing uniform concentration of antibody to ceruloplasmin. Plates were incubated for 24 hours at room temperature (20 degrees C.), allowing for the formation of a precipitin ring for each sample and standard. A standard curve was prepared for each plate. Concentration of ceruloplasmin was plotted against the squared diameter of the precipitin rings for each standard. Serum ceruloplasmin in samples was quantitated by comparing the diameter of the precipitin ring produced by the sample to those produced by the standards.

Erythrocyte Superoxide Dismutase Assay

Preparation of Red Cells. Heparinized blood was centrifuged at 1000 X g for 20 minutes at 4 degrees C.

Blood plasma was removed and frozen for later analysis. White cells were removed with suction and discarded.

Erythrocytes were washed two times with cold 0.9% NaCl and recentrifuged between washes. Approximately one gram of red cells was removed, weighed and lysed with 1.5 ml of water. A 0.5 ml volume of hemolysate was diluted with 1.5 ml ice-cold distilled water and mixed. Chloroform (0.3 ml) and ethanol (0.5 ml) were added; the mixture was vortexed after each addition, then mixed by inverting one minute. Tubes were centrifuged at 1000 X g for 15 minutes and the supernatant solution, containing the enzyme, was removed and transferred to individual plastic vials.

Assay. Activity of Cu superoxide dismutase (ESOD) was determined using a photochemical o-dianisidine-riboflavin assay (Paynter, 1980; Misra and Fridovich, 1977). The assay mixture consisted of 2.7 ml of 50mM potassium phosphate containing 0.1mM ethylenediaminetetraacetate (pH 7.8), 0.1 ml of 6mM o-dianisidine dihydrochloride in water and 0.1 ml of 0.39mM riboflavin in 10mM potassium phosphate (pH 7.8). To this reaction mix, 0.1 to 0.2 ml of red cell extract was added. Following a preincubation of five minutes in subdued light at room temperature, absorbance of the sample at 460nm was read. Samples were then placed beneath the light source (two 15 watt flourescent tubes) and incubated for exactly eight minutes. Absorbance at 460nm was again determined and the net reaction rate calculated.

For each sample, a three-point enzyme dilution curve was constructed, according to the method of Paynter (1980).

Activity in each sample was determined by comparing the slope of the enzyme dilution curve to the slope of the standard curve obtained using purified bovine superoxide dismutase (Sigma Chemical Company) in the assay instead of erythrocyte SOD extract. One activity unit was defined as that activity equivalent to 1 microgram of purified bovine SOD. Activity in samples was calculated based on red cell weight, according to the following formula:

SOD activity/g red blood cells =

SOD activity/microliter supernatant X
1000 microliter/ml X
2.5 ml supernatant/0.5 ml hemolysate X
[volume of red cell sample(ml) +
1.5 ml hemolysate]/
volume of red cell sample(ml)

volume of red cell sample(ml) =

weight of red cell sample(g)/
1.1 g red cell/ml red cell

Statistical Analyses

Blood and saliva data were analyzed by analysis of variance using the General Linear Models procedure of the Statistical Analysis System (SAS). Analysis was done as a split-plot to determine effects of treatment, length of treatment (weeks) and treatment by weeks interaction. Both absolute levels and differences between pre-treatment levels and levels after six and ten weeks of treatment were used for analysis. Two-group difference t-tests were used to

compare effects of treatment for a specific time (after six or ten weeks of treatment).

Effects of length of treatment were compared within each treatment using analysis of variance. Pretreatment measurements of zinc, iron and copper in blood and saliva were used singly as covariates to identify relationships between zinc, iron and copper status and effects of treatment on mineral status. Possible relationships between saliva flow rate and salivary sediment zinc levels were examined using correlation coefficients and covariate analysis.

Dietary data were subjected to analysis of variance to compare intake of selected nutrients over the course of the study within each treatment and averaged over treatments. Two group t-tests were used to compare mean nutrient intakes between treatment groups, and within treatments for each time interval. Possible relationships between nutrient intake and treatment effects on zinc, iron and copper status were examined using covariate analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Description of the Sample

The sample consisted of 18 adult female volunteers. The original sample included 19 women, but one subject was dropped from the study after two weeks due to inability to tolerate the supplements. Age, weight, and pretreatment levels of zinc, iron and copper status measurements for each treatment group are reported in Table III. Raw data for each subject are included in Appendix C.

Nutrient Intake

Mean daily intakes of selected nutrients for each treatment group, averaged over the course of treatment, are reported in Table IV. The two groups differed only in their consumption of vitamin C. Raw data by subject are reported in Appendix C. Nutrient intakes were also compared within treatment groups over the course of the study. Levels prior to treatment were compared with those after six and ten weeks of treatment, and are reported in Tables V and VI. There were no differences in intake for different times in the group receiving zinc and iron. In the zinc only group, no differences existed between pretreatment intakes and

those at ten weeks. Six week levels of vitamin C, fiber and phosphorus did differ from those at 10 weeks for the zinc only group ($P < 0.05$).

TABLE III

AGE, WEIGHT AND PRETREATMENT MINERAL STATUS LEVELS OF SUBJECTS*

Variable	Group Z	Group F-Z
Age (years)	30.8 ± 5.0 (9)	32.8 ± 6.2 (9)
Weight (kg)	60.7 ± 5.0 (9)	57.6 ± 10.2 (9)
Hgb (g/dl)	14.9 ± 1.3 (9)	15.0 ± 0.8 (8)
Hct (vol %)	43.7 ± 2.6 (9)	41.8 ± 1.6 (8)
SOD (mcg/g RBC)	127.7 ± 32.2 (9)	139.5 ± 18.2 (9)
Cp (mg/dl)	29.4 ± 6.1 (9)	31.2 ± 6.0 (9)
Serum Zn (mcg/dl)	84.5 ± 11.1 (9)a	73.8 ± 7.0 (9)b
SSZn (mcg/g dry wt)	78.4 ± 22.3 (9)	101.1 ± 38.8 (7)

* Data are expressed as means ± standard deviation for sample size in parentheses; means in same row followed by a different letter are significantly different ($P < 0.05$).

TABLE IV

COMPARISON OF AVERAGE DIETARY NUTRIENT INTAKE BETWEEN TWO TREATMENTS*

Nutrient	Group Z	Group F-Z
Kcal	1933.8 ± 335.3	1725.8 ± 252.9
prot (g)	70.5 ± 16.6	63.3 ± 15.6
fiber (g)	4.3 ± 1.6	4.4 ± 1.4
vit C (mg)	93.7 ± 26.8a	136.8 ± 42.0b
P (mg)	1181.5 ± 337.4	1097.5 ± 175.0
Ca (mg)	799.6 ± 270.3	756.5 ± 220.1
Mg (mg)	250.7 ± 70.5	243.6 ± 44.0
Fe (mg)	13.5 ± 3.0	11.8 ± 3.5
Zn (mg)	7.8 ± 1.7	6.9 ± 1.4

* Data are expressed as means ± standard deviation; means in the same row followed by a different letter are significantly different ($P < 0.05$).

TABLE V

AVERAGE NUTRIENT INTAKE FOR ZINC TREATMENT GROUP BY TIME PERIOD*

Nutrient	Pretreatment	6 weeks	10 weeks
kcal	1861.8 ± 247.6	2013.2 ± 499.3	2003.5 ± 303.1
prot(g)	66.9 ± 11.1	76.2 ± 23.3	71.0 ± 19.2
fiber(g)	4.5 ± 1.7	4.9 ± 2.1a	3.8 ± 1.4b
vit C(mg)	93.2 ± 36.9	110.3 ± 39.9a	75.8 ± 20.2b
P (mg)	1119.2 ± 299.7a	1312.7 ± 448.5b	1188.8 ± 275.4
Ca (mg)	727.8 ± 269.0	904.5 ± 353.3	806.6 ± 282.9
Mg (mg)	276.1 ± 88.2	246.9 ± 83.4	244.4 ± 51.0
Fe (mg)	13.9 ± 3.4	14.1 ± 4.7	13.2 ± 2.5
Zn (mg)	7.7 ± 2.1	8.2 ± 2.3	7.9 ± 1.2

* Data are expressed as means ± standard deviation; means in the same row followed by a different letter are significantly different ($P < 0.05$).

TABLE VI

AVERAGE NUTRIENT INTAKE FOR IRON-ZINC TREATMENT GROUP BY TIME PERIOD*

Nutrient	Pretreatment	6 weeks	10 weeks
kcal	1658.6 ± 358.4	1849.6 ± 429.5	1669.3 ± 275.0
prot(g)	62.6 ± 17.4	67.7 ± 32.6	59.8 ± 13.4
fiber(g)	4.4 ± 1.3	4.4 ± 1.7	4.3 ± 1.9
vit C(mg)	130.9 ± 51.5	131.6 ± 44.6	147.8 ± 69.9
P (mg)	1109.8 ± 319.2	1108.3 ± 264.3	1074.3 ± 216.8
Ca (mg)	760.8 ± 316.2	762.3 ± 250.8	746.3 ± 197.7
Mg (mg)	244.4 ± 58.0	253.7 ± 65.2	236.0 ± 61.3
Fe (mg)	11.1 ± 2.5	13.4 ± 5.7	10.9 ± 3.4
Zn (mg)	6.9 ± 1.6	7.4 ± 2.6	6.5 ± 2.0

* Data are expressed as means ± standard deviation.

Effect of Treatment on Zinc, Iron and Copper Status

Zinc

Effects of treatment and length of treatment (weeks) on serum zinc levels are reported in Tables VII through IX. Analysis of variance showed a significant effect of length of treatment on serum zinc. Both treatments resulted in significant increases in serum zinc levels (Table VIII). Increases were greater at six and 10 weeks for Group Z than for F-Z, but the difference between the treatments was not significant (P=0.08 at six weeks). There was a greater increment in serum zinc levels at 10 weeks than at six. This is illustrated in Figure 1.

TABLE VII

ANALYSIS OF VARIANCE FOR CHANGE IN SERUM ZINC

Source	df	Mean Square	F	P
Treatment	1	0.0222	0.78	0.39
Error (a)	16	0.0301		
Weeks	1	0.0193	8.18	0.01
Trt X Weeks	1	0.0001	0.06	0.82
Error (b)	12	0.0024		

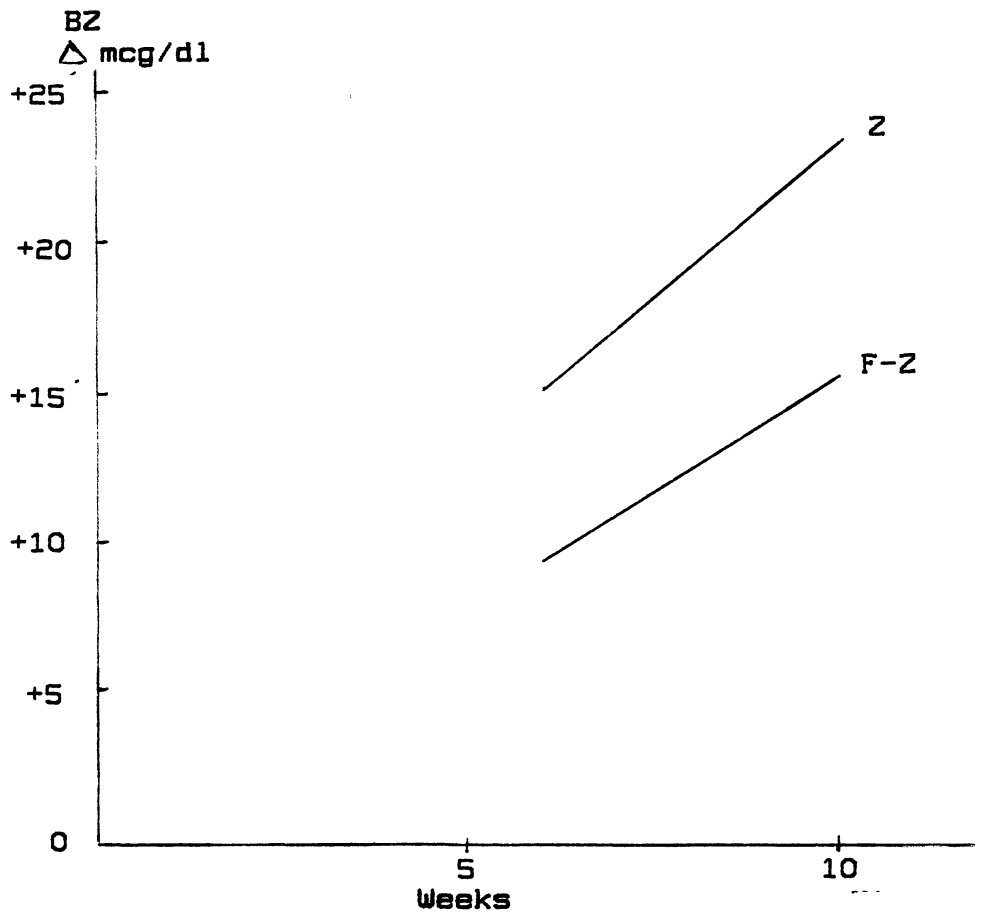

Figure 1. Effect of Treatments on Change in Serum Zinc

TABLE VIII

EFFECT OF TREATMENT AND LENGTH OF TREATMENT ON SERUM ZINC*

	Change in Serum Zinc (mcg/dl)**	
Weeks	Group Z	Group F-Z
6	+ 15.0 ± 5.9a (7)	+ 9.6 ± 3.3a (8)
10	+ 23.5 ± 6.3b (8)	+ 15.8 ± 2.8b (9)

* Data are expressed as means ± standard error of the mean for the number of subjects shown in parentheses.
** Means followed by different letters are significantly different ($P < 0.05$).

TABLE IX

COVARIATE ANALYSIS - SERUM ZINC

	Group Z			Group F-Z		
Covariate	P	R2	Slope	P	R2	Slope
Hgb	0.03	0.40	-4.12	<0.01	0.61	-3.87
Hct	<0.01	0.53	-2.03	0.01	0.56	-2.11
Sfer	0.06	0.33	-0.22	0.84	0.13	0.05
B2	0.26	0.22	-0.06	0.03	0.41	-0.33

Increases in plasma or serum zinc following supplementation at levels similar to that used in the present study are well-documented (Buchanan et al., 1980; Friedman et al., 1980; Freeland-Graves et al., 1982).

Relationships between initial blood levels for zinc and iron and changes in serum zinc with treatment were examined using covariate analysis, and are reported in Table IX. (Also see scattergrams, Appendix D). Initial hemoglobin and hematocrit were significant covariates for both treatment groups, as was initial serum zinc level for Group F-Z ($P<0.05$). The pretreatment level of serum zinc was significantly lower for Group F-Z than for Group Z. As initial hemoglobin or hematocrit increased, the increment in serum zinc occurring with treatment decreased. This trend also occurred for initial serum ferritin status in the zinc group only, with the relationship approaching significance.

Valberg et al.(1984) found no relationship between iron status, measured by serum ferritin, and zinc absorption, measured by retention of [65Zn]zinc chloride. Solomons et al. (1983) examined the relationship between plasma iron level and change in plasma zinc following administration of an oral dose of zinc and iron (25 mg zinc/50 mg iron). A positive correlation existed between plasma iron and the increase in plasma zinc. The authors postulated that in those individuals with iron depletion and a greater tendency

to absorb iron, a greater inhibition of zinc uptake occurred. Data from the present study do not support this theory, since those with lower serum ferritin, hematocrit or hemoglobin had a greater increase in serum zinc than those with higher levels of iron parameters. In studies on mice, iron-deficient animals had a greater capacity to absorb zinc than iron-replete ones (Hamilton et al., 1978; Flanagan et al., 1980).

Effects of treatment and length of treatment on salivary sediment zinc levels are reported in Tables X and XI. Analysis of variance showed a significant interaction between these two variables (Table X). This interaction is illustrated in Figure 2. Salivary sediment zinc levels tended to increase over time with zinc supplements (increase not significant), but decreased significantly over time with iron and zinc supplements ($P<0.05$). At 10 weeks, the difference between the two treatments in change in salivary sediment zinc levels was significant (Table XI).

Salivary sediment zinc has been used, primarily by a group of Texas researchers, to assess zinc status under conditions of zinc supplementation and depletion. Women aged 41-78 had increased levels of salivary sediment zinc after eight weeks of supplementation with 15 or 50 mg zinc/day (Buchanan et al., 1980), but no changes were noted in younger women supplemented with 15-100 mg (Friedman et al., 1980). While salivary sediment zinc increased with

zinc supplementation in the present study, the increase was not significant.

TABLE X

ANALYSIS OF VARIANCE FOR CHANGE IN SALIVARY SEDIMENT ZINC*

Source	df	Mean Square	F	P
Treatment	1	2489.29	2.56	0.13
Error (a)	14	960.29		
Weeks	1	3.85	0.02	0.88
Trt X Weeks	1	1224.27	7.85	0.02
Error (b)	9	155.95		

TABLE XI

EFFECT OF TREATMENT AND LENGTH OF TREATMENT ON SALIVARY SEDIMENT ZINC*

Weeks	Change in Salivary Sediment Zinc (mcg/g dry wt) Group Z**	Group F-Z
6	+ 1.2 ± 11.5 (8)	- 11.0 ± 8.4a (6)
10	+ 16.6 ± 6.8[1] (6)	- 19.5 ± 9.9[2]b(7)

* Data are expressed as means ± standard error of the mean for the number of subjects in parentheses.
** Means followed by different superscripts are significantly different ($P < 0.05$). Numeric superscripts denote differences due to treatment; letters denote differences due to length of treatment.

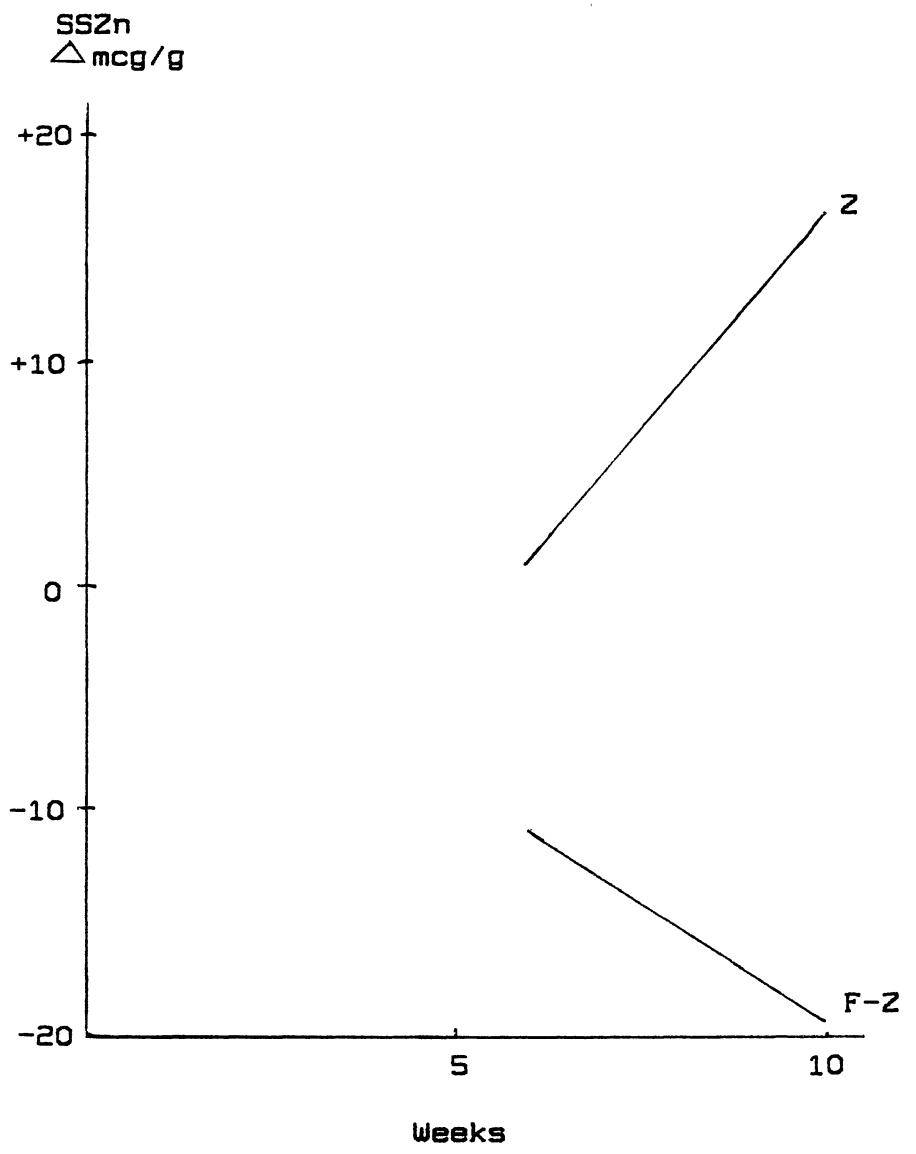

Figure 2. Effect of Treatments on Change in Salivary Sediment Zinc

Subjects receiving both zinc and iron supplements had decreased salivary sediment zinc compared to pretreatment levels. This difference was significant at 10 weeks ($P<0.05$). Freeland-Graves et al. (1981) reported that women undergoing zinc depletion experienced a significant decrease in salivary sediment zinc after three weeks on a low zinc diet. Such a decrease was also noted in women consuming a vegetarian diet for 22 days (Freeland-Graves et al., 1980). Supplementation with iron combined with zinc in the present study may produce changes similar to those experienced by subjects on a low-zinc or vegetarian diet.

The responsiveness of salivary sediment zinc to zinc status or changes in zinc nutriture has not been confirmed. Salivary sediment is composed primarily of exfoliated squamous epithelial cells, with a small quantity of granulocytes, salivary gland acinar cells, and bacteria. Researchers characterizing salivary sediment suggested that it may be more suitable than whole mixed saliva for determination of zinc status because its use eliminates the variations in zinc content due to changes in water concentration of saliva or hydration of the subject (Freeland-Graves et al., 1981).

For subjects in this study, saliva flow rate (ml/min) was significantly correlated with salivary sediment zinc across treatments ($P=0.03$, $R=0.34$). When used as a covariate, mean saliva flow rate was significantly related

to salivary sediment zinc concentration for subjects taking zinc (P=0.02) or zinc and iron (P=0.03), but was not related to changes in salivary sediment zinc over time (see scattergram, Appendix D).

Subjects consuming iron and zinc supplements had a greater saliva flow rate at 10 weeks than pretreatment (P<0.05). Warren et al. (1981) reported a significant effect of flow rate on zinc concentration of parotid saliva. As flow rate increased between days or months, so did zinc concentration. This contrasts with the decrease in salivary sediment zinc accompanying increased saliva flow rate in Group F-Z of the present study. Thus changes in saliva flow rate do not appear to explain the decrease in salivary sediment zinc.

Iron

Effects of supplements on changes in iron status are reported in Tables XII through XVI. Analysis of variance revealed significant effects of treatment on serum ferritin levels (Table XII). Serum ferritin levels decreased with zinc and increased with iron and zinc (Table XIII, Figure 3). Levels at 10 weeks were less than pretreatment for zinc-supplemented subjects, and greater than pretreatment for those supplemented with iron and zinc (P<0.05). A similar trend occurred for hematocrit, but the treatment difference was not significant (Tables XIV and XV).

Hemoglobin did not differ between treatments or with length of treatment (Table XVI).

TABLE XII

ANALYSIS OF VARIANCE FOR CHANGE IN SERUM FERRITIN

Source	df	Mean Square	F	P
Treatment	1	2023.04	12.15	<0.01
Error (a)	15	166.55		
Weeks	1	2.08	0.08	0.78
Trt X Weeks	1	14.41	0.57	0.46
Error (b)	15	25.17		

TABLE XIII

EFFECT OF TREATMENT AND LENGTH OF TREATMENT ON SERUM FERRITIN*

Weeks	Change in Serum Ferritin (ng/ml) Group Z**	Group F-Z
6	-6.6 ± 3.5^{1} (8)	$+7.6 \pm 0.3^{2}$ (9)
10	-8.4 ± 3.9^{1} (8)	$+8.3 \pm 3.3^{2}$ (9)

* Data are expressed as means ± standard error of mean for the number of subjects in parentheses.
** Means followed by a different superscript are significantly different ($P < 0.05$).

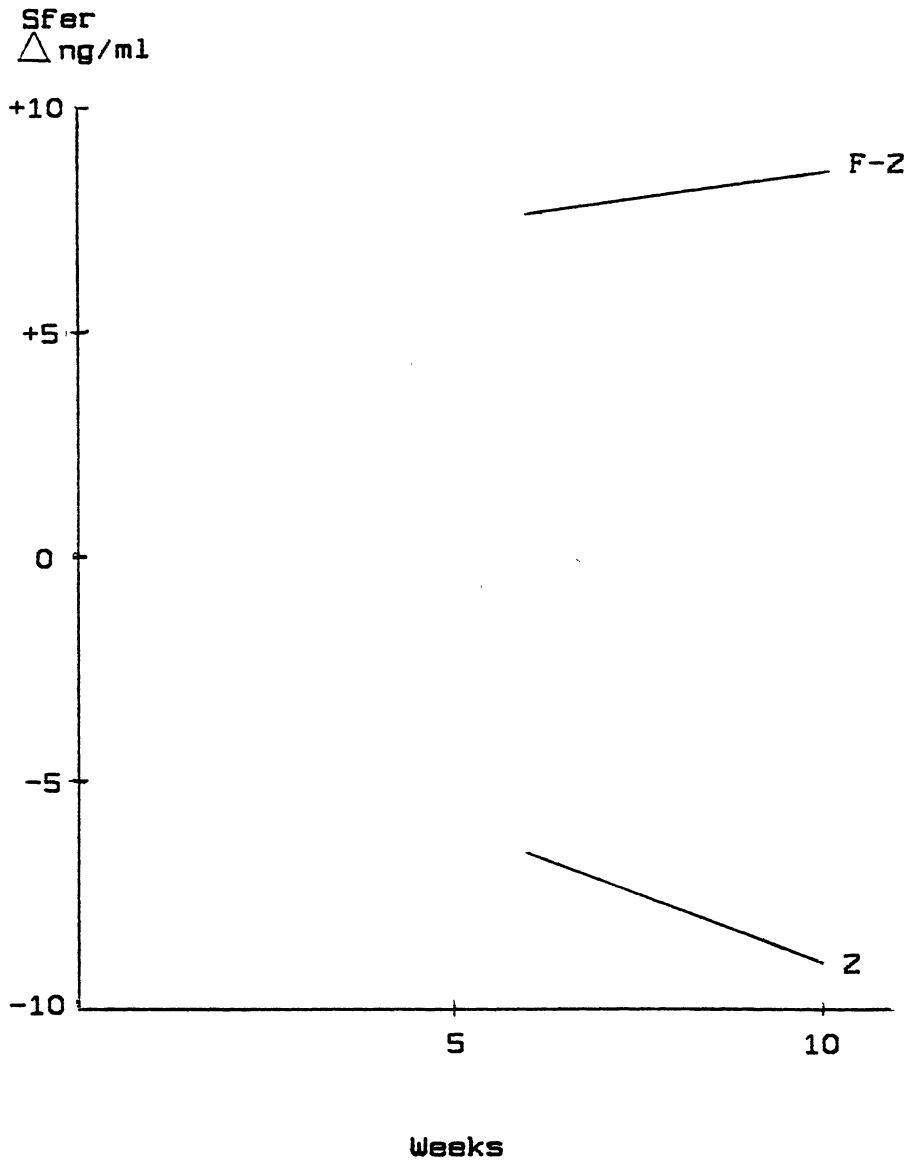

Figure 3. Effect of Treatments on Change in Serum Ferritin

TABLE XIV

ANALYSIS OF VARIANCE FOR CHANGE IN HEMATOCRIT

Source	df	Mean Square	F	P
Treatment	1	40.25	3.91	0.07
Error (a)	15	10.29		
Weeks	1	7.03	3.37	0.09
Trt X Weeks	1	0.28	0.13	0.72
Error (b)	14	2.08		

TABLE XV

EFFECT OF TREATMENT AND LENGTH OF TREATMENT ON HEMATOCRIT*

Weeks	Change in Hematocrit (vol %)** Group Z	Group F-Z
6	- 0.56 ± 0.77 (9)	+ 1.50 ± 0.78 (8)
10	- 1.62 ± 1.02 (8)	+ 0.75 ± 0.94 (8)

* Data are expressed are means ± standard error of mean for the number of subjects in parentheses.
** Means followed by different superscripts are significantly different ($P < 0.05$).

TABLE XVI

EFFECT OF TREATMENT AND LENGTH OF TREATMENT ON HEMOGLOBIN*

Weeks	Group Z	Group F-Z
6	- 0.14 ± 0.24 (9)	- 0.04 ± 0.23 (8)
10	+ 0.05 ± 0.30 (6)	+ 0.11 ± 0.36 (8)

* Data are expressed as means ± standard error of mean for the number of subjects in parentheses.
** Means followed by a different superscript are significantly different ($P < 0.05$).

Mineral status parameters identified by covariate analysis as related to changes in serum ferritin levels are presented in Table XVII (for scattergrams, see Appendix D). Initial serum zinc was a significant covariate for the change in serum ferritin in the zinc group ($P=0.04$), but not in the group receiving iron and zinc. Subjects with higher initial levels of serum zinc experienced greater decrements in serum ferritin than those with lower serum zinc. Similar relationships existed between decrement in serum ferritin and initial levels of hemoglobin, ceruloplasmin and serum ferritin in the zinc-supplemented group. For the group receiving iron and zinc, as initial levels of hemoglobin, hematocrit and ceruloplasmin increased, increment in serum ferritin decreased.

TABLE XVII

COVARIATE ANALYSIS FOR SERUM FERRITIN

Covariate	Group Z P	Group Z R2	Group Z Slope	Group F-Z P	Group F-Z R2	Group F-Z Slope
Hgb	0.02	0.37	-2.20	<0.01	0.66	-5.94
Hct	0.64	0.03	-0.26	<0.01	0.51	-2.58
Cp	<0.01	0.49	-0.63	0.05	0.28	-0.51
Sfer	<0.01	0.61	-0.18	0.35	0.08	-0.05
BZ	0.04	0.34	-0.37	0.56	0.03	0.14

Few studies have considered the effects of zinc supplements on sensitive measurements of iron status in humans. Changes in serum ferritin levels with zinc supplementation have not been evaluated previously. Serum iron was not affected in males supplemented with 22.5 mg zinc/day for four weeks (Hackman and Keen, 1984). Hematocrit decreased in older women (41-78) supplemented with 50 mg zinc/day for eight weeks (Buchanan et al, 1980), but was not affected in younger women (18-40) taking up to 100 mg zinc/day for eight weeks (Freeland-Graves et al., 1982). Iron absorption, measured by increase in plasma iron following an oral dose, was inhibited by zinc when administered to human subjects in a ratio of 2.5:1 iron/zinc (Mills et al., 1985).

Subjects consuming both iron and zinc in this study showed a mean increment of 8.3 ng/ml in serum ferritin after 10 weeks supplementation. Since an iron only group was not included in the design of this study, it is impossible to speculate how such a treatment might differ from the iron-zinc combination in its effect on serum ferritin. When pregnant Burmese women were treated with 60 mg iron as ferrous sulfate for 12 weeks, serum ferritin increased by 7.61 ng/ml (Thane-Toe and Thein-Than, 1982). Supplements were taken six days per week and were consumed after meals. Whether zinc when combined with iron in this study interfered with iron absorption or utilization is impossible to ascertain.

Young Japanese quail had reduced liver iron, hemoglobin and hematocrit when supplemented with zinc at 250 mg/kg (Hamilton et al., 1981). When supplemental iron at 100 mg/kg was provided to the birds with zinc at 500 mg/kg, liver iron did not differ from unsupplemented controls. Birds given iron alone at 100 mg/kg had liver iron levels that were significantly higher than controls.

Copper

Effects of supplementation on measurements of copper status are reported in Tables XVIII through XX. Analysis of variance showed a significant effect of length of treatment on erythrocyte superoxide dismutase levels (Table XVIII). Decreases in ESOD activity occurred in both treatment

groups, and are listed in Table XIX and illustrated in Figure 4. Initial superoxide dismutase level was a significant covariate for change in superoxide dismutase for the zinc group (P<0.01, R2=0.89), but not the iron and zinc supplemented group (for scattergram, see Appendix D). Changes in ceruloplasmin level at six and 10 weeks are reported in Table XX. There were no significant effects of treatment or length of treatment on serum ceruloplasmin.

TABLE XVIII

ANALYSIS OF VARIANCE FOR ERYTHROCYTE SUPEROXIDE DISMUTASE

Source	df	Mean Square	F	P
Treatment	1	71.72	0.71	0.41
Error (a)	16	105.66		
Weeks	1	1050.62	80.52	<0.01
Trt X Weeks	1	17.17	1.32	0.27
Error (b)	15	13.05		

TABLE XIX

EFFECT OF TREATMENT AND LENGTH OF TREATMENT ON ERYTHROCYTE SUPEROXIDE DISMUTASE*

Weeks	Change in ESOD Activity (mcg/g RBC) Group Z**	Group F-Z
6	- 19.5 ± 10.4a (9)	- 33.6 ± 5.2a (9)
10	- 61.4 ± 12.1b (8)	- 65.4 ± 2.8b (9)

* Data are expressed as means ± standard error of mean for the number of subjects in parentheses.
** Means followed by a different superscript are significantly different ($P < 0.05$).

TABLE XX

EFFECT OF TREATMENT AND LENGTH OF TREATMENT ON SERUM CERULOPLASMIN*

Weeks	Group Z	Group F-Z
6	+ 0.27 ± 1.68 (9)	- 0.79 ± 1.31 (9)
10	+ 1.19 ± 1.88 (8)	+ 0.99 ± 2.14 (8)

* Data are expressed as means ± standard error of mean for the number of subjects in parentheses.
** Means followed by different superscripts are significantly different ($P < 0.05$).

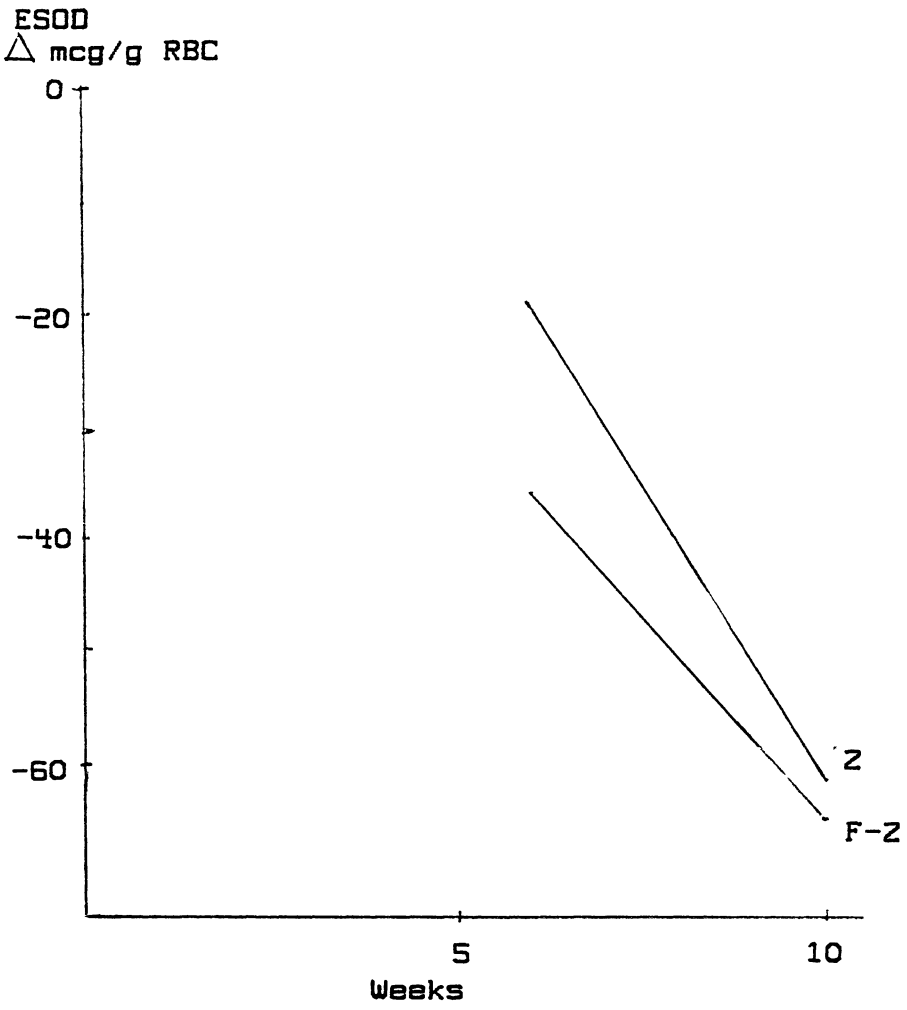

Figure 4. Effect of Treatments on Change in Erythrocyte Superoxide Dismutase

Fischer et al. (1984) reported decreased erythrocyte superoxide dismutase activity in male subjects consuming 50 mg zinc supplements. This decrease is similar to that seen in humans and other species with a copper-deficient diet (Reiser et al., 1985; Klevay, 1984). Liver superoxide dismutase activity decreased linearly in rats with increasing levels of zinc supplementation (L'Abbe and Fischer, 1984b). The decrease reported by Fischer et al. (1984) in men was linear with respect to time. These authors suggested that the decrease may be related to erythrocyte turnover. If copper is added to the apoenzyme only at the time of erythropoiesis, as in sheep, and is not lost during its lifespan, than a decrease in erythrocyte superoxide dismutase activity may only become apparent over time, due to the long half-life of erythrocytes.

The finding of no effects of supplements on changes in ceruloplasmin level was consistent with data reported by Fischer et al. (1984) for men supplemented with 50 mg zinc for six weeks. A zinc supplementation study on rats by the same research group offered a possible explanation for the lack of effect on ceruloplasmin levels (L'Abbe and Fischer, 1984b). Ceruloplasmin in rats responded to increasing levels of zinc in a nonlinear fashion. As the amount of dietary zinc increased, the number of animals having abnormally low ceruloplasmin activity increased, while some animals continued to maintain a normal level. A statistical

calculation was used to estimate the level of zinc in the diet at which half of the animals could be expected to have low ceruloplasmin activity. This was calculated to be 125.4 mg zinc/kg diet.

Nutrient Intake and Changes in Zinc, Iron and Copper Status

Covariate analysis was used to examine relationships between average intake of selected nutrients and changes in zinc, iron and copper status with supplementation. Significant relationships are summarized in Table XXI.

TABLE XXI

NUTRIENT COVARIATES FOR MINERAL STATUS PARAMETERS

Group	Parameter	Nutrient	P	R2
Z	BZ	kcal	0.02	0.55
F-Z	BZ	Ca	0.03	0.39
F-Z	BZ	Mg	0.03	0.40
F-Z	SSZn	kcal	<0.01	0.67
Z	SSZn	Fe	0.04	0.49
Z	ESOD	fiber	0.02	0.56

Relationships identified by these analyses are difficult to explain. All regression lines had a positive slope. Thus, for parameters which increased with length of treatment, such as serum zinc, increased nutrient intake was associated with a greater increase in mineral status. For parameters which decreased with treatment, such as salivary sediment zinc in the iron-zinc group, increased nutrient intake was associated with a lesser decrement in mineral status. In general, better nutritional status (higher intakes) may increase the benefits and lessen the ill effects of supplements. Alternatively, apparent relationships between specific nutrients and mineral status parameters may have resulted from common associations with other variables.

Testing of Hypotheses

This research evaluated the effects of supplements of zinc or zinc with iron on zinc, iron and copper status in adult females. Three objectives and hypotheses were formulated prior to undertaking the research, and are listed in the introduction. These will each be considered here individually; following this, other general conclusions and recommendations will be discussed.

Hypothesis one stated that zinc, iron and copper status measurements will not be affected by supplementation with zinc. Supplementation with zinc did result in a significant increase in serum zinc, and significant decreases in serum

ferritin and erythrocyte superoxide dismutase activity. Salivary sediment zinc increased and hematocrit decreased, but the changes were not significant. Hemoglobin and serum ceruloplasmin were not affected. Thus, for the following status measurements, the first null hypothesis was rejected: serum zinc, serum ferritin, and erythrocyte superoxide dismutase. For salivary sediment zinc, hemoglobin and hematocrit, the first null hypothesis was not rejected.

Hypothesis two stated that following treatment, subjects consuming zinc and iron supplements will not differ in zinc, iron or copper status from those consuming only zinc supplements. For one zinc status parameter, salivary sediment zinc, and one iron status parameter, serum ferritin, there was a significant difference between treatment groups after treatment. Salivary sediment zinc increased with zinc supplements and decreased when iron and zinc were given. For serum ferritin, a decrease occurred with zinc supplements, but consumption of iron and zinc resulted in an increase in serum ferritin. Thus, for these two status parameters, the second null hypothesis was rejected. For the remaining parameters for zinc and iron, and for the two copper status measurements, no differences occurred between treatment groups following treatment. Thus, for serum zinc, hematocrit, hemoglobin, erythrocyte superoxide dismutase, and serum ceruloplasmin, the second null hypothesis was not rejected.

Hypothesis three stated that there will be no relationship between status measurements for zinc, iron and copper prior to supplementation and changes in status measurements following supplementation. Such relationships did exist in some cases. Mineral status prior to treatment was significantly related to changes in serum zinc, serum ferritin and erythrocyte superoxide dismutase activity occurring with treatment.

For subjects receiving only zinc, those with higher pretreatment levels of hemoglobin and hematocrit experienced a lesser increment in serum zinc than those with lower levels. Subjects with better initial iron (serum ferritin and hemoglobin) or zinc (serum zinc) status experienced a greater decrement in serum ferritin than those with lower pretreatment levels for these parameters. Higher initial levels of superoxide dismutase were related to a greater decrement in superoxide dismutase activity with supplementation.

In the iron-zinc treatment group, subjects with higher initial levels of serum zinc, hemoglobin or hematocrit experienced a lesser increment in serum zinc than subjects for whom those parameters were lower prior to treatment. Higher pretreatment levels of hemoglobin or hematocrit were associated with a lesser increment in serum ferritin.

Conclusions

Supplementation with zinc may have been beneficial with regard to zinc status. Serum zinc levels increased, but the increase in salivary sediment zinc was not significant. Since all subjects were in the normal range for serum zinc prior to treatment, the increase occurring with supplementation may be of little practical importance.

The effect of iron in combination with zinc on zinc status is questionable. While addition of iron did not affect the rise in serum zinc occurring with zinc supplementation, it did result in a decrease in salivary sediment zinc levels. Serum zinc, though responsive to changes in zinc intake, is subject to other influences, and thus may not always be indicative of zinc status. Salivary sediment zinc, which has been suggested as a more sensitive parameter, did respond in a negative direction to the iron-zinc supplementation combination. A few subjects then fell outside the range of values for salivary sediment zinc reported in the literature. This parameter has been used only on a very limited basis, however, so the actual range of normal levels for salivary sediment zinc is unknown.

Zinc supplementation did appear to have a detrimental effect on iron status, since serum ferritin decreased with supplement use. Addition of iron apparently overcame this effect, since those subjects consuming both supplements experienced an increase in serum ferritin.

This research confirmed the negative effect of zinc on copper status in humans as measured by erythrocyte superoxide dismutase, which has been reported elsewhere (Fischer et al., 1984). Use of iron with zinc did not alter the effect. The decrease in erythrocyte superoxide dismutase activity was highly significant (P=0.0001). Unfortunately, erythrocyte superoxide dismutase levels occurring following supplementation in this study cannot be compared with norms reported in the literature, since they were calculated based on activity/gram red blood cells, rather than on concentration/gram hemoglobin or units/10^9 red cells as reported elsewhere (Reiser et al., 1985).

These findings suggest that use of zinc supplements is unwarranted unless a state of deficiency has been clearly established.

Results of covariate analysis suggest that homeostatic mechanisms may have influenced how supplements affected mineral status. Generally, subjects with lower levels of status measurements initially experienced greater increases and lesser decrements in zinc or iron status than those with higher pretreatment levels. This may have served to protect those at greater risk against the detrimental influences of supplements.

Data examining relationships between nutrient intake and effects of supplements on mineral status are more difficult to interpret. Existing literature does not offer an explanation for the relationships identified with

covariate analysis. Missing data in the nutritional analysis program used place limitations on any interpretation made. Many foods added to the program had missing mineral values. Copper was not included in the program. This precluded any examination of the relationship between dietary copper and effects on erythrocyte superoxide dismutase activity.

Recommendations

The author recommends the following:

1. Evaluation of the effects of supplementation on a larger sample, with a greater number of subjects, both at lower and upper ends of mineral status measurement ranges prior to treatment. This might help identify the actual risk for individuals with marginal mineral status associated with taking supplements in the manner described.

2. Supplementation for a longer time period. Adaptation of the more sensitive mineral status measurements to supplements did not occur within the time frame of this study. A longer supplement usage period might allow for adaptation.

3. Inclusion of an iron-only supplement group. Effects of iron itself on zinc and copper status could only be inferred from data gathered here.

4. Use of a more complete nutritional analysis

program, or addition of copper and imputed values for other missing nutrient data to the current program.

CHAPTER V

SUMMARY

The purpose of this research was to examine the effects of supplements of zinc or zinc with iron on iron, zinc and copper status in adult females. A supplementation study was conducted in the fall of 1984 on 18 adult female volunteers, aged 25 to 40.

Subjects were ranked by pretreatment levels of serum ferritin (Sfer) and erythrocyte superoxide dismutase (ESOD), and randomly assigned to treatment, such that each treatment group contained subjects with a range of low to high values for Sfer and ESOD. Group Z consumed 50 mg zinc as the gluconate each day. Group F-Z consumed 50 mg iron (as ferrous sulfate) in addition to the zinc each day. Treatment lasted for 10 weeks.

Blood and saliva samples were obtained prior to treatment and after six and 10 weeks of treatment. Effects of treatment on zinc, iron and copper status were evaluated using the following measurements: for zinc, serum zinc and salivary sediment zinc; for iron, serum ferritin, hemoglobin and hematocrit; for copper, erythrocyte superoxide dismutase activity and serum ceruloplasmin.

Dietary intake data were obtained prior to treatment and at six and 10 weeks, using three-day food records. The Health-aide microcomputer program was used to determine the nutrient content of the diets. Intakes of selected nutrients averaged over the course of treatment were compared between treatment groups. Nutrients included were kilocalories, protein, fiber, vitamin C, calcium, magnesium, phosphorus, iron and zinc. Within treatments, initial nutrient intakes were compared with those at six and 10 weeks.

Analysis of variance procedure was used to determine effects of treatment and length of treatment on changes in mineral status parameters from levels prior to treatment. Covariate analyses were conducted to determine relationships between changes in mineral status with treatment and the following variables: pretreatment levels of zinc, iron and copper status parameters; and intakes of selected nutrients averaged over the course of treatment.

Supplementation with zinc resulted in an increase in serum zinc (+23.5 mcg/dl at 10 weeks) and decreases in serum ferritin (-8.4 ng/ml at 10 weeks) and erythrocyte superoxide dismutase activity (-61.4 mcg/g RBC at 10 weeks). Salivary sediment zinc increased, but the increase was not significant. Subjects with higher pretreatment levels of hemoglobin and hematocrit experienced a lesser increment in serum zinc than those with lower levels. Those with better initial iron or zinc status experienced a greater decrement

in serum ferritin than those with lower initial levels. Higher pretreatment levels of superoxide dismutase were related to a greater decrement in superoxide dismutase activity with supplementation.

Supplementation with iron and zinc resulted in increases in serum zinc (+15.8 mcg/dl at 10 weeks) and serum ferritin (+8.3 ng/ml, 10 weeks) and decreases in salivary sediment zinc (-19.5 mcg/g dry weight, 10 weeks) and erythrocyte superoxide dismutase activity (-65.4 mcg/g RBC, 10 weeks). Subjects with higher levels of serum zinc, hemoglobin or hematocrit experienced a lesser increment in serum zinc than subjects for whom those parameters were lower prior to treatment. Higher pretreatment levels of hemoglobin or hematocrit were associated with a lesser increment in serum ferritin.

Significant differences between treatments existed for change in salivary sediment zinc and change in serum ferritin. Differences were significant at 10 weeks for salivary sediment zinc and at six and 10 weeks for serum ferritin.

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APPENDIXES

APPENDIX A

LETTER AND CONSENT FORM

Dear Research Participant,

Thank you for agreeing to participate in this experiment. This research will provide important information on how trace elements interact with each other in the body. By learning how supplements of zinc and iron influence the body's use of these two minerals, we will gain information which can be used to evaluate present supplementation practices and make recommendations for the future.

As a participant in this experiment, you will receive information about your own food intake and nutritional status. You'll also gain the experience of being a research subject, of great value if you are a student.

This experiment will last 11-12 weeks. During this time you will take a mineral supplement each day.

At two to three times during the experiment you will have blood drawn. Only 30 milliliters (about two tablespoons) will be drawn each time. You will also be asked to provide samples of saliva at intervals during the study, and to keep dietary records on several days during the course of the experiment.

Zinc supplements and iron supplements have been used by researchers therapeutically, to treat conditions like anemia and rheumatoid arthritis, as well as in other situations, simply to examine effects of supplementation. Subjects tolerate supplements well, except in a small percentage of cases in which individuals experience minor gastrointestinal upset. This is usually alleviated by taking supplements with food; you will be asked to take supplements with a small snack to minimize discomfort.

I'm very appreciative that you are willing to contribute your time and effort in order that this research may be carried out. Any information you provide will be kept confidential, and at the conclusion of the study, all records associating you as an individual with any data will be destroyed.

As the project leader, I am very interested in your health and welfare during the duration of the experiment. If you have any questions or experience any difficulties, please contact me. You may also contact my advisor, Dr. Mary Alice Kenney, or the Institutional Review Board of Oklahoma State University, which has approved this experiment.

In the event that you experience any discomfort which might be related to the experiment, please let me know, so that appropriate action can be taken.

I hope this will be an interesting and profitable experience for you.

Sincerely,

Kathy Yadrick, Doctoral Student, FNIA

Research on Iron-Zinc Interactions in Adult Females

Consent for Participation

I have received a letter explaining the purpose and procedures of the study on iron-zinc interactions in adult females.

I understand that to be in this study, I agree to

1) Consume the designated capsule each day during the duration of the experiment, under the stated conditions.

2) Report to the OSU Hospital Lab on the designated days to have 30 milliliters (about 2 tablespoons) of blood drawn from my vein, after fasting for 12 hours;

3) Report to the FNIA lab at designated intervals to provide a sample of saliva;

4) Keep records of my food intake on designated days during the study;

5) Provide requested information on age, height, weight, use of medications, menstrual cycle, and other personal information, such as level of exercise, which might affect blood measurements of trace minerals.

I understand that all information about me will be kept confidential, that I can ask questions at any time, that my participation in this research is voluntary, and that I can withdraw from the study at any time. I will contact the project leader if I experience any difficulties that I think might be related to the experimental treatments. I assume whatever risk is involved, but my consent to be in this study does not mean I give up any legal rights or release the persons in charge from liability due to negligence.

I have received a copy of this statement.

With these understandings, I voluntarily agree to be in this study.

_____

Participant Date

_____

Project Director Date

APPENDIX B

FORMS FOR DATA COLLECTION

Kathy Yadrick

Application for Participation in Trace Mineral Study

Name _____ Date_____

Race_____ Birthdate_____

Height_____ Weight_____

Have you ever had

diabetes _____ heart disease _____ other disease or condition of poor health_____

anemia _____ high blood pressure _____

Are you

pregnant _____ taking oral contraceptives _____

taking vitamin or mineral supplements _____

taking any medications _____ if so, what, and how often _____

_____

Do you smoke _____ if yes, what is your usual daily use_____

What was the beginning date of your last menstrual period_____

the usual length of period _____

usual duration of your menstrual cycle_____

describe your usual menstrual flow light_____ medium_____ heavy_____

What is your usual pattern of exercise? Describe briefly the types of exercise you engage in on a regular basis, how often you do them, and how long each time. (e.g. jogging, 3 times/week, 30 minutes)

_____

_____

_____

_____

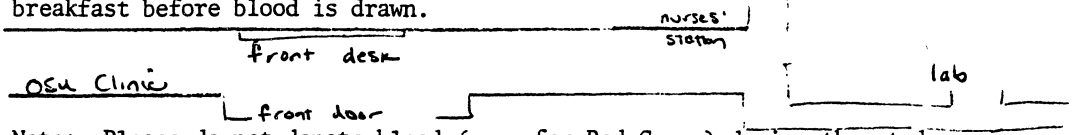
How much has your weight fluctuated in the last six months? _____

Yadrick, 9/12/84

BLOOD COLLECTION

Blood will be drawn three times during the experiment, on September_____, November_____, and December_____. Report to the O.S.U. Hospital lab between 8:00 and 10:00 A.M. on these dates to have your blood drawn. Go directly to the lab; you need not stop at the front desk of the clinic. Tell the medical technologist you are a subject in the nutrition study.

In preparation for having blood drawn, do not eat or drink anything, except water, after midnight the night before blood is to be drawn; do not have breakfast before blood is drawn.



Note: Please do not donate blood (e.g. for Red Cross) during the study.

Directions to hospital/clinic: OSU Hospital is on Farm Rd., north of high rise dorms, east of Colvin Center. There is a patient/visitor parking lot in the rear (north side) of the clinic.

SALIVA COLLECTION

Report to the FNIA lab, HEW 407, between _____ and _____ on _____. Please fast for 8 hours prior to the time saliva is collected. Please bring a toothbrush to the lab with you. In the FNIA lab, you will be instructed to cleanse your mouth thoroughly by brushing teeth, tongue and oral cavity using distilled water, and then rinsing mouth three times with distilled water.

DIETARY RECORDS

Please turn in your dietary records for the previous week when you come to the lab for saliva collection.

Yadrick, 9/12/84

SALIVA COLLECTION - INSTRUCTIONS

Saliva Collection Record Form

This form should be given to each subject as she comes in. It should be filled out by subject with lab assistant's help, checked over and initialed by lab assistant.

Cleansing of oral cavity

Subject should brush teeth, tongue, and entire oral cavity thorougly with toothbrush using distilled water (no toothpaste). If subject has forgotten toothbrush, provide her with one, label with her name, and save in plastic bag in lab drawer for next time.

Following brushing, subject should rinse mouth three times with distilled water. Take water into mouth from cup, swish around in mouth, and spit out.

Saliva collection

Collect fasting saliva samples between 8:30 and 10:30 A.M. Collect in 15 ml plastic, orange-capped centrifuge tubes. Label tubes with subject number.

Subject should spit saliva into centrifuge tube. Record time this begins, to seconds, e.g. 8:30:15. Time begins with first spitting into tube. Have subject continue until tube contains 15 ml. Record time, as above, when 15 ml volume is reached. Instruct subject to note time, using large clock in lab. If subject is unable to salivate 15 ml in reasonable amount of time (should take about 15-20 min), try to collect at least 10 ml. Record volume collected as closely as possible, and time at which saliva collection stopped (last spit). Put labeled tube into beaker in refrigerator.

Yadrick, 9/12/84

DIETARY RECORD

This study permits you to choose the foods you eat, so long as you are consistent with following your regular meal pattern throughout the study. Following are instructions for completing dietary records:

When: Fill in a dietary record on Tuesday, Wednesday and Saturday of Weeks 1, 6 and 10 (September___, October___, December___). Also complete a record for the day before you have blood drawn. Please turn these into the FNIA lab when you report there for saliva collection.

What: Write down everything you eat or drink beginning with when you get up in the morning until you go to bed at night. Try to write things down at the time you eat them; it's easier to remember. Include everything eaten at mealtime as well as between meals.

How: Record what you eat in detail; if it is a casserole or mixed dish, include all the ingredients. Record them in common household units: cups, tablespoons, etc., and indicate the number of units you consumed (e.g. 1 cup of green grapes, ½ cup homemade macaroni and cheese, 6 oz orange juice). Record brand names where possible (e.g. 1 12oz can diet Coke, 1 McDonald's Big Mac). Use weight measurements when this is simpler (e.g. 1 8 oz Nature Valley chocolate chip granola bar).

Try to use the same size cup or glass for beverages all the time that you are keeping food records. This makes it easier to estimate volumes drunk.

Use as many lines of the record as you need to describe a particular food; don't let the form limit you in getting down all the requested information.

SALIVA COLLECTION RECORD

Name_____ Subject number_____

Time begun (first expectoration) _____
record to seconds

Time finished (last expectoration) _____

Saliva volume (to tenth of ml, e.g. 14.9 ml) _____ml

Any recent dental work? If so, describe briefly on back.

For lab personnel (please initial)

_____Saliva collection data complete

_____Dietary records turned in

Name_____

Date_____

Subject No._____

Dietary Record

Time of day	Food	Amount consumed	Where eaten

December 4, 1984

Dear Nutrition Research Participants,

Well, the end is actually near. Thank you so much for being willing to swallow those pills and give me your blood (sweat, tears) and saliva, and for revealing the innermost secrets of your food intake to me. There are just a few more things I'll need from you, and then you'll be able to return to your supplement-less existence.

1. I'll be contacting you in the next day or two to arrange a time for the next blood sample: either Tuesday, Dec. 11 or Wednesday, Dec. 12.

2. Please complete the attached dietary records (days are specified on each form - Dec. 5, 8, 10 and 11) and return them to the lab, 407 HEW, as soon as you have completed them. They can be dropped off or sent by campus mail addressed to me. Also, please turn in any previous dietary records you may have forgotten to turn in before.

3. Please complete the attached form requesting information on how you normally took the supplement and on medication usage.

I would like to express my appreciation to you for your participation by inviting you to a buffet lunch on Tuesday, December 18, at noon, in 403 HEW. At this time, if our judges can get their acts together, we will present awards to the most outstanding subjects. I know finals week will be a busy time for all of you, but I hope you'll be able to squeeze this into your schedule. Come late if you can't make it at 12:00.

Thanks again,
Kathy

Name_____

1. With what food(s), beverage(s) did you usually take the supplement?

   A.M. _____

   P.M. _____

2. Did you take any medications (over-the-counter or prescribed) during the course of the study?

   Medication _____

   Dosage _____

   When did you take it (specific dates best if you can remember)

   _____

APPENDIX C

LIST OF ABBREVIATIONS IN COMPUTER PRINTOUTS AND COMPUTER LISTS OF RAW DATA FOR INDIVIDUAL SUBJECTS

List of Abbreviations in Computer Printouts

Age	- Age (years)
BZn	- Serum zinc (mg/liter)
BZND	- Change in serum zinc (mg/liter)
BZNO	- Pretreatment serum zinc (mg/liter)
Ca	- Calcium intake (mg)
Cp	- Serum ceruloplasmin (mg/dl)
F	- Treatment - zinc + iron
Fe	- Iron intake (mg)
Fiber	- Dietary fiber intake (mg)
Hb	- Hemoglobin (g/dl)
Hct	- Hematocrit (vol %)
HctO	- Pretreatment Hematocrit (vol.%)
HgbO	- Pretreatment Hemoglobin (g/dl)
Ht	- Height (inches)
Kcal	- Caloric intake (kilocalories)
Mg	- Magnesium intake (mg)
Phos	- Phosphorus intake (mg)
Prot	- Protein intake (g)
S	- Measurement at 6 weeks
SFED	- Change in serum ferritin (ng/ml)
SFeO	- Pretreatment serum ferritin (ng/ml)
Sfer	- Serum ferritin (ng/ml)
Sflo	- Saliva flow rate (ml/min)
SOD	- Erythrocyte superoxide dismutase activity (mcg/g red blood cell)
SODD	- Change in SOD (mcg/g red blood cell)
SODO	- Pretreatment SOD activity (mcg/g RBC)
SSZn	- Salivary sediment zinc (mcg/g dry wt)
Sub	- Subject number
T	- Measurement at 10 weeks
Tr, Trt	- Treatment (Z=zinc; F=iron+zinc)
VitC	- Vitamin C intake (mg)
We, Wee	- Time of sample (1=pretreatment, 2=6 weeks, 3=10 weeks)
Wt	- Weight (lbs)
Z	- Treatment - zinc only
Zn	- Zinc intake (mg)

Raw Data for Individual Subjects: Blood and Saliva

sub	wee	trt	Hb	Hct	Sfer	Cp	SOD	BZn	SSZn
01	1	F	14.4	40	41.5	26.7	124	.835	129.4
01	2	F	14.4	44	51.4	23.8	101	.865	140.3
01	3	F	14.8	42	56.5	25.8	68	1.02	136.5
02	1	Z	16.9	46		19.5	127	1.005	86.4
02	2	Z	15.5	45		28.7	114	1.025	132.1
03	1	F	15.8	44	13.3	37.2	153	.805	
03	2	F	14.9	43	17.3	35.4	120	.875	
03	3	F	15.5	43	19.9	30.2	85	.860	
04	1	Z	12.6	39	3.8	24.3	104	0.84	52.6
04	2	Z	11.9	37	3.9	23.4	81	1.245	
04	3	Z	12.3	38	4.2	25.2	55	1.295	49.0
05	1	F	13.4	39	31.9	21.4	163	.69	65.1
05	2	F	13.7	42	48.4	23.2	137	.955	
05	3	F	15.2	45	59.8	29.7	91	.99	71.0
06	1	F	15.8	43	48.2	31.0	137	.71	
06	2	F	15.7	45	36.4	24.7	91	.74	
06	3	F	14.2	40	41.2	22.1	75	.82	
07	1	Z	16.5	47	58.6	32.4	176	.88	71.4
07	2	Z	15.8	46	40.0	30.2	111	.965	46.7
07	3	Z	15.7	45	44.5	39.3	72	1.04	66.5
08	1	F	15.2	42	20.6	28.8	111	.79	58.3
08	2	F	14.3	41	36.2	27.4	101	.79	71.8
08	3	F	15.1	42	28.7	31.7	62	.89	61.1
09	1	F	14.8	42	45.6	34.6	127	.805	167.0
09	2	F	14.6	41	54.8	42.6	68	.91	133.1
09	3	F	14.4	42	58.1	44.5	55	.885	108.1
10	1	F	15.3	42	33.9	34.1	156	.68	102.8
10	2	F	16.1	44	41.8	32.6	137	.74	70.4
10	3	F	15.4	42	39.6	35.6	85	.83	60.9
11	1	F	15.2	42	33.3	40.7	156	.675	110
11	2	F	15.9	46	41.4	38.1	114		103.4
11	3	F	16.2	44	32.8	38.6	88	.94	76.5
12	1	Z	15.4	42	66.3	37.6	146	.864	87.5
12	2	Z	15.1	44	44.8	34.1	111	1.04	86.3
12	3	Z	15.3	42	39	36.1	62	1.085	
13	1	Z	14.8	42	40.4	35.2	133	.865	74.3
13	2	Z	15.7	45	30.8	32.4	91		59.8
13	3	Z	15.4	44	23.1	30.5	75	1.295	109.2
15	1	Z	14.3	44	19.2	24.3	153	.63	48.2
15	2	Z	14.8	42	26.6	28.5	130	.94	66.8
15	3	Z	15.5	42	21.9	25.9	68	.98	69.9
16	1	F			30.6	26.4	130	.648	75.0
16	2	F	14.2	40	39.6	26.0	85	.855	57.7
16	3	F	14.8	43	37.3	31.6	58	.825	56.9
17	1	Z	14.8	42	39.2	35.5	111	.72	95.3
17	2	Z	15.0	44	33.9	32.1	91		94.6
17	3	Z	14.5	43	43.4	46.5	75	.795	120.9
18	1	Z	15.0	46	43.5	28.9	65	.865	68.7
18	2	Z	15.2	43	36.7	24.2	120	.84	107.1
18	3	Z		39	40.2	27.3	68	.8	94.4
19	1	Z	13.8	45	22.0	27.3	140	.935	121.0
19	2	Z	13.8	42	23.9	33.8	130	1.015	69.1
19	3	Z		41	9.3	24.2	62	1.19	

Raw Data for Individual Subjects: Nutrient Intakes and Other Parameters

sub	we	tr	kcal	prot	fiber	vitc	phos	ca	fe	mg	zn	age	ht	wt	sflo
01	1	f	1250	49	3.75	102.2	751	473.8	13.02	190	5.23	35	67	150	.645
01	2	f	2829	147.6	5.04	179.8	1740.8	688.5	20.2	363.2	12.87	35	67	150	.781
01	3	f	1935	71.1	4.48	231.8	1189.5	901.7	17.13	263.2	7.36	35	67	150	.930
02	1	z	1481	54.1	1.36	91.4	447.8	486.8	8.94	100.7	2.67	37	66	145	.62
02	2	z	1141	40.7	1.71	92.1	561	402.8	6.87	113.6	4.85	37	66	145	1.86
03	1	f	1122	50.5	2.84	122.2	836.9	605.9	5.74	158.5	5.28	25	64	140	.973
03	2	f	1336	38.9	1.82	123.5	915.4	646.9	4.79	176.4	5.46	25	64	140	.87
03	3	f	1271	46.8	3.06	54.9	898	745	7.26	155.1	4.7	25	64	140	1.5
04	1	z	1870	57.6	4.53	96.3	1195	686.5	15.99	356	7.7	40	67	127	.307
04	2	z	1649	63.4	4.76	138.5	960.2	662.5	13.22	242.9	7.24	40	67	127	.233
04	3	z	1514	42.2	3.89	83.1	831.4	543.8	9.49	202.4	5.74	40	67	127	.181
05	1	f	1616	51.8	3.28	110.9	1023.4	896.8	8.91	270.4	6.35	40	62.5	103	.219
05	2	f	1898	52.3	5.25	107.7	1047	690	9.92	314.1	6.66	40	62.5	103	.343
05	3	f	1870	44.2	4.06	93.6	999.9	923.8	7.57	229.3	4.66	40	62.5	103	.232
06	1	f	1912	61.95	3.6	132.1	929.1	556	11.42	183.2	6.69	25	67.2	142	.583
06	2	f	1775	65.4	2.84	147.3	1102.4	806	15.34	264.9	9.13	25	67.2	142	.715
06	3	f	1783	64.6	2.29	119.1	1354.1	690.7	9.67	257.5	6.72	25	67.2	142	1.16
07	1	z	2148	84.2	3.39	128.4	1241.2	971.2	17.48	286.7	9.49	30	63.5	156	.454
07	2	z	2299	100.8	3.94	155.1	1468.5	1019.7	15.05	301.3	9.2	30	63.5	156	.545
07	3	z	2070	78.8	3.46	106.9	1303	798.8	14.22	267.9	8.45	30	63.5	156	.73
08	1	f	2237	60.7	4.84	188.5	1523	1033	12.48	313.5	7.83	32	64	111	.421
08	2	f	1978	41.9	3.24	161.4	893.6	592.2	10.3	197.2	3.88	32	64	111	.48
08	3	f	1720	49.3	4.36	239.8	929.8	694.1	9.32	234.4	3.95	32	64	111	.362
09	1	f	1637	59.8	3.94	121.6	892.3	390.7	11.61	234.2	7.9	36	64.5	125	.302
09	2	f	1591	64.8	4.16	116.7	940.6	487.6	10.56	200.1	6.62	36	64.5	125	.234
09	3	f	1673	73.7	4.6	194.5	993.7	588.4	13.18	278.6	8.72	36	64.5	125	.425
10	1	f	1411	58.2	4.34	81.4	1136.3	760.8	11.67	241.8	6.73	35	60	115	.392
10	2	f	1789	75.6	4.17	94.3	966.7	693	15.2	204.4	7.83	35	60	115	.455
10	3	f	1143	50.9	1.8	65.8	747.9	402.8	7.72	118.7	5.36	35	60	115	.406
11	1	f	1771	106.8	6.47	238.5	1697	1409.2	14.21	295.2	10.2	41	63	160	.429
11	2	f	1473	72.1	6.81	198.2	1262	1352.1	23.36	315	8.23	41	63	160	.265
11	3	f	1833	81.8	5.82	137	1402	1071.9	13.81	294	9.38	41	63	160	.396
12	1	z	1950	73.6	4.53	113.2	1096	582.5	14.15	207.3	8.3	31	67	136	.435
12	2	z	2043	80.7	4.51	113.4	1263	593.9	12.26	164.5	6.15	31	67	136	.75
12	3	z	1948	60.7	2.42	68.5	778.4	328.7	10.29	143.5	7.2	31	67	136	.767
13	1	z	1666	73.2	2.98	25.2	1105	522.8	11.28	403.5	9	26	69	136	.943
13	2	z	2039	74.2	5.79	89.1	1419	1056.3	23.2	252.5	9.5	26	69	136	.824
13	3	z	1797	64.7	2.93	69.1	1253	998.5	14.76	251.4	8.33	26	69	136	.818
15	1	z	1854	62	5.04	57.3	931.6	484.5	11.99	234.6	9.18	31	63	120	.313
15	2	z	1612	74.3	2.41	31.8	1033.2	851	9.48	169.3	5.66	31	63	120	.625
15	3	z	2356	91.9	2.12	36.4	1401	1124.7	12.27	265.2	8.06	31	63	120	.288
16	1	f	1971	64.3	6.3	80.9	1199	721.3	10.68	312.8	5.96	26	59	96	.191
16	2	f	1977	50.6	6.46	55.6	1106	904.8	11.17	218.3	5.57	26	59	96	.178
16	3	f	1796	55.6	7.95	193.8	1154	698.3	12.51	293.3	7.89	26	59	96	.162
17	1	z	2004	71.6	6.02	150.2	1532	1282	11.51	305.8	7.54	31	67	130	.454
17	2	z	2619	105.5	8.18	105.1	2044	1603.7	13.47	357.3	10.18	31	67	130	.733
17	3	z	2350	94.7	4.3	72.7	1555	1150	12.48	281.1	9.54	31	67	130	
18	1	z	2204	74.4	5.57	83.7	1263	883.6	13.5	277.1	7	26	65.5	130	.512
18	2	z	2713	99.7	6.92	164.3	1826	1138.9	15.34	340.1	10.63	26	65.5	130	.43
18	3	z	2230	83.6	6.39	81.5	1333	779.8	17.23	306.4	8.91	26	65.5	130	.6
19	1	z	1579	51.3	6.86	93.3	1261	650.2	19.89	313.3	8.46	25	66.5	125	.394
19	2	z	2004	46.4	5.84	103.3	1239	812	17.71	280.5	10.52	25	66.5	125	.429
19	3	z	1763	51.3	5.23	88.2	1056	728.4	14.61	237	7.06	25	66.5	125	.579

APPENDIX D

SCATTER DIAGRAMS

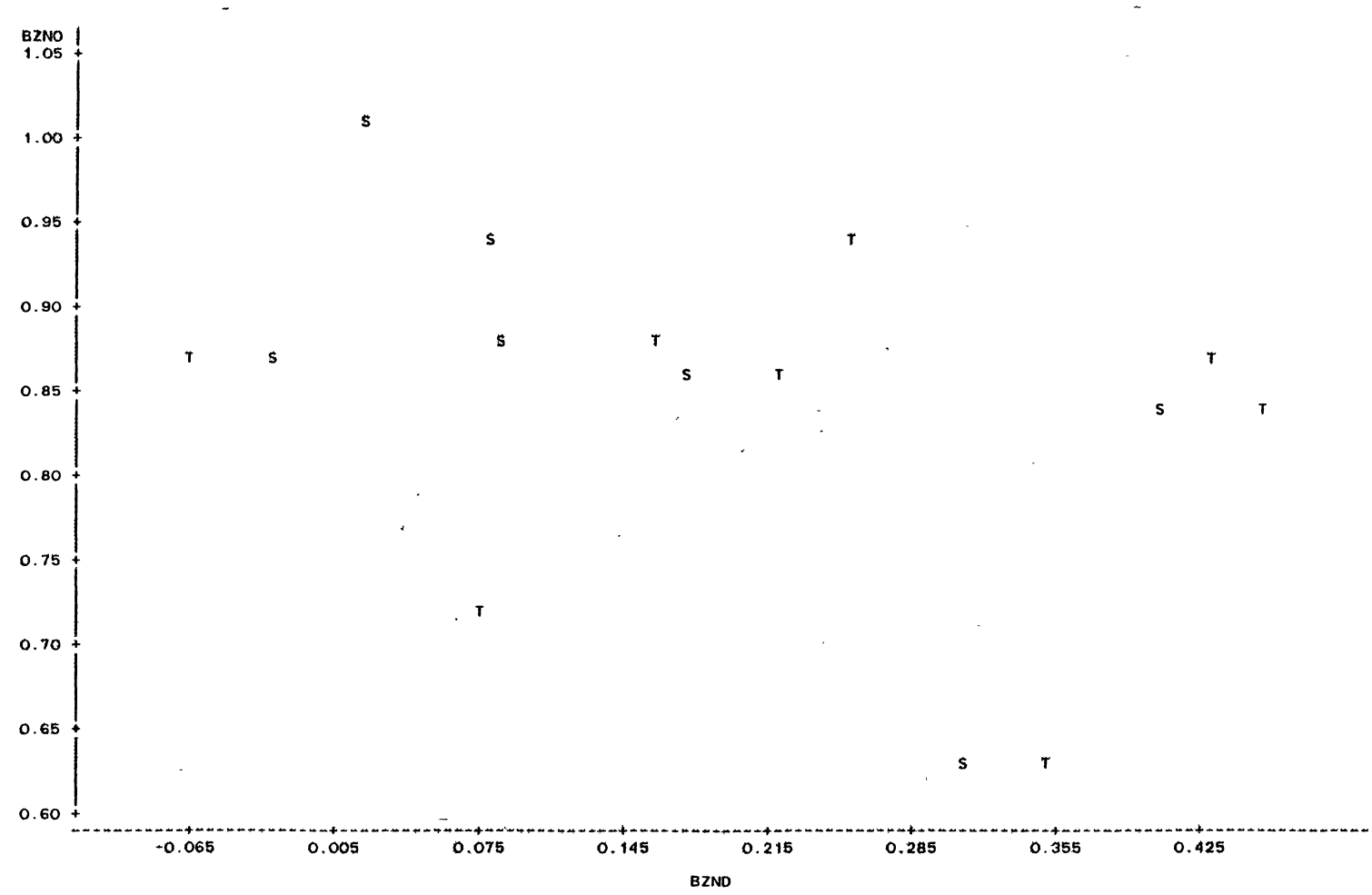


Figure 5. Relationship of Initial Serum Zinc to Change in Serum Zinc - Group Z

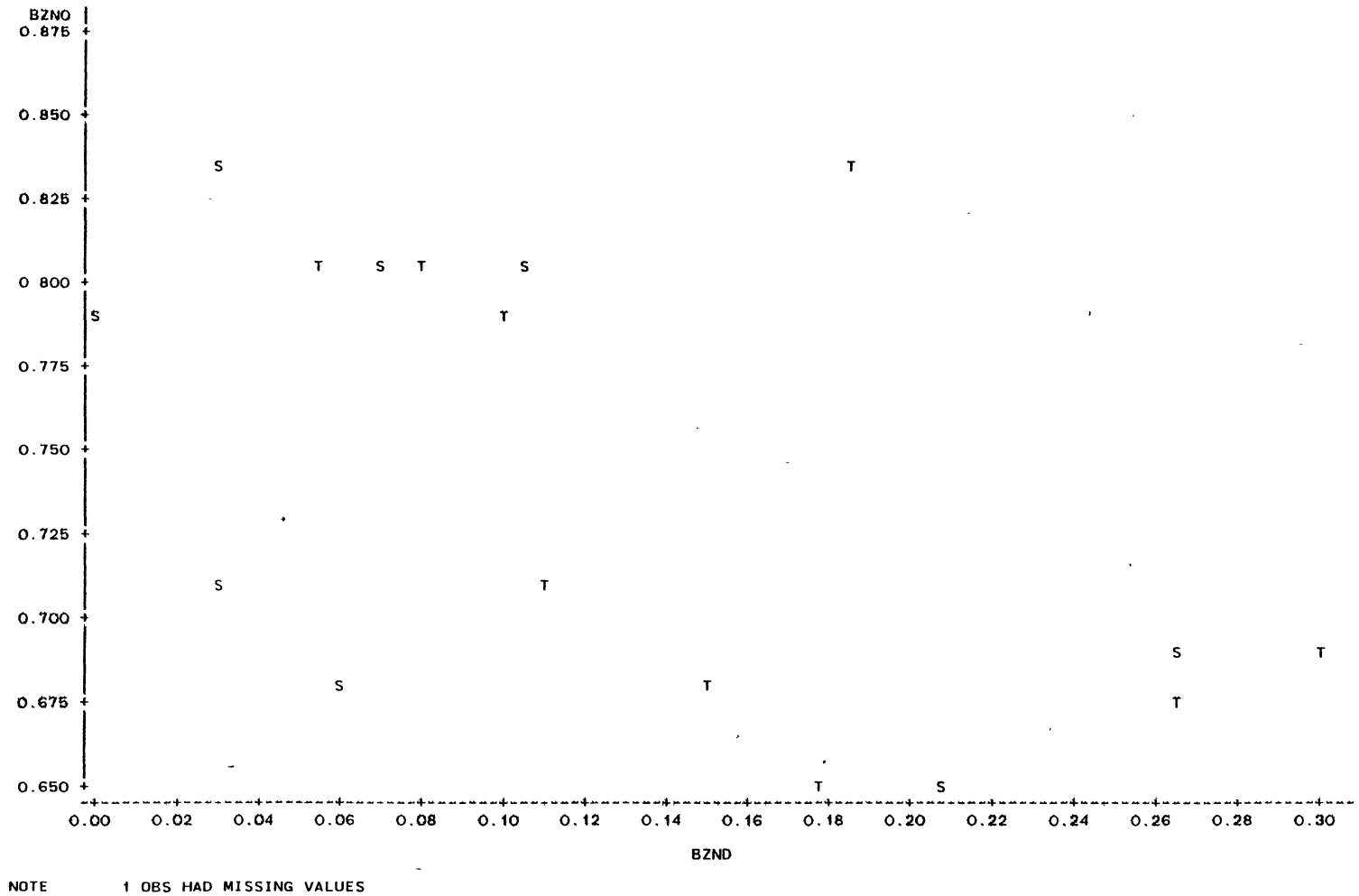


Figure 6. Relationship of Initial Serum Zinc to Change in Serum Zinc - Group F-Z

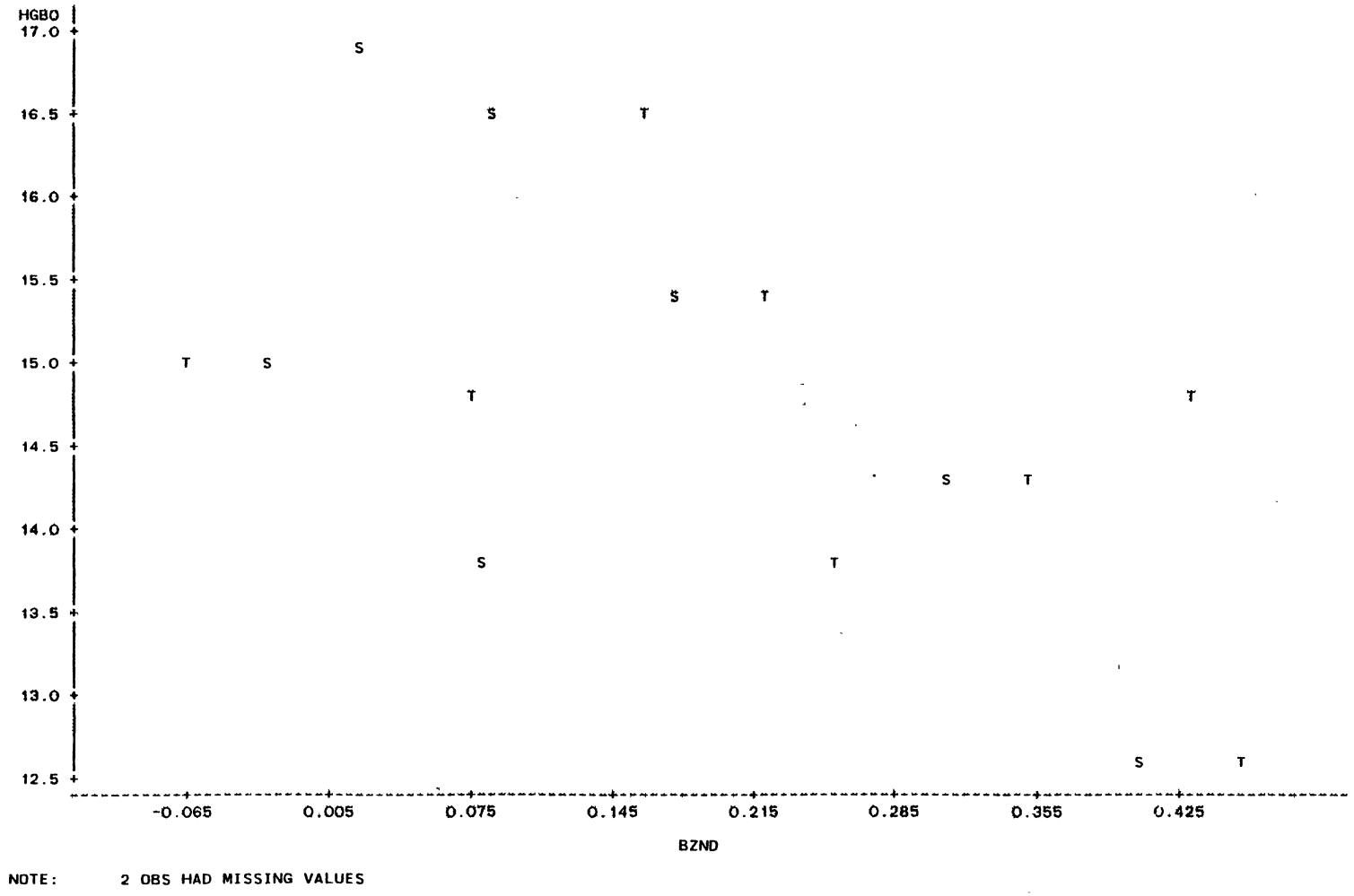


Figure 7. Relationship of Initial Hemoglobin to Change in Serum Zinc - Group 2

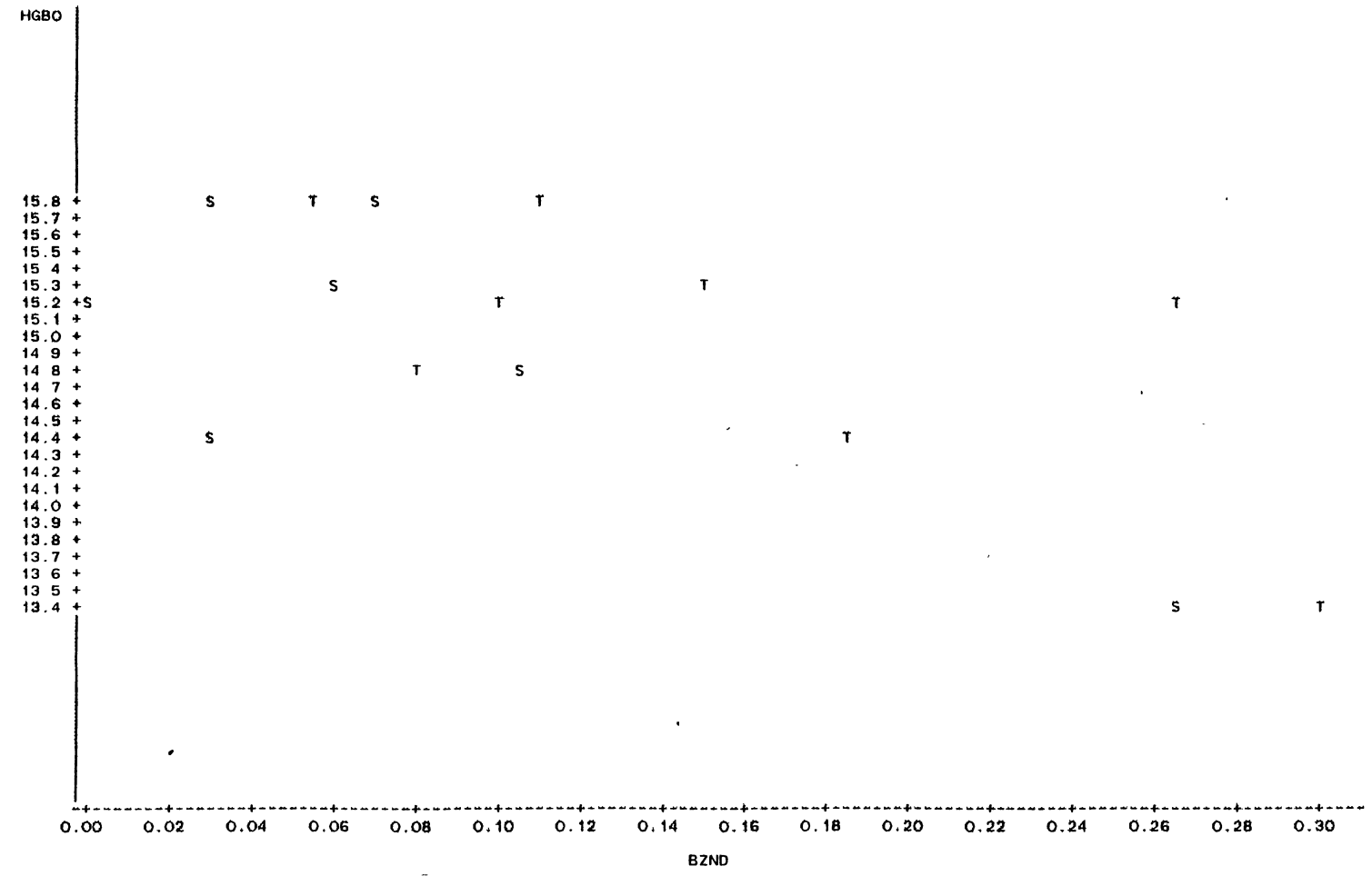


Figure 8. Relationship of Initial Hemoglobin to Change in Serum Zinc - Group F-Z

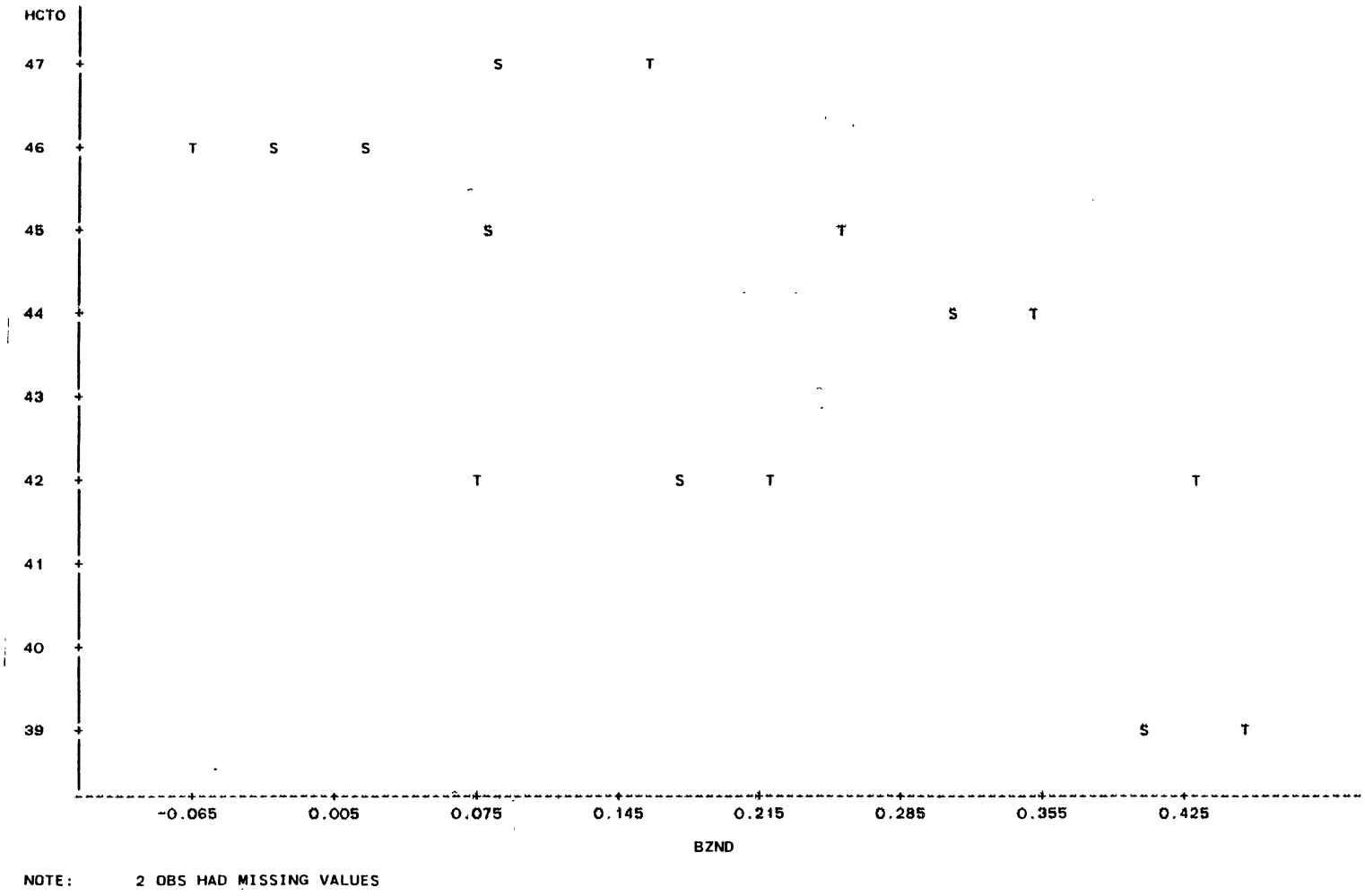
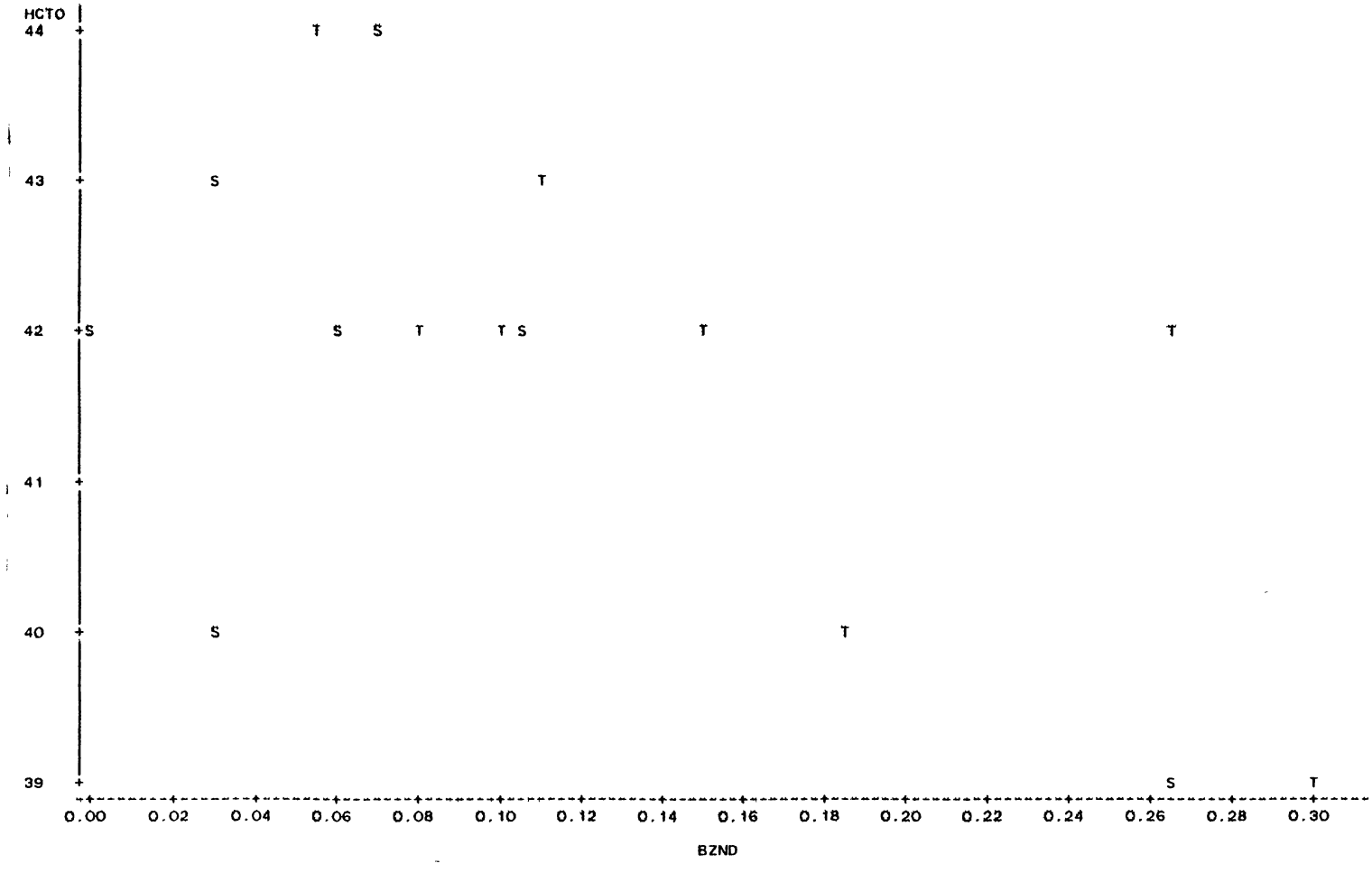


Figure 9. Relationship of Initial Hematocrit to Change in Serum Zinc - Group 2



NOTE: 3 OBS HAD MISSING VALUES

Figure 10. Relationship of Iniial Hematocrit to Change in Serum Zinc - Group F-Z

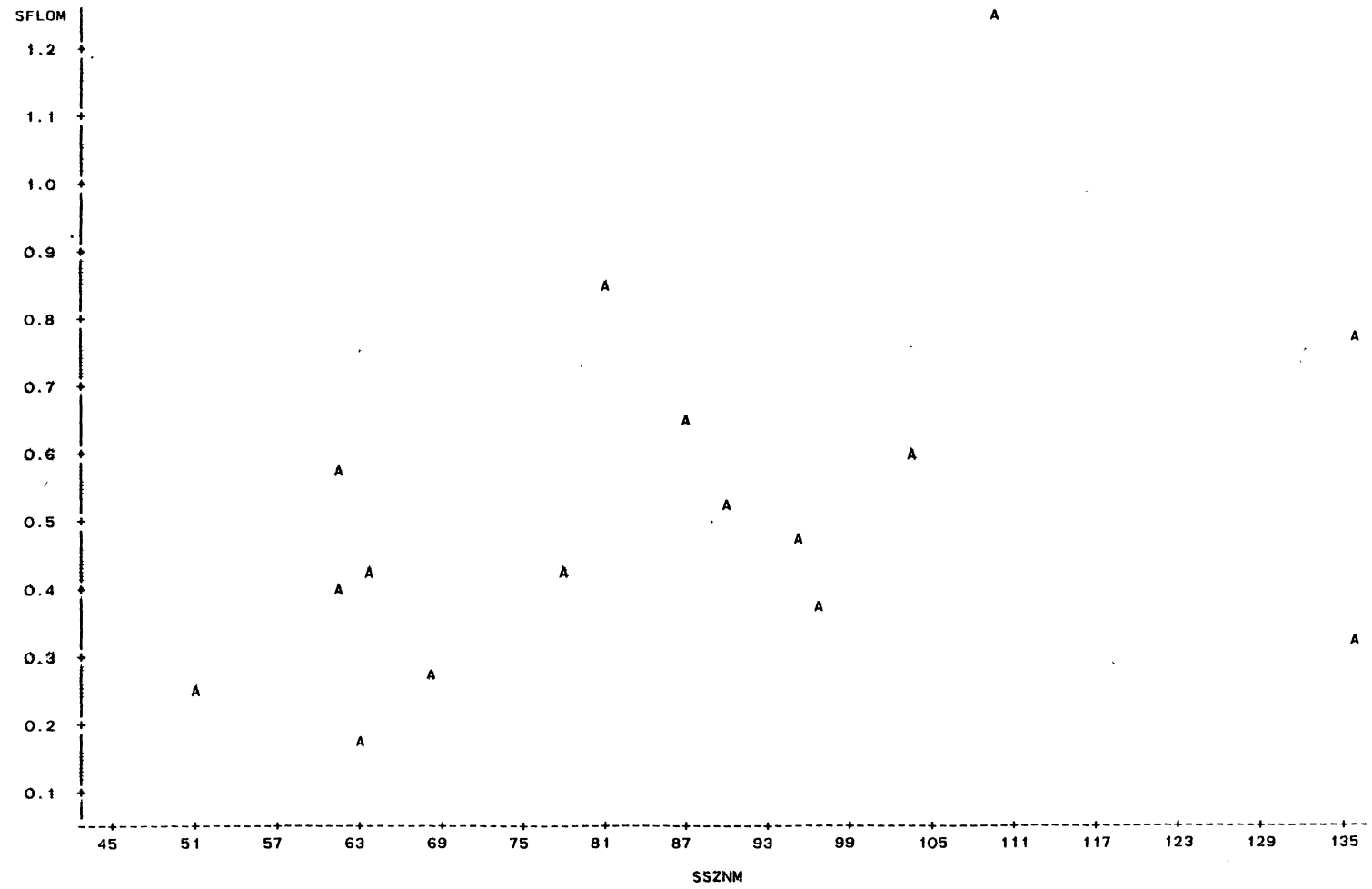


Figure 11. Relationship of Mean Saliva Flow Rate to Mean Salivary Sediment Zinc

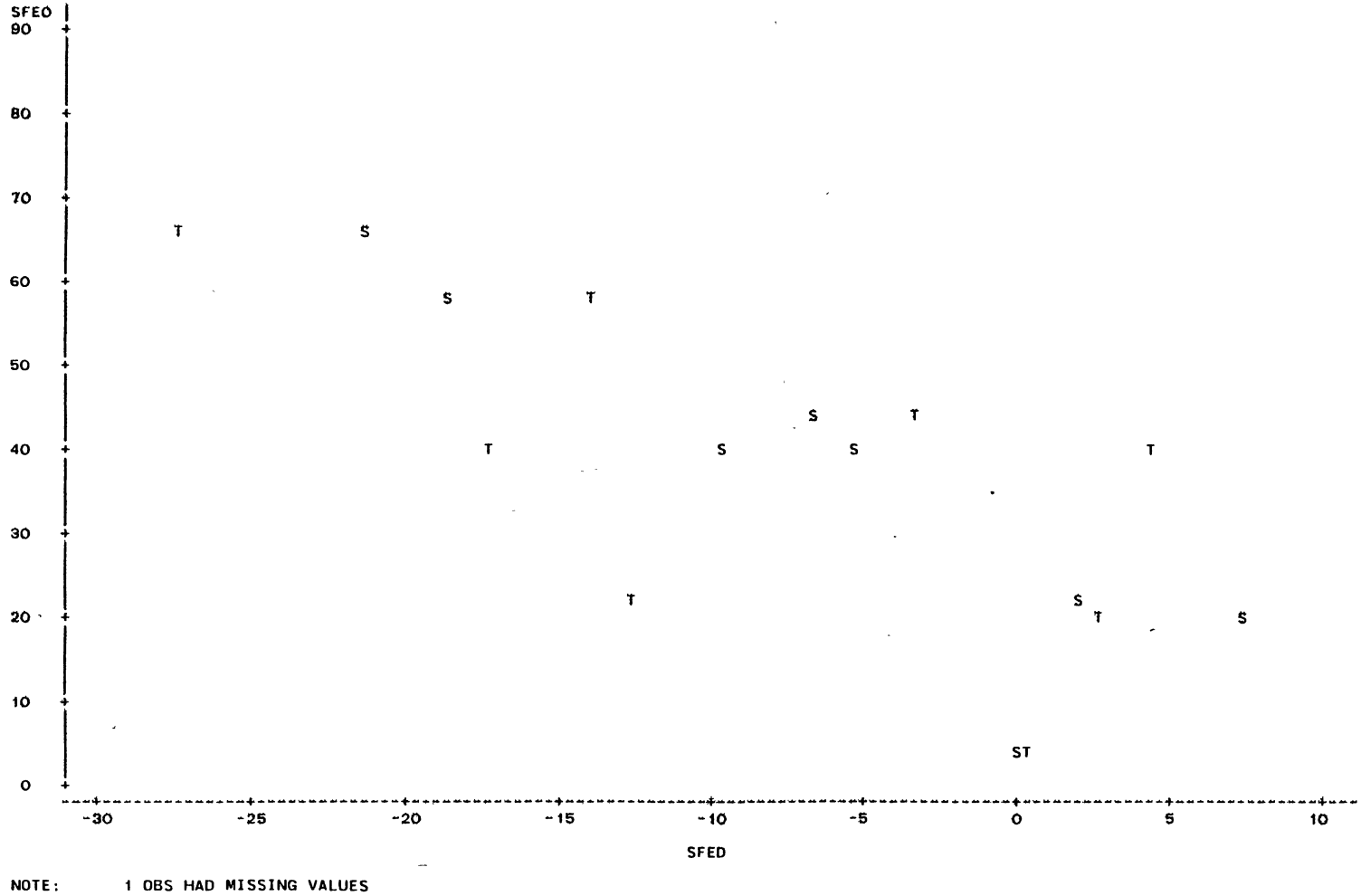


Figure 12. Relationship of Initial Serum Ferritin to Change in Serum Ferritin - Group 2

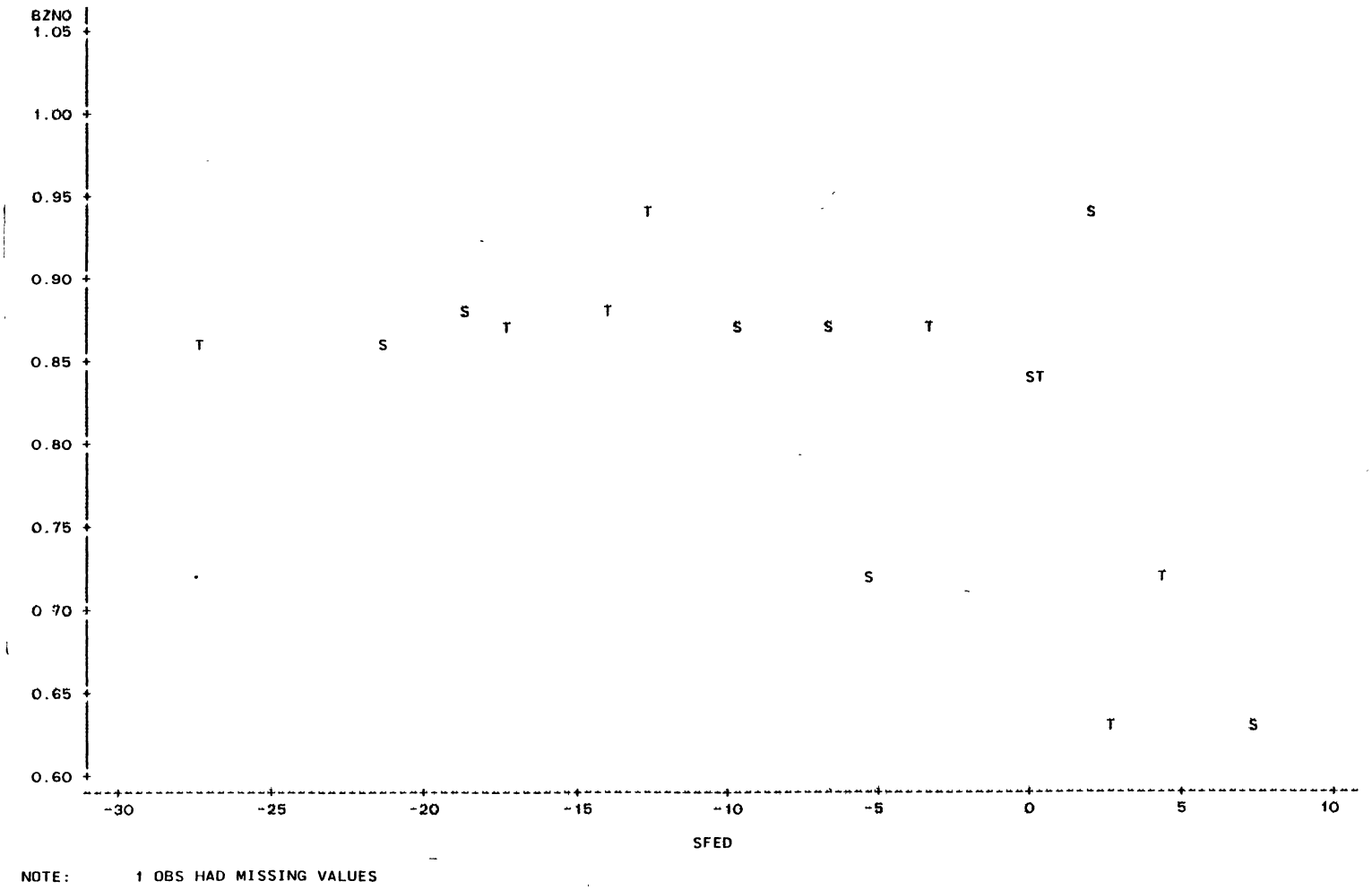
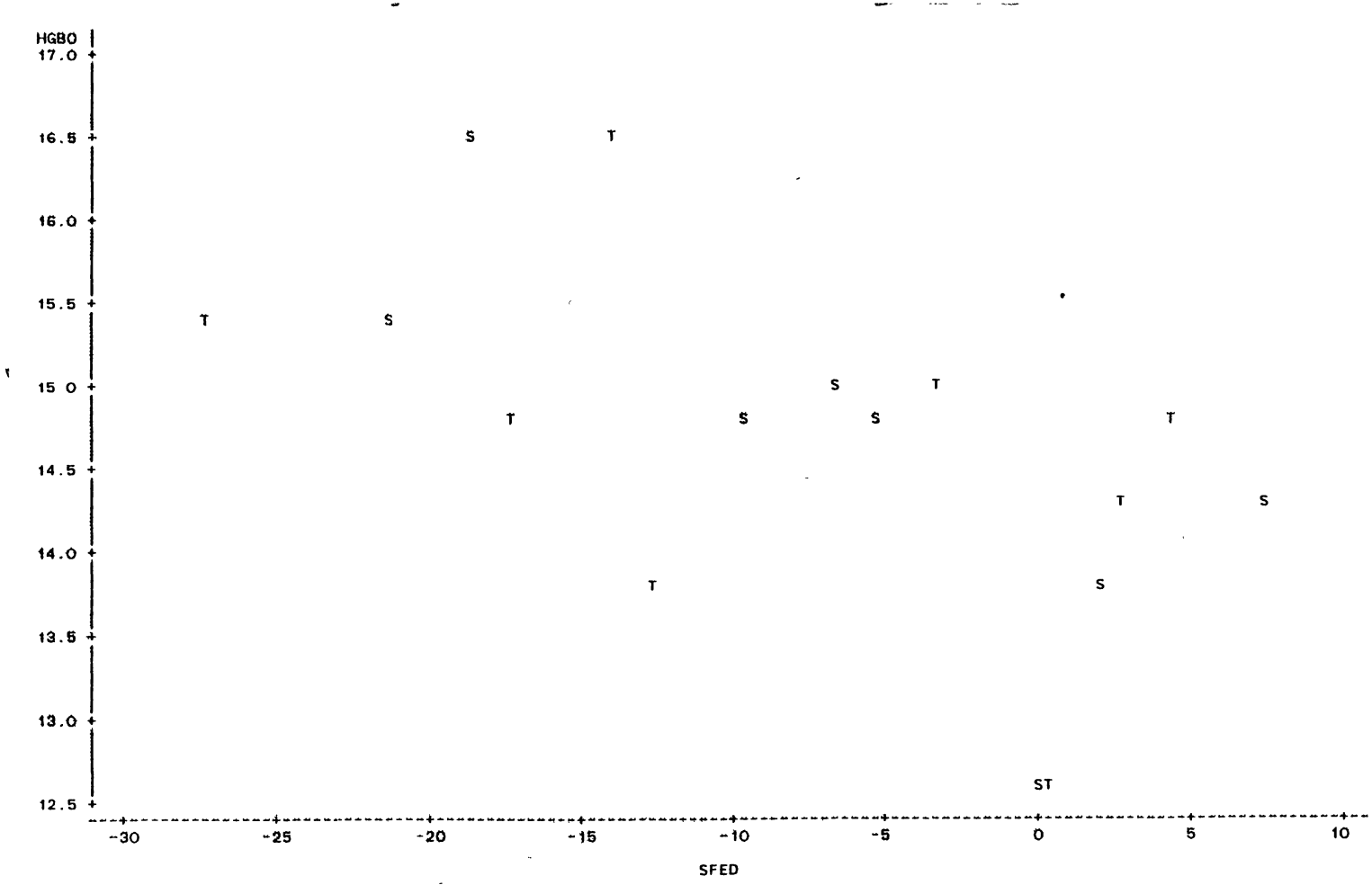


Figure 13. Relationship of Initial Serum Zinc to Change in Serum Ferritin - Group 2



NOTE: 1 OBS HAD MISSING VALUES

Figure 14. Relationship of Initial Hemoglobin to Change in Serum Ferritin - Group 2

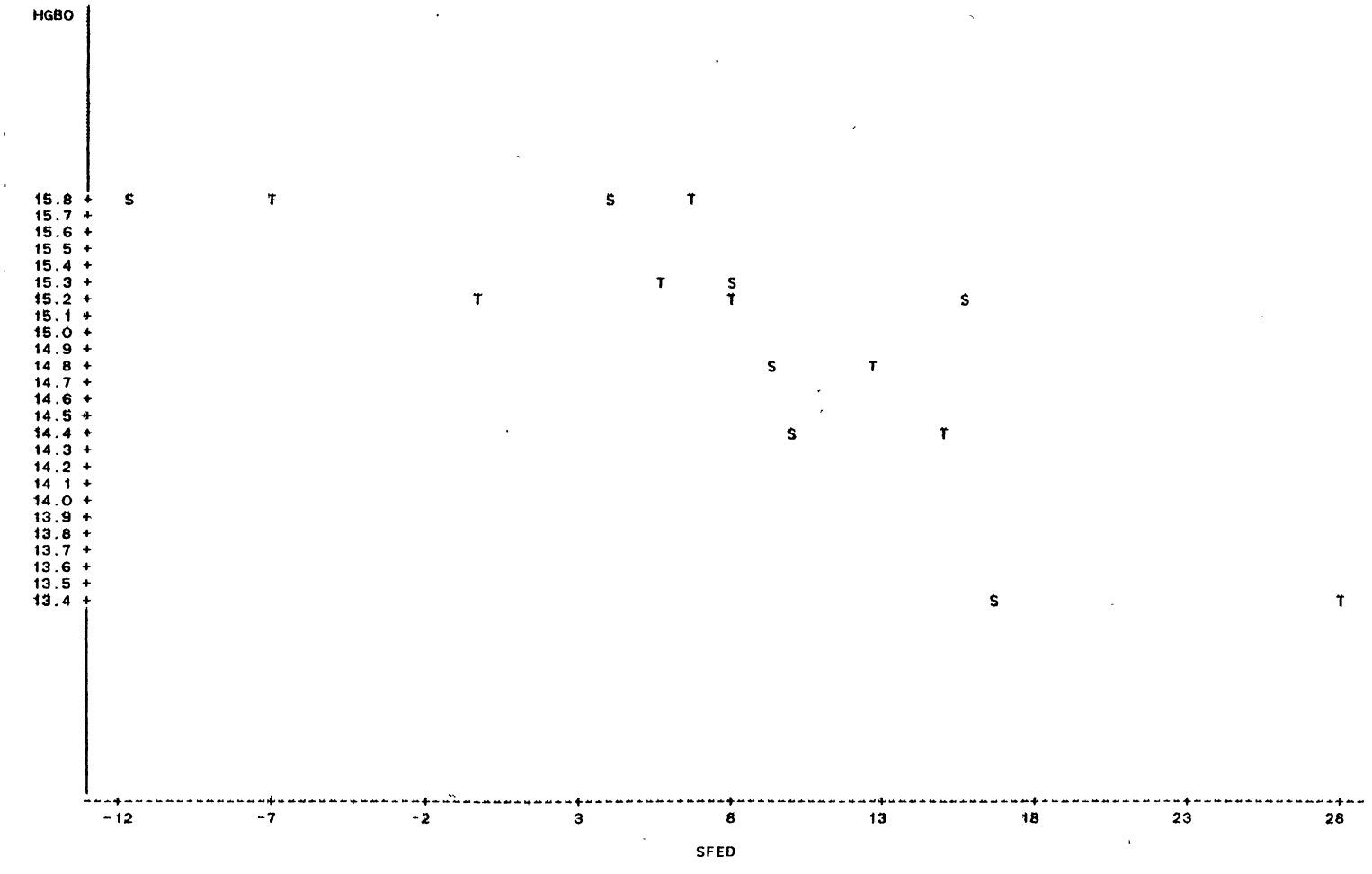


Figure 15. Relationship of Iniial Hemoglobin to Change in Serum Ferritin - Group F-Z

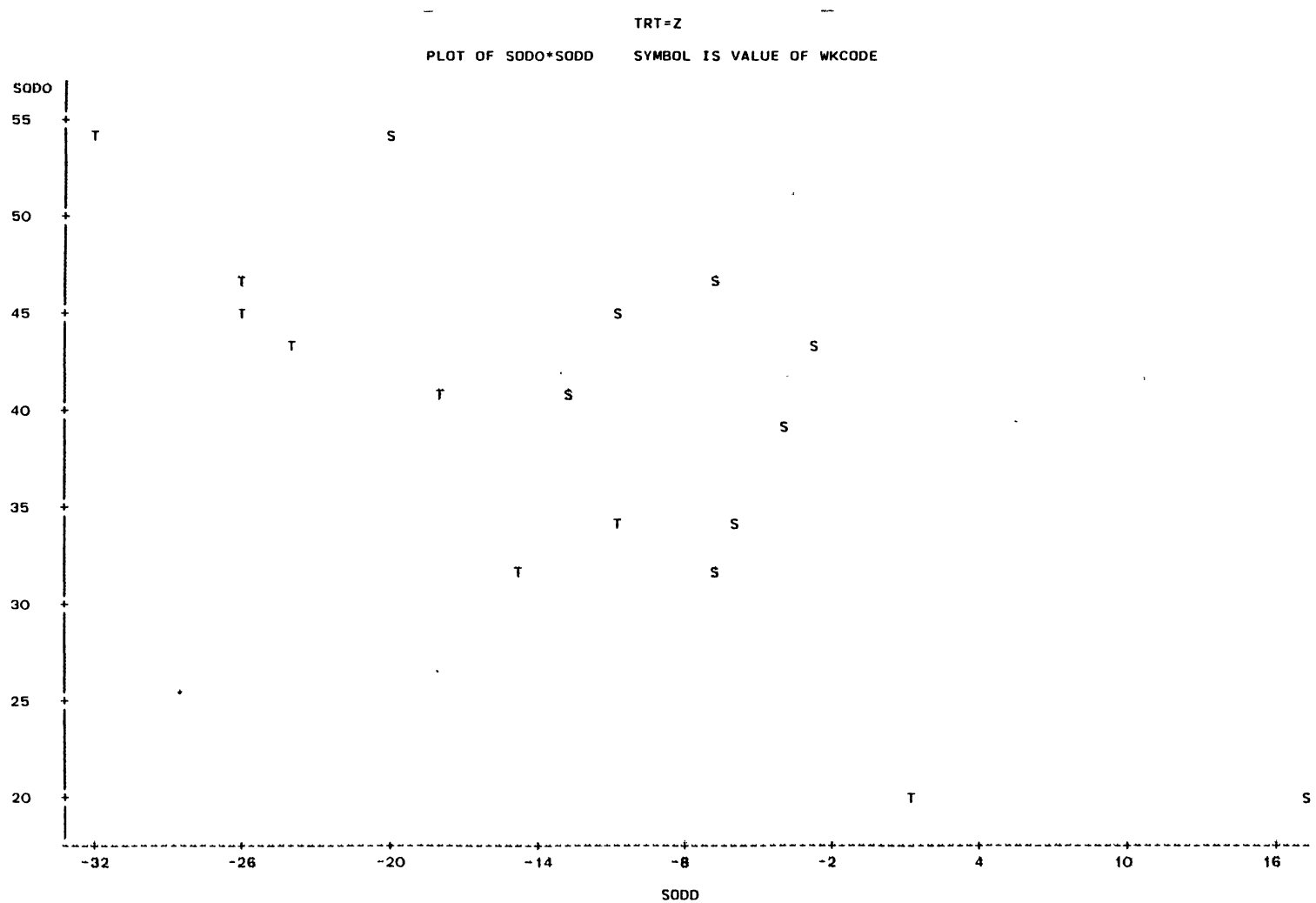


Figure 16. Relationship of Initial Superoxide Dismutase Activity to Change in Superoxide Dismutase Activity - Group Z

VITA

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