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The effect of zinc supplementation on total cholesterol, HDL-cholesterol, the HDL-cholesterol:total cholesterol ratio, HDL_2-cholesterol and HDL_3-cholesterol, serum zinc and copper among participants in a progressive exercise program

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THE EFFECT OF ZINC SUPPLEMENTATION ON TOTAL CHOLESTEROL, HDL CHOLESTEROL, THE HDL CHOLESTEROL:TOTAL CHOLESTEROL RATIO, HDL_2-CHOLESTEROL AND HDL_3-CHOLESTEROL, SERUM ZINC AND COPPER AMONG PARTICIPANTS IN A PROGRESSIVE EXERCISE PROGRAM

by

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APPROVAL PAGE

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The purpose of this dissertation research was to determine the effects of zinc supplementation on total cholesterol, HDL cholesterol, the HDL-cholesterol:total cholesterol ratio, HDL_2 cholesterol, HDL_3 cholesterol, serum zinc and serum copper in a progressive exercise program. Subjects (n=47) in a weight reduction program received either 15 mg zinc (E) or a placebo (C) over a 12 week period. The weight reduction program consisted of 12 lifestyle management workshops and 36 activity sessions. Blood samples were drawn at week 0 and week 12 and analyzed for total cholesterol, HDL cholesterol, HDL_2-cholesterol, HDL_3-cholesterol, copper and zinc. Mile walk times were assessed at both week 0 and week 12 in order to measure fitness. To be consistent, nonparametric statistical tests were used to test all hypotheses. Based on final results, zinc supplementation (15 mg) did not significantly affect lipid status. Significant ($p<0.05$) changes during the study included decreased percent body fat (-1.1 $\pm$1.5 percent body fat difference) among control females, decreased walk time for experimental (-1.23 $\pm$ 1.71 minutes difference) and control (-1.10 $\pm$ 1.10 minutes difference) females, increased HDL-C (5.6 $\pm$ 2.3 mg% difference) for experimental males, increased HDL_2 (7.7 $\pm$ 7.7 mg% difference) for control females, decreased HDL_3 for experimental (-13.9 $\pm$ 12.7 mg% difference) and control (-16.3 $\pm$ 20.4 mg%

difference) females, decreased serum zinc (-37 ± 27 ug/dl difference) for experimental females, decreased serum copper (-33 ± 20 ug/dl difference) for control males and experimental) females (-39 ± 27 ug/dl difference). Significant positive correlations occurred between final serum zinc and both final HDL-C ($r=0.89$; $p=0.007$) and final HDL_2 cholesterol ($r=0.87$; $p=0.01$), between initial serum zinc and initial HDL_3 cholesterol for females ($r=0.47$; $p=0.03$), between the zinc/copper ratio difference and HDL-C:TC ratio difference for control males ($r=0.81$; $p=0.02$). Significant negative correlations were observed between initial walk time and initial HDL-C for females ($r=-0.44$; $p=0.04$) and between initial HDL_3 cholesterol and initial walk time for males ($r=-0.73$; $p=0.03$). In summary, zinc supplementation (15 mg/day) had no significant effect on cholesterol levels. Zinc and copper status decreased for all groups during the weight reduction program. Because of the lack of a sufficient number of returned diet records, it could not be established whether diet, exercise or the combined interaction of diet and exercsie induced the observed decline in zinc and copper status.

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CHAPTER I

INTRODUCTION

An increasing number of Americans are consuming vitamin and mineral supplements, either in doses that approximate the RDA or in "mega dose" (>10 times the RDA) formulations. The potential health benefits of such practices are unproven and largely uninvestigated.

Zinc Supplementation

Zinc supplementation has been recommended to improve wound healing, for disorders of taste and smell, to improve sexual potency, and for the treatment of acne (Walravens, 1971). Recent reports have indicated that daily administration of 440 mg of zinc sulfate (160 mg of elemental zinc) to individuals over age 70 resulted in a marked improvement in such immune parameters as delayed-type hypersensitivity responses to skin testing, number of circulating T cells, and IgG response to tetanus vaccine. (Duchateau, Delepesse, Brijens, 1981). These scientific reports have been translated in the lay press into enthusiastic endorsements of zinc supplementation in either normal or mega doses (Shaklee Zinc Fact Book, 1980). The lay literature especially encourages zinc supplements for those who exercise (Hutcheson, 1981).

Zinc Toxicity

Zinc is considered a safe mineral. Zinc salts are well tolerated and have a large margin of safety. Nevertheless, prolonged oral ingestion may result in toxicity (Murphy, 1970; Moore, 1978). Symptoms of zinc toxicity include anorexia, vomiting, nausea, lethargy, dizziness, diarrhea, muscle pain, and bleeding gastric ulcers (Moore, 1978; Donaldson, 1980; Aggett & Harries, 1979). Some symptoms can occur after the ingestion of a 220 mg capsule (Glover & White, 1977). In addition, zinc may interfere with copper metabolism (Prasad, Brewer, Schoolmaker, & Rabbani, 1978).

Zinc and Lipid Metabolism

Although the mechanisms are unknown, a high zinc to copper ratio is associated with elevated cholesterol in experimental animals (Klevay, 1973). Furthermore, high intakes of zinc reportedly reduce high density lipoprotein cholesterol (HDL-cholesterol) (Hooper, Garry & Goodwin, 1982). HDL-cholesterol is often referred to as "good cholesterol" because the elevated HDL-cholesterol is negatively correlated with an increased incidence of heart disease (Wilson, 1980). While the direct effect of excess Zn:Cu ratio on coronary heart disease has not been established by clinical studies, Klevay's research (1975) suggests that the high Zn:Cu ratio may produce unfavorable changes in cholesterol metabolism; cholesterol is a major risk factor for coronary heart disease.

Purpose of the Study

The overall purpose of this study was to determine the effects of zinc supplementation on total cholesterol, HDL cholesterol, the HDL-C:TC ratio, HDL_2 cholesterol, HDL_3 cholesterol, serum zinc and serum copper in a progressive exercise program.

Rationale of the Study

The rationale for this study is based on the premise that obese subjects are at increased risk of coronary heart disease, hypertension and diabetes. Exercise has been demonstrated by Bazzarre et al. (1985) in conjunction with a nutrition education lifestyle management program to not only be an effective method of promoting weight/fat loss but also to produce favorable alterations in chronic disease risk factors such as cholesterol/lipid profiles and blood pressure.

The results by Kennedy, Failla and Smith (1986) and Chandra and Kutty (1980) suggesting that obese subjects may be at risk of marginal zinc deficiency; results by Spencer, Kramer, Perakis, Norris and Osis (1982) suggesting that weight loss may be associated with the induction of zinc deficiency; and results by Dressendorfer and Sockolov (1980) suggesting that exercise training reduces zinc status all suggest that obese subjects on a diet/exercise/weight loss regimen should receive zinc supplementation or at least be evaluated on an individual basis for signs and symptoms of zinc deficiency. While zinc supplementation of obese subjects during a weight loss program may be advisable, it

is important that the level of supplementation not reverse favorable changes in lipid profiles. No research has been conducted in an obese population during a weight loss program to determine if a low dose zinc supplement would prevent the development of zinc deficiency but not affect the expected favorable changes in lipid profiles.

Objectives

The specific objectives of the 12-week study were the following:

(1) To measure fasting total cholesterol (TC), HDL-C and the HDL-C: TC ratio as well as plasma zinc and copper of experimental and control subjects at weeks 0 and 12.

(2) To measure the effects of activity/fitness on serum zinc status of experimental and control subjects at weeks 0 and 12.

(3) To measure the relationship between serum zinc and TC, HDL-C and HDL-C:TC ratio, HDL_2, HDL_3 and obesity among experimental and control subjects at weeks 0 and 12.

(4) To measure the relationship between serum zinc/copper ratio and the HDL-C:total cholesterol ratio at weeks 0 and 12.

Hypotheses

In the following hypotheses, E represents the experimental subjects participating in a 12 week exercise program who received Zn supplementation (15 mg Zn/day) during the 12 weeks. C represents control subjects receiving no Zn supplementation but participating in the exercise program. E:C represents the

comparative evaluation of significant differences between the means for the experimental subjects and control groups.

Total Cholesterol (TC)

H_1-E: TC is not significantly different at week 0 versus week 12 among experimental subjects.

H_1-C: TC is signficantly different at week 0 versus week 12 among control subjects.

H_1-E:C: TC is significantly different between experimental and control subjects at week 12.

HDL Cholesterol (HDL-C)

H_2E: HDL-C is not significantly different at week 0 versus week 12 among experimental subjects.

H_2C: HDL-C is significantly different at week 0 versus week 12 among control subjects.

H_2-E:C: HDL-C is significantly different between experimental and control subjects at week 12.

HDL Cholesterol:Total Cholesterol (HDL-C:TC)

H_3-E: HDL-C:TC is not significantly different at week 0 versus week 12 among experimental subjects.

H_3-C: HDL-C:TC is significantly different at week 0 versus week 12 among control subjects.

H_3-E:C: HDL-C:TC is significantly different between experimental and control subjects at week 12.

HDL_2 Cholesterol

H_4E: HDL_2 is not significantly different at week 0 versus week 12 among experimental subjects.

H_4C: $HDL_2$is significantly different at week 0 versus week 12 among control subjects.

H_4-E:C: HDL_2 is significantly different between experimental and control subjects at week 12.

HDL_3 Cholesterol

H_5E: HDL_3 is not significantly different at week 0 versus week 12 among experimental subjects.

H_5C: HDL_3 is significantly different at week 0 versus week 12 among control subjects.

H_5-E:C: HDL_3 is significantly different between experimental and control subjects at week 12.

Correlations between Independent Variables and Blood Lipids among Male and Female Participants in a Progressive Exercise Program

H_6: There is a positive association between serum zinc and TC for males and females.

H_7: There is an inverse association between serum zinc and HDL-C for males and females.

H_8: There is an inverse association between serum zinc and HDL-C:TC for males and females.

H_9: There is an inverse association between HDL_2 and serum zinc for males and females.

H_{10}: There is an inverse association between HDL_3 and serum zinc for males and females.

H_{11}: There is an inverse association between obesity and serum zinc for males and females.

H_{12}: There is an inverse association between the serum zinc/copper ratio and the HDL-C: total cholesterol ratio for males and females.

H_{13}: There is an inverse association between activity/fitness and serum zinc for males and females.

Definitions

Control subjects: Subjects participating in a 12 week nutrition education, physical fitness, lifestyle management program (the RESHAPE program) who received a placebo.

Experimental subjects: Subjects participating in the 12 week RESHAPE program who received zinc supplementation (15 mg/day).

CHAPTER II

REVIEW OF LITERATURE

Introduction

The following literature review provides background information on zinc function, deficiency and supplementation. The discussion also addresses characteristics of the study subjects, experimental designs, and the effects of zinc on lipid profiles. Finally, the results and conclusions of the studies included in the literature are reviewed and evaluated.

Zinc Deficiency

The essentiality of zinc (Zn) is based in part on its role as an integral part of a number of metalloenzymes and as a cofactor for regulating the activity of specific zinc-dependent enzymes involved in normal protein, carbohydrate, lipid and nucleic acid metabolism (Roth & Kirchgessner, 1980). Zinc deficiency in experimental animals leads to growth retardation, and deficiency during prenatal life can result in the abnormal development of the embryo and fetus (Swenerton & Hurley, 1968; Hurley, 1970).

Prasad (1979) and, Casey and Hambidge (1980) have reported that zinc deficiency does occur in humans. Nutritionists are concerned about the zinc status of children, teenagers, pregnant women, vegetarians consuming high fiber diets (Swanson & King,

1979; Hambidge, Jacobs & Baum, 1972) and, possibly, endurance athletes (Dressendorfer & Sockolov, 1980). Chandra and Kutty (1980) have reported that obese subjects may be at risk for marginal zinc status; however, clear evidence of inadequate zinc status in the general population is lacking (Swanson et al., 1979). The lack of evidence seems logical since zinc is widely available in food from both plant and animal sources (Freeland & Cousins, 1976).

Men who exercise regularly have a lower risk of coronary heart disease than men who do not exercise (Klevay, 1975). The concentration of zinc (0.93 mg/liter) in sweat (Prasad, Schulert, Sandstead, Miale & Farid, 1963) is approximately 16 times that of copper (0.058 mg/liter). Thus, sweating is associated with a relatively greater loss of zinc than copper, resulting in a lower ratio of plasma zinc to copper (Klevay, 1975).

Klevay (1975) has identified a metabolic imbalance of zinc to copper as a major factor in the etiology of coronary heart diease. This metabolic imbalance is either a relative or absolute deficiency of copper characterized by a high ratio of zinc to copper. The imbalance results in hypercholesterolemia and increased mortality due to coronary heart disease. The elevated zinc:copper ratio results from imbalances in the amounts of zinc and copper consumed (Klevay, 1975). For example, diets associated with a high risk of coronary heart disease are likely to contain a high zinc to copper ratio and to be low in phytic acid and fiber. Both phytic acid and fiber are chelating agents which may prevent a high zinc to copper ratio by reducing zinc bioavailability (i.e., absorption).

Zinc and copper have been measured (Webster, 1965) in the uninjured, presumably healthy, myocardial tissue of victims of myocardial infarction and the myocardial tissue of victims of accidents. No difference was found in the concentration of zinc between the two groups; however, the concentration of copper was lower in tissue from hearts with infarction. Thus, the victims of coronary heart disease may be presumed to have a higher than normal ratio of zinc to copper (Klevay, 1975).

Zinc Supplementation

As discussed in the next section, some nutritionists have become concerned that excessive zinc supplementation may unfavorably alter lipid profiles. These reports raise the possibility that a mineral consumed by millions of individuals daily in multivitamin and mineral supplements (Read & Graney, 1981) may be lowering high density lipoprotein cholesterol, which is associated with a decreased risk of cardiovascular disease (Castelli, Doyle & Gordon, 1977).

Since zinc-deficient animals exhibit impaired reproduction (Hurley & Swenerton, 1966), promoters of zinc supplements claim that large doses increase virility and improve sex drive. Zinc supplements are also promoted because of alleged benefits regarding tissue growth and repair and as a preventive measure to delay various aging processes. No evidence exists that zinc supplementation offers benefits to healthy people with adequate zinc intakes, the RDA being 15 mg/day for most adults (Food and

Nutrition Board of the National Research Council, 1980). Zinc supplementation may be required by young children suffering from the skin disease acrodermatitis enteropathica (Nelder & Hambridge, 1975) and by patients receiving total parenteral nutrition (Arakawa, Tamura, Igarashi, Suzuki & Sandstead, 1976).

Zinc and Cholesterol

The suggestion that dietary levels of zinc and copper play an important role in lipoprotein metabolism was originally forwarded by Klevay (1973; 1975) who suggested that an increase in the ratio of zinc to copper in the diet caused hypercholesterolemia, leading to an increased risk of atherosclerosis. Subsequent work in experimental animals and man has produced conflicting results (Petering, Murthy & O'Flaherty, 1977; Looney & Lei, 1978; Caster & Doster, 1979; Fischer, Giroux & Belonje, 1980; Prasad, 1976; Sandstead, Klevay & Mahalko, 1980; Allen & Klevay, 1978; Freeland-Graves, Han, & Friedman, 1980; Koo & Williams, 1981). Nevertheless, the possibility exists that a mineral consumed by millions of individuals daily in multivitamin and mineral supplements (Read & Graney, 1981) may be lowering high-density lipoprotein (HDL)-cholesterol. HDL-cholesterol is a marker for decreased risk of cardiovascular disease. (Castelli, Doyle & Gordon, 1977).

Mertz (1979; 1982) has concluded that no evidence currently links trace element status directly to cardiovascular disease; rather a number of trace elements apparently influence individual risk

factors for cardiovascular disease. Specifically, zinc supplementation in rats on a copper- and zinc-deficient diet was associated with substantial increases in serum cholesterol. Increasing the copper content of the diet partially counteracted the hypercholesterolemic effect of zinc (Klevay, 1973). The relative importance of zinc "excess" to copper "deficiency" to hypercholesterolemia is a matter of some controversy (Klevay, 1973; Klevay, 1975; Petering et al., 1977; Looney et al., 1978; Caster & Doster, 1979; Allen & Klevay, 1978). Data regarding the influence of zinc intake on blood lipid parameters in humans is more limited. Prasad (1976) reported that an experimental zinc deficiency in two volunteers was associated with decreased serum cholesterol levels, while Sanstead et al. (1980) found experimental zinc deficiency in a single volunteer was associated with increased serum cholesterol.

Other investigators have evaluated whether altered HDL composition is due specifically to zinc deficiency or to reduced food intake (Schneeman, Lacy, Ney, Lefevre, Keen, Lonnerdal & Hurley, 1986). Rats were placed in three groups for 21 days. The three groups were rats fed a zinc-adequate diet (100 ppm) ad libitum, rats fed a zinc-deficient diet (< 1 ppm) ad libitum, and rats fed a zinc-adequate diet equal to the calorie intake of the zinc-deficient group (i.e., pair-feeding). Animals consuming the low zinc diet were zinc deficient. The zinc deficient diet which impaired appetite and reduced calorie intake was associated with lower plasma VLDL triglyceride levels and lower HDL cholesterol levels. The

similarities in the overall pattern of lipoprotein composition between the zinc-deficient and zinc adequate-calorie restricted groups indicate that reduced food intake may account for the differences in lipoprotein composition.

Zinc in Weight Loss, Exercise and Obesity

Excessive zinc loss, induced by weight loss during total starvation, is related to increased plasma levels of zinc (Spencer, Kramer, Perakis, Norris & Osis, 1982). These levels, then, do not reflect zinc status during catabolic states of weight loss. (Spencer et al., 1982). Spencer et al. (1982) arrived at this conclusion after monitoring plasma levels and urinary excretions in 18 total starvation studies carried out over 35 to 80 days in the excessively obese. The authors did not state how many subjects were included in their research. As plasma levels increased to 140 ug% (control levels averaged 100 ug%), urinary zinc excretion reached 4.8 mg/day in contrast to an average of 1.4 mg/day for controls.

The increased plasma and urinary excretion levels Spencer et al. (1982) observed were probably the result of the breakdown of lean body tissue (Klevay, 1975). Lean body tissue is a storage site for zinc (Klevay, 1975). As in the following example, however, different conditions, e.g., intensive training, can increase urinary zinc excretion without increasing serum zinc.

A similar increase in urinary zinc excretion occurred among 30 soldiers studied during a 34-day intensive training exercise (Miyamura, McNutt & Wenkam, 1987). In contrast to Spencer et al.

(1982), serum zinc levels were depressed. According to the investigators, the depressed serum zinc concentrations indicated less zinc available to metabolically active tissue. This depression, they postulated, might "compromise the soldier (or the athlete) who must be at peak performance (physical or mental) at all times" (Miyamura et al., 1987).

The difference between the two studies, i.e., Spencer et al. (1982) and Miyamura (1987), is probably due to two factors. The soldiers probably lost zinc through sweat, while the obese patients increased serum zinc through breakdown of lean body tissue. Lean body tissue is a storage site for zinc (Klevay, 1975).

In a study of experienced runners (Dressendorfer & Sockolov, 1980), serum zinc concentrations decreased with increased training distance. In addition, the redistribution of zinc stores in the body could explain the hypozincemia accompanying exercise. Oh, Deagan and Whanger (1978) observed decreased plasma zinc concentrations during acute endurance exercise in rats. However, this decline was associated with increased synthesis of metallothionein, a zinc-binding protein. Thus, exercise training may stimulate production of zinc-dependent enzymes in the liver and other tissues, thereby lowering circulating zinc levels (Oh, Deagan & Whanger, 1978).

Obesity may affect plasma zinc in both animals and humans (Kennedy, Failla & Smith, 1986; Chandra & Kutty, 1980). Chandra and Kutty (1980), for example, found that 38 percent of obese children and adolescents had a moderately lower serum zinc concentration than did their moderate weight counterparts. Lipid profiles were

not assessed in these studies. In contrast to the previous study, normal serum zinc concentrations were observed in 285 obese patients hospitalized in a department of internal medicine for acute or chronic conditions (Pras, Dayada, Bertrand, Lapalus, Garaffo, Savini & Babeau, 1983). Further research is needed in free-living populations in order to interpret the conflict between these studies.

Human Studies of Zinc Supplementation on Lipid Profiles

While various studies have investigated the relationship of zinc and plasma cholesterol levels among animals (Klevay, 1973; Looney & Lei, 1978; Murthy & Petering, 1976; Helwig, Mulmix & Regenstein, 1978; Eisemann, Pond & Thonney, 1979), only five studies have been reported in the literature since 1980 which focus on the effects of zinc supplementation on lipid profiles of humans (Freeland-Graves, Han, Friedman, Shorey, 1980; Hooper, Visconti, Garry & Johnson, 1980; Goodwin, Hunt, Hooper & Garry, 1985; Crouse, Hooper, Atterbom & Pappenfuss, 1984; Black, Medeiros, Brunett & Welke, 1984).

This final section of the review of literature represents a critical analysis of the design and results of these five research papers. The review includes a brief general description of the findings of each study, a review of the design of these studies (e.g., sample selection), a review of the various supplementation levels used, a review of the various methods used to monitor supplementation compliance, the methods used to evaluate diet, the methods used to collect blood samples, and the procedures used to

analyze lipid profiles, a review of the methods used to evaluate activity/exercise, a review of the methods used to measure body fat, and, finally, a review of the analytical methods used to measure statistically significant effects of zinc supplementation on lipid profiles.

Hooper et al. (1980) studied the effect of administering 440 mg of zinc sulfate (160 mg of elemental zinc) per day for 35 days to 12 healthy adult men. Total cholesterol remained constant, but HDL-cholesterol decreased from 40.5 $\pm$ 6.5 mg/dL before treatment to 33.5 $\pm$ 5.0 mg/dL after treatment ($p=.0001$). While the amount of zinc used in this trial was quite high (~10 times the RDA), the results raised the question of whether lower levels of zinc supplementation might also be associated with decreased HDL-cholesterol.

Freeland-Graves et al. (1980) reported a transient decrease (over two weeks) in both total and HDL-cholesterol in eight women given 100 mg zinc (as zinc acetate) but no change in HDL-cholesterol with lower levels of zinc supplementation (50 mg/dL or less) during the 60-day study period. The level of physical activity of these subjects was not considered.

Crouse et al. (1984) studied, 21 endurance trained men and 23 sedentary men who received either a placebo or 50 mg zinc sulfate daily for eight weeks. Although plasma zinc increased 15 percent, fasting plasma high density lipoprotein cholesterol, total cholesterol, low density lipoprotein cholesterol and triglyceride levels did not change in response to zinc ingestion. The authors

concluded that low-dose zinc supplementation does not affect lipid or lipoprotein values in either endurance-trained or sedentary men.

Goodwin et al. (1985) investigated the association between the level of exercise, ingestion of zinc supplements (>15 mg)/d), and serum high-density lipoprotein (HDL) cholesterol levels in 270 healthy men and women over age 60. After controlling for gender, alcohol intake, and body mass, there was a small but significant positive correlation (partial r=0.26, p=0.005) between the level of exercise and serum HDL cholesterol in the 180 subjects not taking supplemental zinc. There was no correlation for those subjects taking supplemental zinc (r=-0.18, p=0.14). A significant interaction of zinc intake and activity level on HDL cholesterol was noted as a result of multiple regression analysis. In 22 subjects who were ingesting 17.5 to 52.2 mg per day (median 24 mg per day) of supplemental, elemental zinc daily, cessation of zinc supplements for eight weeks was associated with a significant increase in HDL cholesterol levels (2.0 mg/dL, p=0.04). The change in HDL after stopping zinc was positively correlated (r=0.41, p=0.05) with the level of exercise of the subjects. Thus, supplemental zinc ingestion (>15 mg/day) appears to block the exercise-induced increase in serum HDL cholesterol in a healthy population. No associations with total cholesterol were observed.

Black et al. (1988) reported significantly lower HDL cholesterol levels in subjects assigned to the 75 mg group at weeks 6 and 12 when compared to the placebo group and when compared to baseline values at weeks 6, 8 and 12 of the study. Subjects assigned to the

50 mg group had lower serum HDL cholesterol at week 12 than did the placebo group and lower levels at week 12 than at baseline.

Study Parameters

The number of subjects in the five studies was relatively small: 20 (Hooper et al., 1980), 30 (Black et al., 1988), 32 (Freeland-Graves et al., 1980) and 44 (Crouse et al., 1984). Only Goodwin et al. (1985) evaluated a large number of subjects (n=270). Only women were included in one study (Freeland-Graves et al., 1980). Only men were included in two studies (Crouse et al., 1984; Hooper et al., 1980). Goodwin (1985) studied a mixed sample of males and females but did not evaluate any effects of gender in the data analyses.

The ages of the subjects in these studies ranged from relatively young adults (Black et al., 1988; Freeland-Graves et al., 1980; Hooper et al., 1980) to senior adults (Goodwin et al., 1985). Subjects were 18-40 years old in the Freeland-Graves et al. study (1980) and 19-29 years in the Black et al. (1988) study. Hooper et al. (1980) used subjects between 23 to 35 years. Crouse et al. (1984) evaluated subjects 20 to 55 years of age. All subjects in the Goodwin et al. (1985) study were older than 60.

Zinc Dose, Timing and Length of Study

The effects of zinc on lipids may be related to the dose used and the length of each study. The studies lasted 60 days (Freeland-Graves et al., 1980), five weeks (Hooper et al., 1980), eight weeks (Crouse et al., 1984), and five years (Goodwin et al., 1985).

Zinc dose varied among these studies. Dividing subjects into four groups, Freeland-Graves et al. (1980) administered 0, 15, 50 and 100 mg. zinc as zinc acetate. The supplements were ingested daily with the evening meal. Black et al. (1988) administered a placebo to one group, 50 mg zinc to a second group and 75 mg zinc to a third group. Dosage was divided between two capsules taken daily after breakfast with a glass of water for the duration of the study. Hooper et al. (1980), using experimental and control groups, administered 220 mg. zinc sulfate to the experimental group. Subjects took the capsules twice a day with meals. Crouse et al. (1984) administered 50 mg. zinc sulfate. Subjects were instructed to ingest one capsule daily midway between breakfast and lunch. Goodwin et al. (1985) did not adminster zinc supplements. Correlations for the subjects' exercise with serum HDL cholesterol and the self reports of supplemental zinc use during the study were calculated. As an intervention study, the investigators identified 23 subjects who were taking >15 mg a day zinc and who had no change in supplemental intake in six months. These subjects agreed to stop taking zinc supplements or to substitute a multivitamin not containing zinc for an eight week period.

The timing of zinc supplementation varied among the five studies. There is little, if any, available data on whether the timing of supplement use, use with or without meals, or the number of supplements taken per day influences diurnal variation in plasma zinc or copper levels or blood lipids. Nevertheless, drug studies

indicate that dosage use is an important factor that could be a confounding variable in interpreting results between studies.

Supplementation Monitoring

Hooper et al. (1980) stated their subjects received a zinc sulfate capsule twice a day with meals. They implied that the investigators distributed the supplements each day. Black et al. (1988) had subjects take the capsules without monitoring. Crouse et al. (1984) had subjects maintain a daily record of capsule ingestion. On an average, the subjects missed two capsules throughout the entire eight week study. Freeland-Graves et al. (1980), like Hooper et al. (1980), administered supplements directly to the subjects. Goodwin et al. (1985), as mentioned elsewhere, did not adminster zinc but observed HDL-C differences between subjects taking zinc supplements and subjects not taking zinc supplements.

Study Design

Hooper et al. (1980) obtained informed consent from subjects, asked them not to alter alcohol ingestion, exercise, smoking and diet, and they obtained three fasting baseline lipid profiles. Twelve of the 20 subjects received zinc sulfate, and eight subjects received a placebo. Fasting lipid levels were determined on a weekly basis and continued two weeks after the zinc supplementation stopped. A final lipid determination was done 16 weeks after the study began.

Freeland-Graves et al. (1980) randomly divided participants, in a double blind design, into four groups of eight subjects each. The

subjects received either 0, 15, 50 or 100 mg zinc supplements. Blood samples were taken at two week intervals over a 60 day period.

Crouse et al. (1984) divided subjects into endurance trained and sedentary categories. Subjects were matched for age and randomly assigned to either treatment or placebo group. Ten-hour post absorptive blood samples were obtained from each subject on three separate days, and the zinc and placebo capsules were dispensed using a double blind procedure. Blood samples were collected biweekly after an overnight fast throughout the eight week treatment period.

Using self reported information, Goodwin et al. (1985) divided subjects into those who exercised and those who did not exercise; and into those subjects who took zinc supplements and those who did not. Correlation coefficients were then calculated between the level of exercise, zinc supplement use and serum HDL cholesterol. As a separate part of the study, 28 subjects who took supplemental zinc >15mg/dL were identified. Of these, 23 subjects who had no change in supplemental zinc intake in the previous six months agreed to stop zinc supplements or to substitute a multivitamin with mineral preparation not containing zinc. The subjects were free of zinc supplementation for eight weeks.

Black et al. (1988) randomly divided subjects by double blind design into three treatment groups. Subjects in each group received either 50 mg zinc, 75 mg zinc or a placebo tablet. Blood and urine

samples and diet records were collected biweekly. Blood was drawn biweekly after 12 hour fasting.

Diet

Hooper et al. (1980) and Crouse et al. (1984) controlled for diet solely by asking subjects not to alter their usual diet. Goodwin et al. (1985), however, conducted an elaborate procedure. The dietary intake of zinc was assessed from a single three day food record during three successive weekdays. At the end of the three day food recording period, a dietitian visited the subjects' homes to check the completeness of the records. If vitamin and/or mineral supplements were used by the subject, the brand name, contents, and amounts of each nutrient were recorded. All food records were coded and then analyzed by computer for nutrient composition using a nutrient data base obtained from Case Western Reserve University, Cleveland, Ohio. Total zinc intake was calculated as the average daily intake from the three day food record plus the zinc contained in any supplemental mineral preparations each subject was taking.

Freeland-Graves (1980) also used a dietary record. The three day records were "kept throughout the experiment", but the investigators did not mention the frequency of the record. Nevertheless, nutrient intakes were calculated from a computer assisted program of USDA food composition tables. Mean dietary zinc was then added to the amount of supplemental zinc each participant received during the study. This amount was then divided

by the mean dietary copper. The mean Zn/Cu ratios for the four treatment groups as calculated were 3 ± 2, 13 ± 4, 21 ± 8, 64 ± 23.

Black et al. (1988) collected diet history for each subject during the initial interview. Three day diet records were obtained biweekly, and data was coded and analyzed for nutrients by a Nutritionist III computer program.

Blood Sampling

All studies used fasting samples and similar blood collection procedures. Freeland-Graves et al. (1980) had fasting subjects donate 12 ml samples of blood on Tuesday mornings from 7-9 a.m. at two week intervals. Venous blood was withdrawn from the forearm via disposable syringes equipped with 21 gauge stainless steel needles and tranferred to acid washed, polypropylene test tubes. Whole blood was allowed to clot at room temperature for 20 minutes and frozen until subsequent analysis. Heparinized plasma was obtained via centrifugation at 3500 rpm for 10 minutes in a clinical centrifuge. Plasma was either diluted with deionized water for mineral analysis or immediately analyzed for lipid content.

Crouse et al. (1984) collected all fasting blood samples in trace mineral-free vacuum-collecting tubes, and the plasma was stored at -60° C. At the conclusion of the study, the plasma was thawed and analyzed for total cholesterol, HDL cholesterol and triglycerides by techniques specified by Livin and Zak (1964), Lopes-Virella, Stone, Ellis and Colwell (1977) and Kessler and Lederer (1965), respectively.

Goodwin et al. (1985) measured fasting lipid profile at the time of the initial instruction in completing food records. Serum was analyzed for cholesterol, triglycerides and HDL cholesterol as specified by Livin et al. (1964), Kessler et al. (1965) and National Heart, Lung and Blood Institute (1975).

Hooper et al. (1980) obtained three fasting baseline lipid profiles prior to zinc capsule administration. Fasting lipid levels were determined on a weekly basis and continued two weeks after zinc supplementation ended.

A final lipid determination was done 16 weeks after the study began. Fasting serum was analyzed for cholesterol (Livin et al., 1964), triglyceride (Kessler et al., 1965) and high density lipoprotein cholesterol (Lopes-Virella et al., 1977).

Black et al. (1988) drew a morning 12 hour fasting blood sample at baseline and biweekly intervals by venipuncture. The blood was allowed to clot for 60 minutes and centrifuged at 400 x g for 20 minutes. Serum was removed, placed in acid washed tubes and either frozen for mineral analysis or immediately separated into lipoprotein fractions.

In summary, all investigators basically used the same methodology to measure total cholesterol and HDL-C. According to the National Heart, Lung and Blood Institute (1975) data, plasma which was used by Crouse (1984) produces higher values than serum (Goodwin; 1985).

Activity/Exercise Assessment

Hooper et al. (1980), Crouse et al. (1984), Black et al (1988) and Freeland-Graves et al. (1980) had no exercise component and, thus, did no exercise assessment. Goodwin et al. (1985) used an exercise history modified from Cassel (1971). This history provides a score in arbitrary units, based on response to weighted questions about level of physical activity. In general, scores over 20 indicate that the subject is getting some regular form of vigorous aerobic physical exercise, such as jogging or playing tennis. Scores of 10 or 20 are obtained in subjects who regularly (at least three times a week) engage in walking, playing golf or a similar activity. Scores less than 5 indicate a sedentary individual performing little more than house work. Goodwin et al. (1985) found a weak positive correlation between the level of exercise and serum HDL cholesterol in the 180 subjects not taking supplemental zinc ($r=0.26$; $p=0.005$) but not for those subjects taking supplemental zinc ($r=-0.18$; $p=0.14$).

Body Fat Measures

Goodwin et al. (1985) controlled for body mass index, but they did not elaborate on how body mass index affected subjects' cholesterol or zinc status. Freeland-Graves et al. (1980) and Hooper et al. (1980) apparently did not consider body fat in their research. Crouse et al. (1984) included weight and body mass index but didn't provide any evidence of using these variables in interpreting their results. Black et al. (1988) stated only that their selected subjects

were not more than 20 percent above or 10 percent below their ideal body weight as determined by the 1983 Metropolitan Life Insurance Tables.

Statistical Analysis

Goodwin et al. (1985) used SAS for statistical analysis and conducted correlation, partial correlation and multiple regression analysis. The regression model expressed HDL-cholesterol as a linear combination of physical exercise level, total daily zinc intake and the product of physical exercise level and total zinc intake. A two tailed paired t test was used to compare values before and after stopping zinc supplements.

Crouse et al. (1984) analyzed total cholesterol, triglycerides, LDL-cholesterol, HDL cholesterol and zinc data by multiple profile analysis. The mean of the pretreatment measures was considered the baseline value for each dependent variable. To set the Type 1 error at an acceptable level, the Bonferroni procedure was used. This approach adjusts the critical value of the individual tests to yield individual Type 1 errors that sum up to the desired total experimental error. This procedure does not require that the significance criteria for the individual tests be equivalent. They may, in fact, be lower for the hypotheses of greatest theoretical or practical importance.

Freeland-Graves (1980) used a computerized program, STATPACK (Clark and Hunt, 1977), to isolate significant differences in initial, continuous and final concentrations of constitutents in

the blood and diet by two-way analysis of variance. Comparisons of means of grouped data, paired t-tests and simple partial regression analysis were measured for the assessment parameters using the computerized program, OMNITAB (Hogben, Peavy, Varner, 1971).

Black et al. (1988) used a computerized one way analysis of variance with repeated measures program to analyze data. When initial mean baseline values differed between treatments, data were covariate adjusted for baseline values with levels of zinc supplementation and time.

Summary of Study Results

Freeland-Graves et al. (1980) found no uniform response of plasma cholesterol concentrations to altered dietary Zn/Cu ratios. Only subjects with the highest Zn/Cu ratio showed changes in plasma HDL-cholesterol that followed a decline and subsequent increase of plasma cholesterol. As stated previously, the Zn/Cu ratio that was assigned to each group was calculated from the mean dietary zinc added to the supplemental zinc and divided by the mean dietary copper. Dietary means of zinc and copper were based on 24 values for each subject obtained from three day dietary records kept at weekly intervals over the eight week study.

Hooper et al. (1980) observed decreased HDL-cholesterol concentrations following zinc administration. After four weeks of zinc (220 mg zinc sulfate/80 mg elemental zinc) administration, HDL-cholesterol concentrations decreased significantly (mean decline from baseline, 6 mg/dL; $p=0.002$). By the seventh week, HDL-

cholesterol levels decreased 25 percent from baseline (from 40.5 to 30.1 mg/dL; p=0.0001). Sixteen weeks after study initiation (11 weeks after zinc administration stopped) HDL-cholesterol values returned to near-baseline values.

Goodwin et al. (1985) found zinc supplementation abolished the beneficial effects of exercise on increasing serum HDL-cholesterol. In those 184 subjects not taking supplemental zinc, the partial coefficient of correlation between exercise and HDL cholesterol was 0.26 (p=0.005), while the same correlation for those on zinc supplements was -0.18 (p=0.14). The relationship between total zinc intake, physical exercise and HDL-cholesterol showed significant positive interaction. This significant interaction suggested that zinc intake influences the effect of physical activity on HDL-cholesterol. HDL-cholesterol was positively associated with activity level only for those subjects in the lowest zinc intake group.

A statistically significant increase in HDL cholesterol level (2.0 mg/dl, p=0.04) occurred after cessation of zinc supplements. Among subjects taking zinc supplements, the physical activity and change in HDL-cholesterol were positively associated (after stopping supplementation).

There appeared to be a relationship between the level of physical activity and change in HDL-cholesterol after stopping zinc (r=0.41, p=0.05). Those subjects with low physical activity ratings had very small increases or even decreases in HDL-cholesterol after stopping zinc. Subjects with higher physical activity scores had

larger increases in HDL after stopping zinc. Goodwin et al. (1985) went on to state, however, that, while statistically significant, this relationship was weak and could almost entirely be explained by two subjects at the low end of the scale and one subject at the upper end of the scale.

Both Hooper et al. (1980) and Goodwin et al (1985) observed decreased HDL cholesterol concentration in the presence of increased zinc supplementation. Because HDL is apparently the lipoprotein most predictive of coronary artery disease (Castelli, Doyle & Gordon, 1977), a sustained fall in HDL concentration associated with zinc administration might increase the risk of coronary artery disease. These studies seem to indicate that zinc may be atherogenic in man — not because zinc raises serum cholesterol levels in rats, but because it is associated with lowering HDL-cholesterol in man.

Black et al. (1988) found serum total cholesterol, low density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL) and triglycerides unaffected by zinc supplementation. HDL cholesterol in subjects assigned to the 75 mg zinc group were significantly lower at weeks 6 (53 mg%) and 12 (54 mg%) than those for the placebo group. HDL cholesterol in subjects assigned to the 75 mg zinc group were lower at weeks 6 (53 mg%), 8 (51 mg%) and 12 (54 mg%) than at baseline. Subjects assigned to the 50 mg zinc group had lower serum HDL levels at week 12 (55 mg%) than did the placebo group (63 mg%). Subjects assigned to the 50 mg

zinc group had lower serum HDL at week 12 (55 mg%) than at baseline (59 mg%).

The previous studies included a wide range of zinc supplementation. The range varied from 50 mg (Black et al., 1988) to 220 mg (Hooper et al., 1980). The primary question involved in the proposed research was whether low level zinc supplementation (15 mg) maintained/enhanced zinc stores without increasing total cholesterol or decreasing HDL cholesterol. Previous studies have included relatively high levels of supplementation: 220 mg (Hooper et al., 1980), 50 mg (Crouse et al., 1984) and 15 to 100 mg (Freeland-Graves et al., 1980). The study period for Freeland-Graves et al. (1980), Hooper et al. (1980) and Crouse et al. (1984) was 60 days, five weeks and eight weeks, respectively. The dissertation research period was 12 weeks long, and the level of zinc supplementation was in the lowest range of the doses used in these studies.

The previous studies assessed total cholesterol and HDL cholesterol (Crouse et al., 1984; Goodwin et al, 1985; Hooper et al., 1980; Freeland-Graves et al., 1980). The present study, in addition to total cholesterol and HDL cholesterol, included the measurement of HDL_2 cholesterol and HDL_3 cholesterol.

Previous studies monitored supplementation by dispensing capsules daily (Hooper et al., 1980; Freeland-Graves et al., 1980) or having subjects maintain a daily record of capsule ingestion (Crouse et al., 1984). In this disseration research, subject compliance was monitored by the following procedure: an extra two capsules were

included in a 12 weeks' supply of capsules dispensed to subjects. Compliance was measured by whether the allotted number of extra capsules were in each subject's container when containers were returned at the end of the study. Also, subjects completed a calendar given them at the beginning of the study. Each day was checked when the subject ingested the zinc or placebo capsule. Compliance was encouraged during weekly RESHAPE meetings and RESHAPE exercise sessions held three times a week.

Table 1

Summary of Human Studies of Zinc Supplementation on Lipid Profiles

Principal Investigators	Subjects	Study Length	Subject Age	Zinc Level	Response
Freeland-Grave, Han, Friedman & Shorey (1980)	32 women	60 days	18-40	0, 15, 50, 100 mg	No uniform nor sustained response of plasma cholesterol or HDL-C
Garry, Johnson, Hooper & Visconti (1980)	12 males	5 weeks	23-35	440 mg.	HDL-C decreased 25% below baseline 40.5 to 30.1 mg/dL. Total cholesterol, TG, LDL did not change. Zinc ingestion may be atherogenic.
Goodwin, Hunt, Hooper & Garry (1985)	270 men & women	5 years	60 yrs or older	>15 mg.	After controlling for sex, alcohol intake, and body mass, significant positive correlation between exercise and serum HDL-C in subjects not taking supplemental Zn. Zn correlated with low HDL-C.
Crouse, Hooper Atterbom & Pappenfuss (1984)	21 endurance trained men & 23 sedentary men	8 weeks	20-55 yrs.	50 mg.	Fasting plasma high. High density lipoprotein cholesterol, total cholesterol, low density lipoprotein cholesterol cholesterol and triglyceride levels did not change in response to zinc supplementation
Black, Medeiros, Brunett & Welke (1988)	30 white males	12 weeks	19-29 yrs.	0,50, 75 mg	Serum total cholesterol not affected. HDL levels in 75 mg group significantly lower at weeks 6 and 12 than placebo group and lower at weeks 6, 8, 12 than at baseline. Subjects in 50 mg group had lower serum HDL at week 12 than placebo group and lower HDL at week 12 than at baseline.

CHAPTER III

METHODOLOGY

Subjects

Subjects were selected from the 1987-88 fall and spring replications of the RESHAPE program. RESHAPE is a 12-week program which consists of 12 lifestyle management workshops and 36 activity sessions. The activity sessions last 20 to 60 minutes and are conducted on Monday, Wednesday and Friday of each week. The initial activity period is 20 minutes which is increased by five minutes weekly until week nine. The activity period is maintained at 60 minutes for weeks 9-12. Almost 500 subjects have been screened for participation in this program which was first implemented in 1982.

After giving informed consent, volunteers were matched according to their total cholesterol:HDL-C ratio by gender and randomly assigned to two groups, either experimental (E) or control (C). All subjects were questioned to determine whether they were taking any medications. Females were questioned to determine if they were non-gravid and not using oral contraceptive agents. Subjects on medication or oral contraceptives were dropped from participation. Initial parameters of zinc, copper and hematocrit were determined by blood analysis. All lipid variables were determined and diet was measured using a three day food record as

described in a later section. Blood pressure was measured after sitting for at least five minutes.

Experimental Design

After a fasting baseline lipid profile was obtained (week 0), subjects received 84 capsules and were instructed to take one per day for 12 weeks. Capsules were distributed in a double blind fashion. E subjects received capsules containing 15 mg. of zinc sulfate, while C subjects received a placebo. Two methods were used to check patient compliance. The first method was based on counting the number of capsules the subjects returned. An extra two capsules were placed in each subject's capsule container. The subjects were asked to return all unused capsules. The second method was a compliance chart on which subjects checked each day they took their capsules. Fasting lipid, zinc and copper levels were determined at both weeks 0 and 12.

As part of the RESHAPE program, subjects attended 12 lifestyle management workshops and 36 activity sessions. The lifestyle management workshops lasted one and a half hours each and were held on Wednesday evenings each week. Activity sessions consisted of 5-10 minute warm-ups, 20-60 minutes of continuous activity (walking and/or jogging) at 65-75 percent of maximum heart rate (determined by the Karvonen formula), and a 5-10 minute cool-down. The initial activity period was 20 minutes which increased by five minutes weekly until week nine. The activity period was maintained at 60 minutes for weeks 9-12.

Dietary Intake

Prior to zinc supplementation, the dietary intake of zinc was assessed from a single three day food record collected during two successive weekdays and one weekend day (Freeland-Graves et al., 1980; Goodwin et al., 1985). The subjects were instructed on how to keep an accurate three day food record. All food items and portions were recorded on standard forms for each day. Each volunteer was asked to include food brand names, methods of food preparation and recipes for any mixed dish eaten during the period. At the end of the three day recording period, each food record was evaluated for completeness and accuracy. Participants were asked to provide additional information about any unclear descriptions. If the subject used vitamin and/or mineral supplements, the brand name, contents and amount of each nutrient was recorded. All food records were coded and then analyzed by computer for nutrient composition. All food records were coded by food item and the amount consumed. A computerized nutrient data base, Nutranal 3.1 Software Package (1980), was used to calculate the nutrient content of each subject's three day food record.

Bazzarre and Myers (1978) have noted that reliability tests for any dietary method suffer from a number of limitations pertaining primarily to the inability to establish identical conditions in subsequent tests. Obfuscating factors include within-individual and methodological variability. Bazzarre and Yuhas (1983) have stated that the 3-day food record and diet history are more reliable

measures of nutrient intake than the 24 hour recall. They also found that the 3-day food record and diet history correlated better with fasting total cholesterol than did the 24 hour recall. On the basis of these findings, the 3-day food record was recommended for investigations measuring actual food intake.

Fitness Evaluation

Subjects' fitness was determined by the time they took to walk a mile. Mile walk times were taken at both the beginning and end of the program (Cooper, 1977).

Collection and Preparation of Samples

A fasting lipid profile was obtained at the time of the initial instruction in completing food records. Fasting subjects donated 12 ml samples of blood on a single day (e.g., Tuesday) during the morning (7-9 a.m.) at weeks 0 and 12. Venous blood was withdrawn from the forearm via trace mineral free Vacutainers™ with a 21 gauge stainless steel needle. Whole blood was allowed to clot at room temperature for 20 minutes and the serum was frozen until subsequent analysis. Heparinized plasma was obtained via centrifugation at 3500 rpm for 10 minutes in a clinical centrifuge. Plasma was frozen and later analyzed for lipid content. Serum samples (weeks 0 and 12) were stored at -70° C, and samples for all subjects were assayed in duplicate in the same run under coded designation. Serum was analyzed for concentrations of total cholesterol and HDL (Sigma Diagnostics, 1986). Duplicate samples

were re-analyzed if the percent difference was greater than two percent.

Body Fat Measures

Subjects were also assessed using Body Mass Index and skinfold measurements. Body Mass Index (BMI) or Quetelet Index is derived by the following:

$$\frac{\text{weight (kg)}}{\text{height (m)}^2}$$

The BMI is compared to values in the following table:

Minimum body mass index in obesity

Frame Size	Men	Women
small	25.4	24.7
medium	27.0	27.0
large	29.9	29.9

Source: Adapted from Zeman, P.J.: Clinical Nutrition and Dietetics. Lexington, MA: D.C. Heath and Co., 1983.

Values of obesity greater than the table values are indicative of obesity. BMI may not adequately reflect variations in body fat (American Dietetic Association, 1986). Critics (Garn, Leonard and Hawthorne, 1986) have charged that, since stature, i.e., standing height, is one component of BMI, BMI may be stature dependent over part of the age range. For example, in the first National Health and

Nutrition Examination Survey (NHANES I) correlations between stature and BMI approximate 0.30 for children, shift during adolescence and become negative in adult years. In addition, the use of stature as a divisor suggests that BMI may be affected by relative leg length or relative sitting height. NHANES data also indicate that children, adolescents or adults with short legs for their height have higher BMI values, which indicates the extent to which BMI is a measure of body build or body proportions. Finally, the use of weight as the numerator suggests that the BMI, like weight itself, may reflect both lean tissue and fat tissue to a comparable degree. The latter issue is the main limitation of the BMI. One cannot distinguish excess weight due to excess body mass from excess weight due to excess body fat.

The measurement of skinfolds at various appropriate body sites should reflect the body's storage fat (Katch and McArdle, 1983). Skinfold measurements can be used to assess an individual's change in percent body fat in weight reduction programs, and before and after physical conditioning. Advantages of the skinfold measurement technique are that little equipment is necessary and the procedure can be performed quickly with simple interpretation. If measurements are taken correctly, and the data obtained are applied to the appropriate equation based on age and sex, the percentage body fat determined from the skinfolds correlates well with results using hydrostatic weighing (Pollock, M.L. and Jackson, A.S., 1984). The main limitation of using skinfolds is that internal

body fat stores are estimated on the basis of measures of superficial body fat (Blair, Haskell, Ho, 1985).

Mineral Analysis

Zinc and copper concentrations in blood were analyzed by atomic absorption spectrophotometry. Samples were diluted with deionized water to appropriate concentrations. Blank solutions were prepared in a similar manner to reveal any detectable mineral contamination from the procedure. A Perkin-Elmer Model 290-B atomic absorption spectrophotometer equipped with a three slot burner head, a single element hollow cathode ray lamp and an automatic readout were used to read mineral concentrations (Freeland-Graves et al., 1980).

Cholesterol and HDL Cholesterol Analysis

The procedure involved cholesterol measurement as published by Allain, Poon, Chan, Richmond, and Fu (1974). The procedure generated oxidation products by cholesterol oxidase following cholesterol ester hydrolysis by cholesterol esterase. The use of these enzymes was coupled with the p-hydroxybenzenesulfonate and 4-aminoantipyrine chromogenic system. The reactions employed were as follows:

1. Cholesterol Esters $\xrightarrow{\text{Cholesterol Esterase}}$ Cholesterol + Fatty acids
2. Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholest-4-en-3-one + H_2O_2
3. $2H_2O_2$ + 4-Aminoantipyrine + p-Hydroxybenzene-sulfonate $\xrightarrow{\text{Peroxidase}}$ Quinoneimine Dye + $4H_2O$

In the last step, hydrogen peroxide reacted with 4-aminoantipyrine with p-hydroxybenzenesulfonate in the presence of peroxidase to yield a quinoneimine dye, which had a maximum absorbance at 500 nm. The amount of color produced was directly proportional to the concentration of total cholesterol in the sample.

Cholesterol and other lipids were present in the form of lipoproteins. Diagnostic significance has been attached to the cholesterol concentrations associated with high density lipoproteins (HDL), which appeared inversely related to the incidence of coronary artery disease (Castelli et al., 1977). The procedure for determination of HDL cholesterol was based on selective precipitation of low density lipoproteins (Lopes-Virella, Stone, Ellis, & Colwell, 1977). HDL-cholesterol was determined enzymatically by the method described by Allain et al. (1974). HDL_2 and HDL_3 were determined by a simple precipitation procedure as described by Lewis et al. (1982).

Statistical Analysis

Statistical analysis was accomplished with the aid of SAS (Statistical Analysis System), version 79.6. Because the data were not normally distributed for several variables for males, nonparametric statistical tests were used to test all hypotheses. To compare medians between each control and experimental group at week 0 and week 12, the paired comparisons t-test was used for all variables. The Wilcoxon test using median difference scores was used to determine if the changes from week 0 to week 12 were

significantly different between the control (C) and experimental (E) groups. Spearman correlation was used to measure associations between zinc status and specific variables. A significance criterion of $p<0.05$ was used for all tests.

CHAPTER IV

RESULTS

The results of this research are summarized in Tables 2 through 18 and Appendices A-1 through A-15. In reviewing the preliminary results, the data were not normally distributed for several variables for males. To be consistent, nonparametric statistical tests were used to test all hypotheses. To compare medians between each control and experimental group at week 0 and week 12, the paired comparisons t-test was used for all variables. The Wilcoxon test using median difference scores was used to determine if the changes from week 0 to week 12 were significantly different between the control (C) and experimental (E) groups. Because nonparametric statistics were used, median values are presented in the tables. All Wilcoxon scores and related statistics for comparing medians between groups at each week are reported in the appendices.

Participation

Forty-seven subjects were recruited. There were five experimental male subjects, seven control male subjects, 17 experimental female subjects and 18 control female subjects. Dropouts occurred in each group. Dropouts included two E and two C male subjects, five E and nine C female subjects. Twenty-nine subjects remained until the end of the study. Approximately 37

percent of the original population was not available for final data analysis. The primary reasons for dropping out were not evaluated. In the final group, only four subjects returned dietary data at the end of the program, and eleven subjects did not participate in final walk/run evaluation.

Because of the high percentage of dropouts in each subgroup from week 0 to week 12, the descriptive statistics for the entire sample are presented in the appropriate appendices. The data presented in the tables where comparisons between week 0 and week 12 are made represent data for subjects available from both data collection periods.

The format for reporting data includes an evaluation of descriptive traits of E and C groups, both male and female, i.e., age, height, weight. This section is followed by data for skinfold measures, percent body fat, body mass index and the prevalence of obesity among the groups. The data for the lipid profiles of the sample and serum and dietary zinc and copper for the sample are reported. Finally, the results for the evaluation of the presence of associations between selected variables are presented. The sample size for each subgroup varies for fitness because not all subjects completed the fitness test.

In the following text, results are reported with an accompanying probability statistic only if the results are significant, i.e., $p \leq 0.05$. In all other cases, no probability statistic will appear. Probability levels are reported in appropriate tables and appendices.

Subject Monitoring

As stated previously, two methods were used to check patient compliance. The first method was based on counting the number of capsules the subjects returned. An extra two capsules were placed in each subject's capsule container. The subjects were asked to return all unused capsules. The second method was a compliance chart on which subjects checked each day they took their capsules. Of the 16 subjects returning the calendar, each took the capsules every day. Ninety percent of the subjects returned their capsule containers. Each of the returned containers contained two capsules.

Descriptive Traits of Sample

Age (E:43 ± 12 years vs. C:41 ± 13 years), height (E:69 ± 4 inches vs. C:69 ± 3 inches) and initial weight (E:212 ± 79 pounds vs. C:233 ± 47 pounds) were similar for E and C males (Table 2). Age (E:40 ± 11 years vs. C:39 ± 13 years), height (E:66 ± 3 inches vs. C:65 ± 2 inches) and initial weight (E:166 ± 27 pounds vs. C:161 ± 33 pounds) were not significantly different for E and C females (Table 2).

Measures of Obesity

Final weight (E:208 ± 28 pounds vs. C:230 ± 56 pounds), initial skinfold (E:79 ± 31 mm vs. C:88 ± 20 mm), final skinfold (E:75 ± 26 mm vs. C:77 ± 29 mm), initial percent body fat (E:29 ± 10 vs. C:32 ± 4), final percent body fat (E:31 ± 9 vs. C:32 ± 3), initial body mass

Table 2

Age, Height, and Weight of Study Population of Week 0 (Medians and Standard Deviations)

	Males		Females	
Groups	Zinc	Placebo	Zinc	Placebo
(n)	(3)	(5)	(12)	(9)
Age (years)	43 ± 12	41 ± 13	40 ± 11	39 ± 13
Height (inches)	69 ± 4	69 ± 3	66 ± 3	65 ± 2
Weight (lbs)	212 ± 79	233 ± 47	166 +27	161 ± 33

index (BMI) (E:31 ± 6 vs. C:32 ± 7), and final BMI (E:27 ± 5 vs. C:29 ± 7) were not significantly different between E and C males (Table 3). Final weight (E:156 ± 19 pounds vs. C:151 ± 35 pounds), initial skinfold (E:111 ± 29 mm vs. C:93 ± 41 mm), final skinfold (E:91 ± 29 mm vs. C:85 ± 35 mm), initial percent body fat (E:39 ± 4 vs. C:37 ± 6), final percent body fat (E:36 ± 6 vs. C:35 ± 6), initial body mass index (BMI) (E:26 ± 4 vs. C:27 ± 8), and final BMI (E:24 ± 4 vs. C:26 ± 8)(Table 3) were not significantly different between E and C females.

Table 3

Descriptive Statistics of Study Population at Weeks 0 and 12 (Medians and Standard Deviations)

	Males		Females	
Groups	Zinc	Placebo	Zinc	Placebo
(n)	(3)	(5)	(12)	(9)
Initial Weight (Lbs.)	210 ± 38	245 ± 52	166 ± 22	159 ± 41
Final Weight (Lbs.)	208 ± 28	230 ± 56	156 ± 19	151 ± 35
Initial BMI	31 ± 6	32 ± 7	26 ± 4	27 ± 8
Final BMI	27 ± 5	29 ± 7	24 ± 4	28 ± 8
Initial Skinfold (mm)	79 ± 31	88 ± 20	111 ± 29	93 ± 41
Final Skinfold (mm)	75± 26	77 ± 29	91 ± 29	85 ± 35
Initial Percent Body Fat	29 ± 10	32 ± 4	39 ± 4	37 ± 6
Final Percent Body Fat	31 ± 9	32 ± 3	36 ± 6	35 ± 6

Body mass index was divided into three categories: normal, overweight and obese (Table 4). These categories were defined as follows:

$$\text{normal weight} < 25 \text{ kg-m}^{-2}$$
$$\text{overweight} = 25\text{-}29.9 \text{ kg-m}^{-2}$$
$$\text{obesity} \geq 30 \text{ kg-m}^{-2}$$

According to these criteria, 43 percent of women and 25 percent of men were initially classified as normal weight/fat (Table 4). Sixty-three percent of men and 23 percent of women were classified as obese.

From week 0 to week 12, the percentage of males classified as normal weight decreased from 25 percent at week 0 to 12.5 percent at week 12; the percentage of males classified as overweight increased from 12.5 percent to 50 percent; and the percentage of males classified as obese decreased from 62.5 percent to 37.5 percent (Table 4). The percentage of females classified as normal weight increased from 42.9 percent at week 0 to 66.7 percent at week 12; the percentage of females classified as overweight decreased from 33.3 percent to 19.0 percent; and the percentage of females classified as obese decreased from 23.8 percent to 14.3 percent (Table 4).

Table 4

Incidence of Excess Adiposity among Initial Study Population at Week 0

Group	Males n (%)		Females n (%)	
	Week 0	Week 12	Week 0	Week 12
BMI[1]:				
normal	2 (25.0)	1 (12.5)	9 (42.9)	14 (66.7)
overweight	1 (12.5)	4 (50.0)	7 (33.3)	4 (19.0)
obese	5 (62.5)	3 (37.5)	5 (23.8)	3 (14.3)
% Body Fat[2]:		[3]		
normal	1 (12.5)	0	0	1 (4.8)
overweight	1 (12.5)	6 (75.0)	1 (4.7)	2 (9.5)
obese	6 (75.0)	2 (25.0)	20 (95.0)	18 (85.7)

1. BMI= $\frac{\text{weight (kg)}}{\text{height (m)}^2}$ Overweight is defined as 25-29.9 kg-m^{-2} weight for height. Obesity is defined as greater than or equal to 30 kg-m^{-2} weight for height.

2. Percent body fat estimated from sum of four skinfolds using the equation of Durnin and Womersley (1973). Overweight defined as 20-28 percent for males and 22-30 percent for females. Obesity is greater than 28 percent for males and greater than 30 percent for females.

Percent body fat was estimated from the sum of four skinfolds using the equation of Durnin and Womersley (1973). The categories were defined as follows:

% Body Fat	Males	Females
Normal	<20 percent	<22 percent
Overweight/fat	20-28 percent	22-30 percent
Obese	>28 percent	>30 percent

The percentage of males classified as normal decreased from 12.5 percent to 0 percent; the percentage of males classified as overweight increased from 12.5 percent to 75 percent; and the percentage of males classified as obese decreased from 75 percent to 25 percent. The percentage of females classified as normal increased from 0 percent to 4.8 percent; the percentage of females classified as overweight increased from 4.7 percent to 9.5 percent; and the percentage of females classified as obese decreased from 95 percent to 85.7 percent (Table 4).

Body weight decreased for males (E: -5.6 $\pm$ 11.7 pounds vs. C: -6.2 $\pm$ 2.1 pounds) and females (-8.6 $\pm$ 3.4 pounds vs. -6.5 $\pm$ 1.1 pounds), but not significantly (Table 5). Similarly, body fat decreased among males (E:-1.1 $\pm$ 0.8 percent vs. C:-0.5 $\pm$ 3.3 percent) and E females (-1.4 $\pm$ 5.4 percent) but, again, not significantly. The percent body fat among C females decreased significantly (-1.1 $\pm$ 1.5 percent;$p<0.006$).

Table 5

Paired Comparions T-Test of Body Weight and Body Fat
Mean Differencs Scores and Standard Deviations

Variables	Males		Females	
(n)	Zinc (3)	Placebo (5)	Zinc (12)	Placebo (9)
Body Weight Difference (lb) (Initial - Final)	-5.6 ± 11.7	-6.2 ± 2.1	-8.6 ± 3.4	-6.5 ± 1.1
p-value	0.42	0.36	0.18	0.21
% Body Fat Difference (Initial - Final)	-1.1 ± 0.8	-0.5 ± 3.3	-1.4 ± 5.4	-1.1 ± 1.5
p-value	0.42	0.74	0.31	0.006

Fitness

There was no statistically significant difference in fitness level as measured by initial mile walk time between males (E: 14.8 ± 3.4 minutes vs. C: 15.8 ± 0.7 minutes) nor between females (E: 16.0 ± 2.2 minutes vs. 14.8 ± 1.6 minutes), and there was no statistically significant difference in fitness level as measured by final mile walk time between males (E: 14.8 ± 2.9 minutes vs. C: 14.6 ± 1.2 minutes) nor between females (E: 14.4 ± 1.4 minutes vs. 14.2 ± 1.1 minutes)(Table 6). As measured by the paired comparison t-test, mile walk times for neither E nor C males improved significantly (E:-1.70 ± 1.73 minutes vs. C: -0.45 ± 1.34 minutes). Both E and C females improved significantly (E:-1.23 ± 1.71 minutes; p=0.03) vs. (C: -1.10 ± 1.10 minutes; p=0.03) (Table 7).

Lipid Profile of Sample

The following research hypotheses were tested: (The numbers presented in parentheses are the medians and standard deviations or mean difference scores and standard deviations.)The data for blood lipids are presented in Tables 8-10.

Table 6

Initial and Final Walk time of Study Population (Medians and Standard Deviatios)

Groups	Males		Females	
(n)	Zinc (3)	Placebo (5)	Zinc (12)	Placebo (9)
Initial Walk Time (min)	14.8 + 3.4	15.8 ± 0.7	16.0 ± 2.2	14.8 ± 1.6
Final Walk Time (min)	14.8 + 2.9	14.6 ± 1.2	14.4 ± 1.4	14.2 ± 1.1

Table 7

Paired Comparisons T-Test of Fitness (Mean Difference Scores and Standard Deviations)

Variable	Males		Females	
(n)	Zinc (3)	Placebo (5)	Zinc (12)	Placebo (9)
Difference Fitness (Final-Initial)	-1.70 ± 1.73	-0.45 ± 1.34	-1.23± 1.71	-1.10± 1.10
p-value	0.40	0.49	0.03	0.03

Table 8

Initial and Final TC, HDL-C and HDL-C:TC ratio of Study Population (Medians and Standard Deviations)

	Males		Females	
Groups (n)	Zinc (3)	Placebo (5)	Zinc (12)	Placebo (9)
Initial Total Cholesterol (mg%)	253 ± 56	220 ± 41	200 ± 40	225 ± 27
Final Total Cholesterol (mg%)	258 ± 13	197 ± 53	200 ± 50	226 ± 34
Initial HDL Cholesterol (mg%)	37 ± 10	47 ± 8	57 ± 15	66 ± 19
Final HDL Cholesterol (mg%)	40 ± 10	57 ± 7	49 ± 17	55 ± 17
Initial HDL-C:TC	.16 ± .06	. 21 ± .06	. 29 ± .06	.29 ± .10
Final HDL-C:TC	.18 ± .06	.25 ± .16	.26 ± .10	.26 ± .09

Table 9

Initial and Final HDL_2 and HDL_3 of Study Population (Medians and Standard Deviations)

Groups (n)	Males		Females	
	Zinc (3)	Placebo (5)	Zinc (12)	Placebo (9)
Initial HDL_2 Cholesterol (mg%)	2 ±2	9±9	13 ±9	8 ±2
Final HDL_2 Cholesterol (mg%)	7±10	15 ±9	16 ±14	15 ±8
Initial HDL_3 Cholesterol (mg%)	39±9	42 ±21	50 ±13	58±20
Final HDL_3 Cholesterol (mg%)	39±0	42 ±11	33 ±14	44±12

Table 10

Paired Comparisons T-Test of Lipid Variables (Means and Standard Deviations)

Variable	Males		Females	
(n)	Zinc (3)	Placebo (5)	Zinc (12)	Placebo (9)
Total Cholesterol Difference (Final-Initial)	-6.0 ± 49.5	-22.6 ± 23.7	1.5 ± 42.3	2.6 ± 31.4
p-value	0.90	0.10	0.90	0.80
HDL Difference (Final-Initial)	5.6 ± 2.3	10.6 ± 13.9	-3.9 ± 9.1	-8.4 ± 23.2
p-value	0.05	0.17	0.16	0.30
HDL-C:TC Difference (Final-Initial)	0.02 ± 0.03	0.02 ± 0.16	-0.01 ± 0.07	0.02 ± 0.13
p-value	0.37	0.21	0.40	0.41
HDL_2 Difference (Final-Initial)	5.6 ± 11.7	6.2 ± 12.6	2.8 ± 12.4	7.7 ± 7.7
p-value	0.49	0.33	0.45	0.01
HDL_3 Difference (Final-Initial)	-0.3 ± 9.7	-0.2 ± 24.0	-13.9 ± 12.7	-16.3 ± 20.4
p-value	0.95	0.98	0.002	0.04

Total Cholesterol (TC)

H_1-E: TC was not significantly different at week 0 versus week 12 among experimental subjects.

There was no significant difference in the mean difference scores for total cholesterol between week 0 and week 12 among either experimental males (-6.0 ± 49.5 mg%) or experimental females (1.5 ± 42.3 mg%)(Table 10). This hypothesis was accepted.

H_1-C: TC was significantly different at week 0 versus week 12 among control subjects.

There was no significant difference in total cholesterol mean difference scores between week 0 and week 12 among either control males (-22.6 ± 23.7 mg%) or control females (2.6 ± 31.4 mg%)(Table 10). This hypothesis was rejected.

H_1-E:C: TC was not significantly different between experimental and control subjects at week 0 and 12.

Total cholesterol was not significantly different between experimental and control males at either week 0 (E:253 ± 56 mg% vs. C: 220 ± 41 mg%) or week 12 (E: 258 ± 13 mg% vs. C:197 ± 53 mg%) cholesterol values (Table 8). Total cholesterol was not significantly different between experimental and control females at either week 0 (E: 200 ± 40 mg% vs. C: 225 ± 27 mg%) or week 12 (E:200 ± 50 mg% vs.C: 226 ± 34 mg%). This hypothesis was accepted.

HDL-Cholesterol (HDL-C)

H_2E: HDL-C was not significantly different at week 0 versus week 12 among experimental subjects.

HDL-C increased significantly from week 0 to week 12 among experimental males (5.6 ± 2.3 mg%; p=0.05). No statistically significant difference occurred, however, for experimental females (-3.9 ± 9.1 mg%)(Table 10). This hypothesis was rejected for males and accepted for females.

H_2C: HDL-C was significantly different at week 0 versus week 12 among control subjects.

HDL-C was not significantly different between week 0 and week 12 among either control males (10.6 ± 13.9 mg%) or control females (-8.4 ± 23.2 mg%)(Table 10). This hypothesis was rejected for both males and females.

H_2-E:C: HDL-C was not significantly different between experimental and control subjects at week 0 and 12.

Total HDL-C was not significantly different between experimental and control males at week 0 (E:37 ± 10 mg% vs. C:47 ± 8 mg%) or week 12 (E:40 ± 10 mg% vs. C:57 ± 7 mg%)(Table 8). HDL-C was not significantly different between experimental and control females at week 0 (E:57 ± 15 mg% vs. C:66 ± 19 mg%) or week 12 (E:49 ± 17 mg% vs. C:55 ± 17mg%). This hypothesis was accepted for males and females.

HDL Cholesterol:Total Cholesterol (HDL-C:TC)

H_3-E: The HDL-C:TC ratio was not significantly different at week 0 versus week 12 among experimental subjects.

The HDL-C:TC ratio was not significantly different between week 0 and week 12 among either experimental males (0.02 ± 0.03) or experimental females (-0.01 ± 0.07)(Table 10). This hypothesis was accepted for males and females.

H_3-C: The HDL-C:TC ratio was significantly different at week 0 versus week 12 among control subjects.

The HDL-C:TC ratio was not significantly different between week 0 and week 12 among either control males (0.02 ± 0.16) or control females (0.02 ± 0.13)(Table 10). This hypothesis was rejected for males and females.

H_3-E:C: The HDL-C:TC ratio was not significantly different between experimental and control subjects at week 0 and 12.

The HDL-C:TC ratio was not significantly different between experimental and control males for week 0 (E:0.16 ± 0.06 vs. C:0.21 ± 0.06) or week 12 (E: 0.18 ± 0.06 vs. C: 0.25 ± 0.16)(Table 8). The HDL cholesterol: total cholesterol ratio was not significantly different between experimental and control females for week 0 (E:0.29 ± 0.06 vs. C: 0.26 vs. 0.10) or week 12 (E:0.29 ± 0.10 vs. C:0.26 ± 0.09)(Table 8). This hypothesis was accepted.

HDL$_2$ Cholesterol

H_4E: HDL$_2$ was not significantly different at week 0 versus week 12 among experimental subjects.

HDL$_2$ increased from week 0 to week 12 for experimental males (5.6 ± 11.7 mg%) and experimental females (2.8± 12.4 mg%) but not significantly (Table 10). This hypothesis was accepted.

H_4C: HDL$_2$ was significantly different at week 0 versus week 12 among control subjects.

HDL$_2$ did not increase significantly from week 0 to week 12 (6.2 ± 12.6 mg%) for control males. HDL$_2$ increased significantly for control females from week 0 to week 12 (7.7 ± 7.7 mg%; p=0.01)(Table 10). This hypothesis was rejected for males and accepted for females.

H_4-E:C: HDL$_2$ was not significantly different between experimental and control subjects at week 0 and week 12.

HDL$_2$ was not significantly different between experimental and control males at week 0 (E:2 ± 2 mg% vs. C:9 ± 9 mg%) or week 12 (E:7 ± 10 mg% vs. C: 15 ± 9 mg%)(Table 9). HDL$_2$ was not significantly different between experimental and control females at week 0 (E:13 ± 9 mg% vs. C: 8 ± 2 mg%) or week 12 (E:16 ± 14 mg% vs. C:15 ± 8 mg%). (Table 9). This hypothesis was accepted.

HDL$_3$ Cholesterol

H_5E: HDL$_3$ was not significantly different at week 0 versus week 12 among experimental subjects.

HDL_3 was not significantly different at week 0 versus week 12 for experimental males (-0.3 ± 9.7 mg%) (Table 10). HDL_3 decreased significantly from week 0 to week 12 for experimental females (-13.9 ±12.7; p=0.002). This hypothesis was accepted for males and rejected for females.

H_5C: HDL_3 was significantly different at week 0 versus week 12 among control subjects.

HDL_3 did not decrease significantly from week 0 to week 12 for control males (-0.2 ± 24.0 mg%)(Table 10). HDL_3 decreased significantly from week 0 to week 12 for control females (-16.3 ± 20.4; p=0.04). This hypothesis was accepted for females and rejected for males.

H_5-E:C: HDL_3 was not significantly different between experimental and control subjects at week 0 and week 12.

HDL_3 was not significantly different between experimental and control males for week 0 (E:39 ± 9 mg% vs. C: 42 ± 21 mg%) or week 12 (E: 39 ± 0 mg% vs. C:42 ± 11 mg%)(Table 9). HDL_3 was not significantly different between experimental and control females for HDL_3 at week 0 (E:50 ± 13 mg% vs. C:58 ± 20 mg%) or week 12 (E: 33 ± 14 mg% vs. C:44 ± 12 mg%)(Table 9). This hypothesis was accepted.

Serum and Dietary Zinc and Copper Zinc

Initial dietary zinc (E:9.6 ± 5.8 mg/day vs. C:11.7 ± 2.4 mg/day), initial serum zinc (E:120 ± 35 ug/dl vs. C:90 ± 32 ug/dl) or final

serum zinc (E:70 ± 31 ug/dl vs. C:93 ± 3 ug/dl)(Table 11) were not significantly different between experimental and control males. Initial dietary zinc (E:8.9 ± 4.0 mg/day vs. C:8.9 ± 3.3 mg/day), initial serum zinc (E:115 ± 36 ug/dl vs. C:100 ± 22 ug/dl) or final serum zinc (E:91 ± 18 ug/dl vs. C:91 ± 27 ug/dl)(Table 11) were not significantly different between experimental and control females.

Serum Zinc. Normal serum zinc was defined as 101-139 ug/dl; marginal serum zinc was defined as 80-100 ug/dl; and deficient serum zinc was defined as <80 ug/dl (Table 12). According to these criteria, by the end of the 12 weeks, the percentage of males classified as normal decreased from 50.0 percent at week 0 to 13.0 percent at week 12; the percentage of males classified as marginal increased from 25.0 percent to 50.0 percent; and the percentage of males classified as deficient increased from 25.0 percent to 37.0 percent (Table 12). The percentage of females classified as normal decreased from 52.4 percent to 14.0 percent; the percentage of females classified as marginal increased from 33.3 percent to 43.0 percent; and the percentage of females classified as deficient increased from 14.3 percent to 43.0 percent (Table 12).

Table 11

Initial and Final Mineral Profile of Study Population (Medians and Standard Deviations)

	Males		Females	
Groups	Zinc	Placebo	Zinc	Placebo
(n)	(3)	(5)	(12)	(9)
Initial Dietary Zinc (mg)	9.6 ± 5.8	11.7 ± 2.4	8.9 ± 43.0	8.9 ± 3.3
Initial Serum Zinc (ug/dl)	120 ± 35	90 ± 32	115 ± 36	100 ± 22
Final Serum Zinc (ug/dl)	70 ± 31	93 ± 3	91 ± 18	91 ± 27
Initial Dietary Copper (mg)	2 .0 ± 2.6	2.2 ± 2.1	2.0 ± 2.3	2.0 ± 2.4
Initial Serum Copper (ug/dl)	110 ± 26	120 ± 15	124 ± 38	146 ± 38
Final Serum Copper (ug/dl)	80 ± 17	110 ± 13	94 ± 14	98 ± 43

Table 12

Zinc Distribution Among Study Population at Week 0

	Males (n=8)		Females (n=21)	
	Initial	Final	Initial	Final
Serum Zinc:[1]				
Normal (101-139 ug/dl)	4 (50.0%)	1 (13.0 %)	11 (52.4 %)	3 (14.0 %)
Marginal (80-100 ug/dl)	2 (25.0 %)	4 (50.0 %)	7 (33.3 %)	9 (43.0 %)
Deficient (< 80 ug/dl)	2 (25.0 %)	3 (37.0 %)	3 (14.3 %)	9 (43.0 %)
Dietary Zinc:[2]				
≥ RDA (≥15 mg/day)	0		2 (9.5 %)	
Marginal (<15->12 mg/day)	2 (25 %)		1 (4.8 %)	
Deficient (<12 mg/day)	6 (75 %)		18 (85 %)	

[1] Grant, A. and DeHoog, G. (1985). Nutritional Assessment Guidelines. Seattle, WA.: Northgate Station.

Mean serum zinc status decreased during the 12 week exercise program (Table 13). The decrease in zinc status was not significant for experimental males (-46 ± 66 ug/dl), control males (-22 ± 33 ug/dl) or control females (-14 ± 27 ug/dl). Zinc status decreased significantly for experimental females (-37 ± 27 ug/dl; p=0.007)(Table 13).

Table 13

Paired Comparisons T-Test of Zinc and Copper (Mean Difference Scores and Standard Deviations)

Variable	Males		Females	
	Zinc	Placebo	Zinc	Placebo
(n)	(3)	(5)	(12)	(9)
Serum Zinc Difference				
(Final-Initial)	-46± 66	-22 ± 33	-37 ± 27	-14 ± 27
p-value	0.35	0.27	0.007	0.25
Serum Copper Difference				
(Final-Initial)	-33 ± 15	-33 ± 20	-39 ± 27	-19± 34
p-value	0.06	0.04	0.004	0.21

Dietary Zinc. Dietary zinc levels were defined as follows:

normal > 15 mg/day

marginal = 12-15 mg/day

deficient < 12 mg/day

At the beginning of the program, the zinc intake of 9.5 percent of the women was equal to or more than the RDA. The zinc intake for all the men was less than the RDA. The intake of 25 percent of the men and 4.8 percent of the women was marginal. The intake of 85 percent of the women and 75 percent of the men was deficient (Table 12) .

Only four study participants returned dietary records at the end of the program. This lack of response prohibited an evaluation of final dietary zinc and copper at the end of the 12 week program.

Copper

Initial dietary copper (E:2.0 ± 2.6 mg/day vs. C:2.2 ± 2.1 mg/day), initial serum copper (E:110 ± 26 ug/dl vs. 120 ± 15 ug/dl) or final serum copper (E:80 ± 17 ug/dl vs. C:110 ± 13 ug/dl)(Table 11) was not significantly different between experimental and control males. Initial dietary copper (E:2.0 ± 2.3 mg/day vs. 2.0 ± 2.4 mg/day), initial serum copper (E:124 ± 38 ug/dl vs. C:146 ± 38 ug/dl) or final serum copper (E:94 ± 14 ug/dl vs. C:98 ± 43 ug/dl)(Table 11) were not significantly different between experimental and control females. Again, as with dietary zinc, the lack of dietary records

prohibited analysis of final dietary copper intake for the E and C groups.

Serum Copper. Normal serum copper levels were defined as 75-150 ug/dl; marginal serum copper levels were 45-75 ug/dl; and deficient serum copper levels were <45 ug/dl (Table 14). Compared to these criteria, by the end of the 12 weeks, the percentage of males classified as normal decreased from 100 percent to 75 percent; the percentage of males classified as marginal increased from 0 percent to 12.5 percent; and the percentage of males classified as deficient increased from 0 percent to 12.5 percent. The percentage of females classified as normal decreased from 100 percent to 81.0 percent; the percentage of females classified as marginal increased from 0 percent to 5.0 percent; and the percentage of females classified as deficient increased from 0 percent to 14.0 percent(Table 14).

Dietary Copper. Dietary copper levels were defined as follows:

normal > 2 mg/dl

marginal = 1-2 mg/day

deficient < 1 mg/day

According to these criteria, 50 percent of males and 38.1 percent of females had normal copper intake. The intake of 12.5 percent males and 52.4 percent of females was marginal. Finally, the intake of 37.5 percent of males and 9.5 percent of females was deficient (Table 14).

Table 14

Copper Distribution among Study Population at Week 0

	Males (n=8)		Females (n=21)	
Serum Copper:[1]	**Initial**	**Final**	**Initial**	**Final**
Normal (75-150 ug/dl)	8 (100 %)	6 (75.0 %)	21 (100 %)	17 (81.0%)
Marginal (75-45 ug/dl)		1 (12.5 %)		1 (05.0%)
Deficient (< 45 ug/dl)		1 (12.5 %)		3 (14.0%)
Dietary Copper:[2]				
≥ RDA (≥ 2 mg/day)	4 (50.0 %)		8 (38.1%)	
Marginal (<2->1 mg/day)	1 (12.5%)		11 (52.4%)	
Deficient (<1 mg/day)	3 (37.5%)		2 (9.5 %)	

1 Grant, A. and DeHoog, G. (1985). Nutritional Assessment Guidelines. Seattle, WA.: Northgate Station.

2 Recommended Dietary Allowances (9th edition). Normal dietary copper status is defined as greater than or equal to 2 mg/day copper. Marginal copper status is defined as less than 99-80% RDA. Deficient copper status is defined as less than 80% RDA.

Copper status decreased for experimental males (-33 ± 15 ug/dl; p=0.06) which approached significance (Table 13). Copper status decreased significantly for control males (-33 ± 20 ug/dl; p=0.04) and experimental females (-39 ± 27 ug/dl; p=0.004). Copper status did not decrease significantly for control females (-19 ± 34 ug/dl).

Zinc, Copper and Blood Lipids

The following hypotheses were tested to identify any relationships between zinc, copper and blood lipids:

H_6: There was a direct association between serum zinc and TC for males and females.

No statistically significant correlations were observed for total cholesterol and initial serum zinc (r=0.02) or for total cholesterol and final serum zinc (r=-0.55) (Table 14) for males. No significant correlations were observed between total cholesterol and initial serum zinc (r=0.09) or for total cholesterol and final serum zinc (r=0.41) for females. This hypothesis was rejected.

H_7: There was an inverse association between serum zinc and HDL-C for males and females.

For males, HDL-C and initial serum zinc (r=-0.25)(Table 15) were not significantly related, but HDL-C and final serum zinc (r=0.89; p=0.007) were significantly related. No significant associations were observed between HDL-C and initial serum zinc

(r=-0.13) or HDL-C and final serum zinc (r=-0.14) for females. This hypothesis was rejected.

Table 15

Spearman Correlation Coefficients of Lipid Variables (Initial and Final Values) and Initial and Final Zinc

Variable	Male Comparison		Female Comparison	
	Initial Serum Zinc	Final Serum Zinc	Initial Serum Zinc	Final Serum Zinc
(n)	(8)	(8)	(21)	(21)
Total Cholesterol	0.02	-0.55	0.09	0.41
p value	0.95	0.19	0.71	0.06
HDL Cholesterol	-0.25	0.89	-0.13	-0.14
p value	0.54	0.007	0.58	0.57
HDL-C:TC	-0.42	0.70	-0.32	-0.09
p value	0.32	0.07	0.14	0.70
HDL_2 Cholesterol	0.19	0.87	0.11	-0.06
p value	0.64	0.01	0.60	0.81
HDL_3 Cholesterol	0.20	-0.07	0.47	0.23
p value	0.64	0.87	0.03	0.36

H_8: There was an inverse association between serum zinc and HDL-C:TC for males and females.

No significant correlations were observed between the initial HDL-C: TC ratio and initial serum zinc (r=-0.42) or for the correlation between final HDL-C: TC ratio and final serum zinc (r=0.70)(Table 15) for males. For females, no significant correlations were observed between the initial HDL-C: TC ratio and initial serum zinc (r=-0.32), or the final HDL-C: TC ratio and final serum zinc (r=-0.09). This hypothesis was rejected.

H_9: There was no inverse association between HDL_2 and serum zinc.

No significant correlation was observed between HDL_2 cholesterol and initial serum zinc (r=0.19), but HDL_2 and final serum zinc were significantly correlated (r=0.87; p=0.01)(Table 15) for males. No significant correlations were observed for HDL_2 cholesterol and initial serum zinc (r=0.11) or HDL_2 and final serum zinc (r=-0.06) for females. This hypothesis was accepted.

H_{10}: There was no significant association between HDL_3 and serum zinc.

No significant correlations were observed for HDL_3 cholesterol and initial serum zinc (r=0.20) or HDL_3 cholesterol and final serum zinc (r=-0.07)(Table 15) for males. No significant correlations were observed for HDL_3 cholesterol and final serum zinc (r=0.23), but HDL_3 cholesterol and initial serum zinc were significantly

correlated (r=0.47; p=0.03) for females. This hypothesis was accepted for males and for HDL_3 and final serum zinc for females. The hypothesis was rejected for HDL_3 and initial serum zinc for females.

H_{11}: There was an inverse association between obesity and zinc.

There was no statistically significant correlation among males for initial serum zinc and BMI (r=0.07), for initial serum zinc and skinfold (r=0.37), for initial serum zinc and percent body fat (r=0.46), for initial serum zinc and total body weight (r=0.09). There was no statistically significant relationship between final serum zinc and BMI (r=-0.32), final serum zinc and skinfold (r=-0.39), final serum zinc and percent body fat (r=-0.28), or final serum zinc and total body weight (r=0.00)(Table 16).

There were no statistically significant correlations among females for initial serum zinc and BMI (r=-0.10), for initial serum zinc and skinfold (r=0.09), for initial serum zinc and percent body fat (r=-0.04), for initial serum zinc and total body weight (r=-0.26), for final serum zinc and BMI (r=-0.01), for final serum zinc and total body weight (r=-0.004), for final serum zinc and skinfold (r=0.10), or for final serum zinc and percent body fat (r=-0.02)(Table 16). This hypothesis was rejected for males and females.

H_{12}: There was an inverse association between the zinc/copper ratio and the HDL-C: total cholesterol ratio.

Table 16

Spearman Correlation of Serum Zinc and Body Composition Parameters (Initial and Final Values) for Males and Females

Variable	Male Comparison		Female Comparison	
	Initial Serum Zinc	Final Serum Zinc	Initial Serum Zinc	Final Serum Zinc
(n)	(8)	(8)	(21)	(21)
BMI	0.07	-0.32	-0.10	- 0.01
p-value	0.87	0.48	0.67	0.97
Skinfold	0.37	-0.39	0.09	0.10
p-value	0.35	0.37	0.71	0.69
% Body Fat	0.46	-0.28	-0.04	- 0.02
p-value	0.25	0.53	0.84	0.92
Total Body Weight	0.09	0.00	-0.26	- 0.004
p-value	0.84	1.00	0.26	0.98

There were no significant inverse correlations between the zinc/copper ratio and HDL-C:total cholesterol ratio for experimental males (r=0.50) or for experimental females (r=-0.22)(Table 17). There were no significant inverse correlations between the zinc/copper ratio and HDL-C:total cholesterol ratio for control

males (r=0.81; p=0.02) or control females (r= 0.08). This hypothesis was rejected.

Table 17

Spearman Correlation Coefficients of Zn/Cu Ratio Differences and HDL-C:TC Ratio Differences

Variable	Male Comparison		Female Comparison	
	Zn/Cu Ratio Difference (Zinc)	Zn/Cu Ratio Difference (Placebo)	Zn/Cu Ratio Difference (Zinc)	Zn/Cu Ratio Difference (Placebo)
(n)	(3)	(5)	(12)	(9)
HDL-C:TC Ratio Difference	0.50	0.81	-0.22	0.08
p-value	0.20	0.02	0.32	0.72

H_{13}: There was an inverse association between activity/fitness and zinc.

No significant inverse correlation was observed between zinc and fitness as measured by the mile walk administered at the beginning and end of the 12 week exercise program (Table 18). No significant correlation was observed for males between serum zinc and initial walk time (r=-0.14) or between serum zinc and final walk time (r=0.65). For female subjects, no significant correlation was observed between serum zinc and initial walk time (r=0.13) or

between serum zinc and final walk time (r=-0.01)(Table 18). This hypothesis was rejected for males and females.

Table 18

Spearman Correlation Coefficients for Initial and Final Walk Times

Variable	Males		Females	
	Initial Walk Time	Final Walk Time	Initial Walk Time	Final Walk Time
(n)	(8)	(8)	(21)	(21)
Total Cholesterol	0.59	0.10	0.33	0.34
p-value	0.11	0.81	0.13	0.14
HDL Cholesterol	-0.55	-0.03	-0.44	0.23
p-value	0.15	0.93	0.04	0.33
HDL_2 Cholesterol	0.21	-0.52	0.28	0.33
p-value	0.61	0.22	0.20	0.16
HDL_3 Cholesterol	-0.73	-0.18	-0.04	0.02
p-value	0.03	0.69	0.83	0.92
Serum Zinc	-0.14	0.65	0.13	- 0.01
p-value	0.73	0.11	0.56	0.97
Body Fat	0.46	-0.28	-0.04	-0.02
p-value	0.25	0.53	0.84	0.92

CHAPTER V

DISCUSSION

High levels of zinc supplementation have been associated with increased total cholesterol and decreased HDL cholesterol (Klevay, 1973). No research is available on the effects of zinc supplementation on HDL_2-cholesterol and HDL_3-cholesterol in a population of overweight/obese subjects. The present investigation was begun to establish the effects of low level zinc supplementation on total cholesterol, HDL cholesterol, HDL_2-cholesterol and HDL_3-cholesterol. The low level of zinc supplementation (15 mg) was the same dose used in most vitamin supplements. Since the supplement dose was quite low, few statistically significant differences in lipid profiles following zinc supplementation were expected. Consequently, the exercise training was expected to favorably alter lipid profiles among the placebo group, but probably not among the experimental (zinc supplemented) group.

This discussion will focus on the findings according to the specific hypotheses tested and the relationship of these hypotheses to previous findings reported in the literature. This discussion includes a review of the problems experienced in implementing the study and the limitations encountered in evaluating the results.

As established in the preceding results, experimental and control subjects, according to each gender evaluated, did not differ significantly in age, height, weight, body mass index, sum of skinfolds and percent body fat at the beginning of the 12 week exercise/zinc supplementation regimen (Tables 2 and 3). By the end of the program, there were no significant differences between experimental and control subjects, male and female, for final weight, final skinfold, final percent body fat or final BMI (Table 3). By the end of the 12 week program, subjects lost weight and body fat. Only control females lost a percentage of body fat that was statistically significant(-1.1 $\pm$ 1.5; p=0.006)(Table 5). Fitness level, as measured by mile walk time, improved significantly only among E and C females (E:-1.23 $\pm$ 1.71 minutes; p=0.03) vs. (C:-1.10 $\pm$ 1.10 minutes; p=0.03)(Table 7).

Serum zinc declined for all subjects but not significantly for three of four groups. For E females, the decrease was statistically significant(-37 ug/dl $\pm$ 27; p=0.007)(Table 14). Normal serum zinc was defined as 101-139 ug/dl; marginal serum zinc was defined as 80-100 ug/dl; and deficient serum zinc was defined as <80 ug/dl (Table 12). According to these criteria, by the end of the 12 weeks, the percentage of males classified as normal decreased from 50.0 percent to 13.0 percent; the percentage of males classified as marginal increased from 25.0 percent to 50.0 percent; and the percentage of males classified as deficient increased from 25.0 percent to 37.0 percent (Table 12). The percentage of females

classified as normal decreased from 52.4 percent to 14.0 percent; the percentage of females classified as marginal increased from 33.3 percent to 43.0 percent; and the percentage of females classified as deficient increased from 14.3 percent to 43.0 percent(Table 12).

At the beginning of the program, the zinc intake of 9.5 percent of the women was equal to or more than the RDA. The zinc intake for all the men was less than the RDA. The intake of 25 percent of the men and 4.8 percent of the women was marginal. The intake of 85 percent of the women and 75 percent of the men was deficient (Table 12) .

Fifty percent of males and 38.1 percent of females had normal copper intake. The intake of 12.5 percent males and 52.4 percent of females was marginal. Finally, the intake of 37.5 percent of males and 9.5 percent of females was deficient (Table 13).

The decrease in copper status was not significant for experimental males (-33 $\pm$ 15 ug/dl) or control females (-19 $\pm$ 34 ug/dl). Copper status decreased significantly for control males (-33 $\pm$ 20 ug/dl; p=0.04), and experimental females (-39 $\pm$ 27 ug/dl; p=0.004)(Table 14).

Hypothesis Testing

On the basis of the statistical analysis obtained for each hypothesis presented in the results, the following conclusions were developed for the specific lipid variables evaluated. In the

following section, each hypothesis is reviewed and compared to previously cited, relevant studies.

Lipids

Total Cholesterol (TC).

H_1-E: Total cholesterol levels were not significantly different between week 0 and week 12 among either experimental males or experimental females (Table 10).

H_1-C: Total cholesterol levels were not significantly different between week 0 and week 12 among either control males or control females (Table 10).

H_1-E:C: Cholesterol values at weeks 0 and 12 were not significantly different between experimental and control males (Table 8). Total cholesterol at week 12 was not significantly different between experimental and control females.

These results are consistent with Crouse et al. (1984), Freeland-Graves et al. (1980), Hooper et al. (1980) and Black et al. (1988) who found zinc supplementation had no significant effect on total cholesterol.

A high percentage of the subjects had elevated cholesterol. This may have been a result of their sedentary lifestyle, their diet or their overweight.

HDL Cholesterol (HDL-C).

H_2E: HDL cholesterol levels increased significantly (5.6 ± 2.3 mg%; p=0.05) between week 0 and week 12 among experimental males, but did not increase significantly for experimental females (Table 10).

H_2C: HDL cholesterol levels were not significantly different between week 0 and week 12 among either control males or control females (Table 8).

H_2-E:C: HDL-C was not significantly different between experimental and control males for weeks 0 and 12 (Table 10). HDL-C was not significantly different between experimental and control females for week 12.

These results differ from Hooper et al. (1980), Goodwin et al. (1985) and Black et al. (1988). Hooper et al. (1980), using a 220 mg supplement, found HDL-C decreased 25 percent below baseline, i.e., from 40.5 to 30.1 mg/dl among experimental subjects. Goodwin et al. (1985) found low HDL-C among those subjects on supplemental zinc (>15 mg/day). Black et al. (1988) found HDL-C significantly lower ($p<0.05$) at weeks 6 (53 mg%), 8 (51 mg%), and 12 (54 mg%) among subjects taking 75 mg zinc per day during the 12 week study than at week 0 (63 mg%). Among subjects taking 50 mg zinc per day, Black et al. (1988) found experimental subjects had lower serum HDL-C levels at week 12 (55 mg%) than control subjects (63 mg%).

HDL-C:TC Ratio.

H_3-E: HDL-C:TC levels were not significantly different between week 0 and week 12 for either experimental males or experimental females (Table 10).

H_3-C: HDL-C:TC level was not significantly different between week 0 and week 12 for either control males or control females (Table 10).

H_3-E:C: The HDL cholesterol:total cholesterol ratio was not significantly different between experimental and control males at weeks 0 and 12 (Table 8) nor between experimental and control females for weeks 0 and 12. None of the other researchers reported HDL-C:total cholesterol ratios.

Because researchers have only recently begun to explore the following variables, e.g., HDL_2 and HDL_3 cholesterol, some of the variables considered in the following hypotheses have not been considered in any of the studies previously cited. Nevertheless, since zinc has elicited changes on HDL-C (Hooper et al., 1980; Freeland-Graves, 1980; Goodwin, et al., 1985; Black et al., 1988), an investigation of the effect of zinc on HDL_2 and HDL_3 cholesterol seems warranted.

HDL_2 Cholesterol.

H_4E: HDL_2 was not significantly different between week 0 and week 12 for experimental males or experimental females (Table 10).

H_4C: HDL_2 was not significantly different for control males but significantly increased (7.7 ± 7.7 mg%; p=0.01) for control females between week 0 and week 12 (Table 10).

H_4-E:C: HDL_2 was not significantly different between experimental and control males at weeks 0 or 12 (Table 9). HDL_2 was not significantly different between experimental and control females at weeks 0 and 12.

HDL_2 increased significantly only for control females. Nevertheless, median HDL_2 increased for all groups. This effect was anticipated since HDL_2 increases in response to exercise (Goldberg & Elliot, 1985). Control females did not take a zinc supplement that might have suppressed HDL_2 levels (Hooper et al, 1980; Freeland-Graves et al., 1980; Goodwin et al., 1985; Black et al., 1988). Zinc supplementation may have been responsible for the lack of a statistically significant increase in HDL_2 among experimental females or males. However, since the relative magnitude of change observed for males was similar to that observed for C females, the lack of a significant change was probably a reflection of the smaller sample size and two-fold greater variability in the C and E male groups.

HDL_3 Cholesterol.

H_5E: HDL_3 was not significantly different at week 0 versus week 12 for experimental males (Table 10). HDL_3 decreased significantly (-13.9 ± 12.7 mg%; p=0.002) between week 0 and week 12 for experimental females.

H_5C: HDL_3 was not significantly different at week 0 versus week 12 for control males (Table 9). HDL_3 decreased significantly (-16.3 $\pm$ 20.4 mg%; p=0.04) between week 0 and week 12 for control females.

H_5-E:C: HDL_3 was not significantly different between experimental and control males for weeks 0 and 12 (Table 9). HDL_3 was not significantly different between experimental and control females for HDL_3 weeks 0 and 12.

Median HDL_3 remained relatively constant for males but decreased significantly for both E and C females. Thus, the decrease occurred in spite of zinc supplementation. Exercise, diet, weight loss or the combined effect of exercise, diet and weight loss may have contributed to the change. Suntsov, Zhukovskii, Poleskii and Kundraykova (1987) found that hyperinsulinemia decreases HDL_3. They stated that derangements in the metabolism of HDL_3 would likely be associated with a high risk of coronary heart disease development especially among patients with diabetes mellitus. Therapeutic measures aimed at reducing insulin secretion were recommended for the normalization of lipid metabolism including HDL_3. Exercise and weight loss positively affect diabetes mellitus (Zeman, 1983). Fitness improved and body weight decreased for subjects in this dissertation research. Therefore, it would seem that diet probably had more to do with the reduction of HDL_3 than fitness or body weight. In the absence of final dietary assessment,

however, the extent to which diet affected HDL_3 can only be speculated .

H_6: Neither total cholesterol and initial serum zinc nor total cholesterol and final serum zinc were significantly correlated among either male or female subjects (Table 10).

No study specifically considered this correlation. However, Freeland-Graves et al. (1980) observed a weak correlation of whole blood zinc with plasma cholesterol ($r=-0.17$; $p<0.03$) in their sample of 32 subjects.

While Klevay did not conduct human studies in which the relationship between total cholesterol and serum zinc was evaluated by correlation calculation, he has stated that high levels of zinc can contribute to hypercholesterolemia. Apparently, in this dissertation research, the level of zinc supplementation was inadequate to affect either serum zinc or, total cholesterol.

H_7: For males, HDL-C and initial serum zinc were not significantly correlated, but HDL-C and final serum zinc were significantly correlated ($r=0.89$; $p=0.007$). Neither HDL-C and initial serum zinc nor HDL-C and final serum zinc were significantly correlated for females (Table 15).

The significant positive correlation observed among the males is in contrast to a negative correlation for plasma zinc versus HDL-C ($r=-0.22$; $p<0.005$) reported by Freeland-Graves et al. (1980). In this study, however, the significant positive correlation occurred among males whereas Freeland-Graves et al. (1980) evaluated females. In

this dissertation research, the small number of subjects compared to the 32 females studied by Freeland-Graves et al. (1980) may have been a confounding variable. The -0.22 correlation reported by Freeland-Graves et al. (1980) is a very weak correlation.

The following hypotheses have not been considered in any of the studies previously cited:

H_8: Neither the correlation of HDL-C:TC ratio and initial serum zinc nor the correlation of HDL-C:TC ratio and final serum zinc were significant for males or females (Table 15).

H_9: HDL_2 cholesterol and initial serum zinc were not significantly correlated for males or females (Table 15). HDL_2 cholesterol and final serum zinc were significantly correlated ($r=0.87$; $p=0.01$) for males, but HDL_2 cholesterol and final serum zinc were not significantly correlated for females (Table 15).

H_{10}: HDL_3 cholesterol and initial serum zinc and HDL_3 cholesterol and final serum zinc were not significantly correlated for males (Table 15). HDL_3 cholesterol and final serum zinc were not significantly correlated for females, but HDL_3 cholesterol and initial serum zinc were significantly correlated ($r=0.47$; $p=0.03$) for females.

H_{11}: Neither initial nor final serum zinc were correlated with BMI, skinfold, percent body fat or total body weight for either males or females (Table 16). None of the other studies considered correlations of these variables.

Only Crouse et al. (1984) measured body mass index and body weight. These measures were used to test for differences among the groups participating in the study. The investigators found that one of the groups in the study, the endurance trained subjects, had a lower body mass index ($p<0.01$) and weighed less ($p<0.01$) than another group in the study, the sedentary group. The investigators did not attempt to correlate BMI or body weight with serum zinc.

As stated previously (Spencer et al., 1982; Miyamura et al., 1987; Kennedy et al., 1986; Chandra et al., 1980; Pras et al., 1983), body fat affects zinc status. Specifically, from studies such as Spencer et al. 1982), there is the anticipation of increased serum zinc with weight loss. However, as in this dissertation research, the exercise necessary for significant weight loss can also be accompanied by zinc loss through sweat, perhaps enough to sufficiently lower serum zinc (Miyamura et al., 1987).

As stated previously, subjects were assessed by both body mass index and skinfold measures. BMI may not adequately reflect variations in body fat. As critics have charged, stature, i.e., height is one component of BMI, and BMI may be stature dependent over part of the age range (Garn et al., 1986). Measures of skinfolds at various appropriate body sites should reflect the body's storage fat (Katch and McArdle, 1983). Skinfold measurements can be used to assess an individual's change in percent body fat in weight reduction programs and before and after physical conditioning. Thus, of the measures used, skinfold measures and the percent body fat figures

derived from skinfold measures probably reflect body fat stores more accurately than BMI.

H_{12}: The zinc/copper ratio and the HDL-C:total cholesterol ratio were not significantly inversely correlated for experimental males, experimental females or control females (Table 17). Control males, however, had a statistically significant positive correlation ($r=0.81$; $p=0.02$).

H_{13}: Neither serum zinc and initial walk time nor serum zinc and final walk time were significantly inversely correlated for males or females (Table 18).

Zinc supplementation had no consistent statistically significant effect on lipid profiles in the present study. This lack of statistical significance is consistent with the findings reported by Freeland-Graves et al. (1982). Administering a range of zinc supplementation, the investigators in that study found relatively high pharmacologic doses (100 mg) lowered HDL-C in four weeks, but no changes were noted with lower levels of zinc ($\leq$ 50 mg/day).

In the Black et al. (1988) study, HDL cholesterol levels in subjects assigned to the 75 mg zinc group were significantly lower than those for the placebo group at weeks 6 (E:53 mg% vs. C:61 mg%) and 12 (E:54 mg% vs. C:63 mg%). HDL cholesterol levels in subjects assigned to the 75 mg zinc group were lower at weeks 6 (53 mg%), 8 (51 mg%), and 12 (54 mg%) than at baseline (63 mg%). Subjects in the 50 mg group had lower serum HDL-C levels at week 12 (55 mg%) than did the placebo group (63 mg%).

Crouse et al. (1984) concluded that "low dose zinc supplementation was not associated with significant alterations in lipid lipoprotein levels in either trained or sedentary persons." They did not evaluate HDL_2 or HDL_3 cholesterol. The lack of statistically significant results of this study seem to support the conclusions of Crouse et al. (1984), viz., that low dose (15 mg.) zinc supplementation does not significantly affect HDL-C.

After administering 50 mg. zinc to E and C sedentary and endurance trained subjects, Crouse et al. (1984) found no statistical significance between experimental and control groups. None of the interactions between the zinc dose and the remaining independent factors were significant. Their findings suggested that the power of the statistical test may have been limited by the large variability associated with plasma zinc concentration or by sample size. A longer period of zinc administration, they said, or a trial with larger numbers of participants might have produced significant results.

As for increasing study length, of the studies cited, only the study by Goodwin et al. (1985) was conducted for a longer period, i.e., five years. The Black et al. (1988) study was conducted for 12 weeks, the same time span as the present investigation.

HDL cholesterol, HDL_2 cholesterol, HDL_3 cholesterol values and HDL-C:TC ratio showed positive, statistically significant correlations with final serum zinc among the females (Table 15). No previous study has investigated the effects of either zinc

supplementation or exercise on HDL_2 or HDL_3. Thus, even in the absence of statistical significance, this study, by its design, has provided some information on the effect of zinc supplementation on HDL_2 and HDL_3 among an exercising population.

In a previous RESHAPE study (Bazzarre, Evans, Kinard & Truslow, 1985), 24 percent of participants dropped out of the program by the end of 12 weeks. Twelve percent dropped out before completing the first two weeks of the program, and the remaining 12 percent dropped out prior to the eighth week of the study. Males were 40.2 $\pm$ 9.8 years and females 36.8 $\pm$ 10.7 years. Body weight decreased significantly from 200 $\pm$ 18 lbs. to 191 $\pm$ 19 lbs. ($p \leq 0.010$) for males and from 158 $\pm$ 27 lbs. to 153 $\pm$ 25 lbs. ($p \leq 0.001$) for females. Percent body fat decreased from 29 $\pm$ 5 percent to 25 $\pm$ 4 percent ($p \leq 0.001$) for males and from 38 $\pm$ 5 percent to 35 $\pm$ 5 percent ($p \leq 0.01$) for females. Collectively, total cholesterol for the sample of 93 subjects decreased from 197 $\pm$ 38 mg% to 186 $\pm$ 37 mg% ($p < 0.01$); HDL-C increased from 52 $\pm$ 14 mg% to 53 $\pm$ 14 mg%; HDL-C:TC ratio increased from 0.27 $\pm$ 0.08 to 0.29 $\pm$ 0.08 ($p \leq 0.01$).

Overall, participants in this dissertation research, compared to the previous report by Bazzarre et al. (1985), were slightly older and heavier, had similar body fat and HDL-C:TC ratio, had higher total cholesterol and varied as to HDL-C levels. Their changes over time were not as pronounced as changes among previous participants. Their dropout rate (34 percent) was greater than that for the previous participants (24 percent). As stated previously,

subjects in this dissertation research were older, fatter, more overweight and had higher cholesterol levels than subjects in the study by Bazzarre et al. (1985). In their previous research, Bazzarre et al. (1985) noted that individuals who had poor attendance were more overweight and more overfat than the other RESHAPE participants. One possible explanation for the higher dropout rate in this dissertation research, then, was that, because of their greater weight and fat, these participants were more prone to dropping from the program.

The median total cholesterol of subjects in this study was either borderline-high or high according to levels set by the Adult Treatment Panel of the National Cholesterol Education Program (U.S. Dept. of Health and Human Services, 1987). These blood cholesterol levels were as follows: (1) desirable (less than 200 mg%), (2) borderline-high (200 to 239 mg%), (3) high (240 mg% and above). According to these criteria, initial median cholesterol levels were quite high for E males (253 $\pm$ 56 mg%), C males (220 $\pm$ 41 mg%) and C females (225 $\pm$ 27 mg%). E females had borderline-high cholesterol levels (200 $\pm$ 40 mg%). By the end of the program, only C males had reduced their cholesterol to desirable levels (197 $\pm$ 53 mg%). In the previous RESHAPE study (Bazzarre et al., 1985), both initial and final cholesterol levels, collectively, were within desirable range according to the previously mentioned standards.

According to the Adult Treatment Panel of the National Cholesterol Education Program (U.S. Department of Health and

Human Services, 1987), epidemiological studies have shown that people with high blood cholesterol have a greater chance of developing coronary heart disease than do people with lower levels of cholesterol, and the chances of developing coronary heart disease increase in proportion to the amount the cholesterol is elevated, especially for values over 200 mg%. In the United States, people with a blood cholesterol of 240 mg% or higher have more than two times the risk of developing heart disease as do those with a level of under 200 mg%. About 25 percent of adults in the United States have blood cholesterol levels over 240 mg% and more than half of U.S. adults have levels over 200 mg%.

Overall, subjects lost weight and body fat (Table 5), and serum zinc and copper decreased. The weight loss could have been a response to the diet-exercise program: all subjects decreased walk times (Table 7). Walk times improved significantly for all females, but not for males. The improved performance suggested that the subjects achieved a training effect. Thus, both weight loss and exercise could have contributed to zinc loss.

As previously stated, Klevay (1975) has acknowledged that the concentration of zinc (0.93 mg/liter) in sweat is 16 times that of copper (0.058 mg/liter). He has also noted that increased physical activity increases sweating, causes a greater loss of zinc than copper, and results in a lower ratio of zinc to copper.

Klevay (1975) reported that the ratio of zinc to copper of human muscle and bone are 56 and 120, respectively. Thus, under the

stimulus of exercise, far more zinc than copper must be incorporated into muscle and bone from amounts absorbed from the intestinal tract. Relatively less zinc than copper is then likely to be available to the other organs such as the liver. The improved fitness and decreases in weight/fat loss would, therefore, be consistent with decreased serum copper and zinc provided the absence of satisfactory zinc-copper supplementation.

Klevay (1975) noted that diets high in fat and sucrose and low in vegetable fiber, which have been associated with a high risk of coronary heart disease, contained a high zinc to copper ratio. Diets low in fat and sucrose and high in fiber, in addition to having a low zinc to copper ratio, contain substances such as fiber that reduce the degree to which zinc is absorbed from the intestinal tract. The subjects' increased intake of dietary fiber could also explain the decrease in zinc and copper status.

According to Klevay (1975), the concentration of copper in the serum of men increases in a linear manner between the third and seventh decades of life. These data are consonant with a selective mortality due to coronary heart disease of the individuals at each age with a higher ratio of zinc to copper which restricts the measurements of copper to those who survive. In this study, male subjects were obese with elevated cholesterol levels. These subjects, then, were at risk for heart disease.

According to Fischer et al. (1980), zinc competes with copper for binding to metallothionein and consequently at higher dietary

levels, less copper is absorbed. Fischer et al. (1980), in studies with rats, have determined that copper and zinc, at levels likely to be present in the normal mixed North American diet, can affect serum mineral concentration but have no effect on serum cholesterol levels. Helwig et al. (1979) investigated the effect of adding different amounts of copper and zinc to the diet of laying hens. They found no effect on either serum or egg cholesterol levels. Caster and Doster (1979) fed rats diets in which the zinc/copper ratio ranged from 2 to 220. No significant significant effect on mean plasma cholesterol was observed. Thus, some animal studies suggest zinc/copper ratios may not affect lipid profile. This lack of effect is a contradiction of the research reported by Klevay (1979).

Zinc status could have declined as a function of the combined effect of exercise and diet. As stated previously, subjects in the Miyamura et al. (1987) study had depressed zinc levels following exercise. Subjects in the Dressendorfer et al. (1980) study had depressed zinc levels following exercise. In that study, serum zinc decreased with greater aerobic training. Dressendorfer et al. (1980) stated that the redistribution of zinc stores in the body could explain hypozincemia during exercise. Animal studies (Oh et al., 1978) indicate exercise induces production of the zinc-binding protein metallothionein. Thus, exercise training could stimulate production of zinc dependent enzymes in the liver and other tissues and lower circulating zinc levels. As elsewhere stated, diet could affect the zinc status by having included foods with zinc binding

agents. Perhaps subsequent research should include analysis of covariance of these variables to determine if their combined effect could account for the serum zinc reduction.

Subsequent research might also include animal models. Animal research would provide an opportunity to rigorously control diet, graded levels of zinc and copper, specific exercise regimens of varying duration, the use of normal weight and genetically obese rodents, and various weight reduction programs. The animal model would also permit researchers to evaluate functional changes in specific tissues.

Limitations

This study had two major limitations: the use of free living subjects and the failure of most subjects to return final dietary records. Because subjects were free living, direct capsule monitoring was not possible. To have monitored supplement intake only when the subjects attended the daily workout sessions would have limited supplementation to three days a week or less, depending on subject attendance. The study investigator regularly attended the three day a week subject exercise sessions and weekly workshop meetings. Subjects were urged, at these sessions and workshops, to take their supplement and record supplement compliance on charts provided them. Chart data indicated they consistently complied. In addition, approximately 90 percent of subjects returned capsule containers at the end of the study. All of

the returned containers contained an extra two capsules. The extra two capsules that had been added as a check of subject compliance was a good monitoring system. The ideal approach would be to require subjects to take the supplement in the presence of the staff members.

Also, as stated previously, only four study participants returned dietary records at the end of the program. This lack of response prohibited evaluating final dietary fiber, zinc and copper. These dietary records could have indicated whether dietary habits might explain the decrease in serum zinc. For example, subjects in RESHAPE workshops were encouraged to increase fiber and decrease dietary fat intake. Both measures could reduce zinc status.

Black et al. (1988) required biweekly diet records but acknowledged the difficulty of monitoring dietary habits among subjects such as those in the present study. They stated that, among free living subjects, factors including diet patterns not recorded for each three day period could contribute to variations in expected results. Thus, even if subjects had completed a number of diet records, the possibility exists that diet could affect subjects' zinc status and still go undetected. Thus, a rigorous controlled feeding study would have been the only means of monitoring dietary intake compliance.

Subsequent research might include some form of monetary incentive. This monetary incentive might effectively insure subjects' adherence and compliance to study criteria and completion.

A monetary incentive would be predicated on both the return of final dietary records (if that was a part of the program), satisfactory compliance with supplement/placebo ingestion and completion of all tests.

In summary, 15 mg/day zinc supplementation had no apparent effect on lipid profile. Nevertheless, this supplementation was not enough to prevent a decrease in zinc status. Thus, individuals considering a weight reduction program of diet and exercise should be aware of the potential of decreased zinc status. Previous research (Freeland-Graves et al., 1980; Black et al., 1988; Goodwin et al., 1985) has indicated the effect of lower levels of zinc supplementation on lipid profile, but only Goodwin et al. (1985) has included an exercise component at these lower levels. None of the previous investigators evaluated the effect of weight loss, exercise and low level zinc supplementation on lipid profiles. More research is needed to define the effect of exercise and weight loss on zinc status.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A sample of 47 (12 male and 35 female) participants in a 12 week exercise program (RESHAPE) were divided into experimental (E) and control (C) subjects. E subjects received zinc supplementation (15 mg) to assess the effects of supplementation on total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), HDL-C:TC ratio, HDL_2 cholesterol, HDL_3 cholesterol, serum zinc and copper at week 0 and 12. Additional objectives included measuring the effects of obesity and exercise on TC, HDL-C, HDL-C:TC ratio, HDL_2 and HDL_3, and serum zinc and copper at week 0 and 12. Hypotheses generated from these objectives were tested. Results indicated the following:

Percent body fat significantly decreased among control females. Walk time significantly decreased for control and experimental females.

HDL-C significantly increased for experimental males (5.6 $\pm$ 2.3; p=0.05), while HDL_2 significantly increased for control females (7.7 $\pm$ 7.7; p=0.01). HDL_3 decreased significantly among control(-13.9 $\pm$ 12.7; p=0.002) and experimental(-16.3 $\pm$ 20.4; p=0.04) females.

For control females, HDL_2 (7.7 mg% ± 7.7; p=0.01) significantly increased, and HDL_3 (-16.3 mg% ± 20.4; p=0.04) significantly decreased. The latter change was not expected.

Serum zinc significantly decreased for experimental females (-37 ± 27 ug/dl; p=0.007). Serum copper significantly decreased for both control (-33 ± 20 ug/dl; p=0.04) males and experimental females (-39 ± 27 ug/dl; 0.004).

Final serum zinc was positively correlated with final HDL-C (r=0.89; p=0.007) and final HDL_2 cholesterol (r=0.87; p=0.01) for males. Initial serum zinc was positively correlated with initial HDL_3 cholesterol for females (r=0.47; p=0.03). The zinc/copper ratio difference was positively correlated with HDL-C:TC ratio difference for control males (r=0.81; p=0.02). Initial walk time and initial HDL-C were negatively correlated for females(r=-0.44; p=0.04). Initial HDL_3 cholesterol and initial walk time were negatively correlated for males (r=-0.73; p=0.03).

A number of hypotheses were confirmed when, as predicted, no change occurred over time. No significant change occurred over time for experimental subjects for TC, HDL-C:TC, and HDL_2. No significant change occurred over time for HDL-C among experimental females or HDL_3 among experimental males. No significant correlations occurred between lipid variables and initial and final serum zinc except for the following: final serum zinc correlated significantly with final HDL-C (r=0.89; p=0.007) and HDL_2

cholesterol (r=0.87; p=0.01) for males. For females, initial serum zinc correlated with initial HDL_3 cholesterol (r=0.47; p=0.03).

No statistically significant correlation was observed between HDL_2 cholesterol and serum zinc for females or between HDL_3 cholesterol and initial or final serum zinc for males. Finally, no correlation occurred between measures of obesity and zinc.

Original assumptions had included both a significant improvement in walk times and a significant weight loss among subjects. The data supported these two assumptions: both E and C females signficantly reduced their walk times (E:-1.23 $\pm$ 1.71 minutes; p=0.03) vs. (C:-1.10 $\pm$ 1.10 minutes; p=0.03), and control females significantly reduced percent body fat (-1.1 $\pm$ 1.5 percent; p=0.006).

Serum zinc status had originally been assumed to remain constant or possibly improve in the supplemented group. Nevertheless, the possibility existed that the combined effects of a weight reduction program and changes in diet, e.g., increased fiber, might have contributed to a decrease in zinc status. This reduction in serum zinc did, in fact, occur.

This study was limited by a small sample size; the lack of compliance with protocol, e.g., return of final dietary records and the use of free living subjects. These limitations have been acknowledged elsewhere and remedies have been suggested. Subsequent research dealing with zinc supplementation and lipid profile should consider these factors and attempt to counteract

them. More active recruitment might also be used to increase the number of subjects.

The implications of the present research are that zinc supplementation (15 mg/day) should have no deleterious effect on cholesterol levels. However, exercise and diet may affect zinc status (Miyamura et al., 1987; Dressendorfer et al., 1980; Oh et al., 1978) The extent to which either exercise or diet alone or together could affect zinc status is indeterminate from this research. Nevertheless, persons wishing to initiate a weight loss program involving exercise and diet should consider the effect exercise and diet may have on serum zinc, take precautions to monitor serum zinc status, and, if advised by a physician, take the measures necessary to raise serum zinc to appropriate levels.

None of the previous research has considered either the effect of weight loss, exercise and zinc supplementation on lipid profile or the effect of exercise and weight loss on zinc status. Moreover, no research has specifically focused on the effect of these variables, i.e., weight loss, exercise and zinc supplementation on obesity. Further research, therefore, is needed in these areas.

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APPENDIX

Table A-1

Wilcoxon Two Sample Test by Zinc Supplementation: Age, Height, Weight

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
		MALES			
Age	43	41	26.5	0.38	0.70
(n)	(5)	(7)			
Initial Height	69	69	32.0	0.00	1.00
(n)	(5)	(7)			
Initial Body Weight	212	233	23.0	-0.09	0.92
(n)	(5)	(7)			
Final Body Weight	208	230	23.0	-0.09	0.92
(n)	(3)	(5)			
		FEMALES			
Age	40	39	279.5	-0.60	0.50
(n)	(17)	(18)			
Initial Height	66	65	355.0	1.60	0.10
(n)	(17)	(18)			
Initial Body Weight	166	161	301.00	0.10	0.91
(n)	(17)	(18)			
Final Body Weight	156	151	23.0	-0.09	0.92
(n)	(12)	(9)			

Table A-2

Descriptive Statistics of Study Population at Week 0 (Medians and Standard Deviations)

	Males		Females	
Groups (n)	Zinc	Placebo	Zinc	Placebo
Initial Weight (Lbs.)	212 ±79	233 ±47	166 ±27	161 ±33
(n)	(5)	(7)	(17)	(18)
Final Weight (Lbs.)	208 ±28	230 ±56	156 ±19	151 ±36
(n)	(3)	(5)	(12)	(9)
Initial BMI	31 ±8	32 ±6	26 ±5	27 ±6
(n)	(5)	(7)	(17)	(18)
Final BMI	27 ±3	29 ±7	24 ±4	26 ±8
(n)	(3)	(5)	(12)	(9)
Initial Skinfold (mm)	81 ±40	89 ±19	105 ±31	99 ±35
(n)	(5)	(7)	(17)	(18)
Final Skinfold (mm)	75 ±23	77 ±29	91 ±27	85 ±36
(n)	(3)	(5)	(12)	(9)
Initial Percent Body Fat	28 ±8	32 ±4	39 ±5	37 ±5
(n)	(5)	(7)	(17)	(18)
Final Percent Body Fat	29 ±8	32 ±2	38 ±5	37 ±5
(n)	(3)	(5)	(12)	(9)

Table A-3

Wilcoxon Two Sample Test by Zinc Supplementation of Males: Percent Body Fat, Skinfold, BMI

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
Initial Skinfold	81	89	29.0	-0.49	0.62
(n)	(5)	(7)			
Final Skinfold	75	77	18.5	-0.25	0.81
(n)	(4)	(5)			
Initial Percent Body Fat	31	32	34.0	0.16	0.87
(n)	(5)	(7)			
Final Percent Body Fat	31	32	17.0	-0.61	0.54
(n)	(4)	(5)			
Initial BMI	31	32	21.0	-0.47	0.63
(n)	(4)	(7)			
Final BMI	27	29	10.0	-0.89	0.37
(n)	(3)	(5)			

Table A-4

Wilcoxon Two Sample Test by Zinc Supplementation of Females: Percent Body Fat, Skinfold, BMI

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
Initial Skinfold (n)	105 (17)	99 (18)	317.0	0.36	0.72
Final Skinfold (n)	91 (12)	85 (9)	317.0	0.36	0.72
Initial Percent Body Fat (n)	39 (17)	37 (18)	310.5	0.13	0.89
Final Percent Body Fat (n)	38 (12)	37 (9)	264.5	-0.25	0.80
Initial BMI (n)	26 (17)	27 (18)	294.0	-0.10	0.92
Final BMI (n)	24 (12)	26 (9)	80.0	-0.44	0.65

Table A-5

Initial and Final Lipid Profile of Study Population (Medians and Standard Deviations)

Groups (n)	Males		Females	
	Zinc	Placebo	Zinc	Placebo
Initial Total Cholesterol (mg%)	228 ± 46	220 ± 38	192 ± 42	217 ± 42
n	(5)	(7)	(17)	(18)
Final Total Cholesterol (mg%)	258 ± 13	197 ± 53	200 ± 50	226 ± 34
n	(3)	(5)	(12)	(9)
Initial HDL Cholesterol (mg%)	33 ± 8	42 ± 7	51 ± 14	52 ± 17
n	(5)	(7)	(17)	(18)
Final HDL Cholesterol (mg%)	40 ± 10	57 ± 7	49 ± 17	55 ± 17
n	(3)	(5)	(12)	(9)
Initial HDL-C:TC	.15 ± .06	. 21 ± .05	. 29 ± .08	30 ± .10
n	(5)	(7)	(17)	(18)
Final HDL-C:TC	.15 ± .5	.25 ± .16	.26 ± .10	.25 ± .10
n	(3)	(5)	(12)	(9)

Table A-6

Initial and Final Lipid Data of Study Population (Medians and Standard Deviations)

Groups (n)	Males		Females	
	Zinc	Placebo	Zinc	Placebo
Initial HDL_2 Cholesterol (mg%)	4 ± 2	5 ± 9	10 ± 9	8 ± 4
n	(5)	(7)	(17)	(18)
Final HDL_2 Cholesterol (mg%)	7 ± 10	15 ± 9	16 ± 14	15 ± 8
n	(3)	(5)	(12)	(9)
Initial HDL_3 Cholesterol (mg%)	37 ± 9	35 ± 19	46 ± 12	54 ± 20
n	(5)	(7)	(17)	(18)
Final HDL_3 Cholesterol (mg%)	39 ± 0	42 ± 11	33 ± 14	44 ± 12
n	(3)	(5)	(12)	(9)

Table A-7

Wilcoxon Two Sample Test of Lipid Variables by Zinc Supplementation of Males

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
Initial Total Cholesterol	228	228	38.5	0.89	0.37
(n)	(5)	(7)			
Final Total Cholesterol	258	221	20.0	1.80	0.15
(n)	(3)	(5)			
Initial HDL Cholesterol	33	42	20.5	-1.89	0.06
(n)	(5)	(7)			
Final HDL Cholesterol	40	57	9.50	- 1.05	0.13
(n)	(3)	(5)			
Initial HDL-C:TC Ratio	15	22	21.0	-1.78	0.07
(n)	(5)	(7)			
Final HDL-C:TC Ratio	15	25	8.00	-1.50	0.22
(n)	(3)	(5)			

Table A-8

Wilcoxon 2-Sample Test by Zinc Supplementation of Males

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
Initial HDL_2 (n)	4 (5)	5 (7)	21.0	-1.56	0.12
Final HDL_2 (n)	1 (3)	12 (5)	10.0	26.00	0.30
Initial HDL_3 (n)	37 (5)	35 (7)	30.5	0.00	1.00
Final HDL_3 (n)	39 (3)	42 (5)	12.0	-0.30	0.70

Table A-9

Wilcoxon Two Sample Test of Lipid Variables by Zinc Supplementation of Females

Variable	Medians for		Wilcoxon	Approximate	
	Zinc	Placebo	S Value	Z Score	P value
Initial Total Cholesterol	192	217	266.00	-1.30	0.19
(n)	(17)	(18)			
Final Total Cholesterol	200	226	113.50	0.99	0.20
(n)	(12)	(9)			
Initial HDL Cholesterol	51	52	293.50	-0.39	0.69
(n)	(17)	(18)			
Final HDL Cholesterol	49	55	111.00	0.82	0.57
(n)	(12)	(9)			
Initial HDL-C:TC Ratio	26	30	314.00	0.25	0.80
(n)	(17)	(18)			
Final HDL-C:TC Ratio	26	25	94.00	0.32	0.73
(n)	(12)	(9)			

Table A-10

Wilcoxon 2-Sample Test of Median Lipid Scores for Females

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
Initial HDL_2 (n)	10 (16)	8 (17)	262.0	0.33	0.58
Final HDL_2 (n)	13 (12)	12 (9)	102.00	0.17	0.85
Initial HDL_3 (n)	46 (17)	54 (18)	280.5	-0.83	0.40
Final HDL_3 (n)	33 (12)	44 (9)	118.5	1.35	0.37

Table A-11

Lipid status according to level of obesity for males and females at week 0 (Medians and Standard Deviations)

	BMI[1]			% Body Fat[2]		
	<25	25-29.9	≥30	normal	overweight	obese
			MALES:			
Total Cholesterol (mg%)	270 ±71 (2)	249 ±27 (2)	213 ±35 (8)	220 (1)	229 ±1 (2)	230 ±50 (9)
HDL-C (mg%)	42 ±13 (2)	43 ±14 (2)	41 ±7 (8)	52 (1)	46 ±9 (2)	40 ±7 (9)
HDL-C:TC ratio	16 ±9 (2)	17 ±7 (2)	19 ±4 (8)	23 (1)	20 ±4 (2)	19 ±5 (9)
HDL_2 (mg%)	2 ±2 (2)	4 ±1 (2)	6±8 (8)	50 (1)	42 ±7 (2)	6±8 (9)
HDL_3 (mg%)	40 ±13 (2)	46 ±1 (2)	38 ±17 (8)	1 (1)	4 ±1 (2)	38 ±17 (9)
			FEMALES:			
Total Cholesterol (mg%)	214 ±31 (12)	198 ±37 (12)	197 ±56 (11)		213 ±63 (2)	202 ±42 (33)
HDL-C (mg%)	64 ±16 (12)	53 ±13 (12)	51 ±15 (11)		77 ±28 (2)	55 ±15 (33)
HDL-C:TC ratio	30 ±8 (12)	27 ±9 (12)	29 ±13 (11)		35 ±2 (2)	28 ±10 (33)
HDL_2 (mg%)	10 ±8 (12)	6±5 (12)	11 ±6 (11)		9±1 (2)	9±7 (33)
HDL_3 (mg%)	57 ±17 (12)	55 ±17 (12)	47 ±15 (11)		74 ±19 (2)	52 ±16 (33)

1. BMI= $\frac{\text{weight (kg)}}{\text{height (m)}^2}$ Overweight is 25-29.9 $kg\text{-}m^{-2}$ weight for height. Obesity is greater than or equal to 30 $kg\text{-}m^{-2}$ weight for height.

2. Percent body fat estimated from sum of four skinfolds (Durnin and Womersley, 1973). Overweight is 20-28 percent and 22-30 percent for males and females, respectively. Obesity is greater than 28 percent and greater than 30 percent for males and females, respectively.

Table A-12
Serum zinc status according to level of obesity for males and females at week 0 (Means and Standard Deviations)

	BMI[1]			% Body Fat[2]		
	<25	25-29.9	≥30	normal	overweight	obese
MALES						
Dietary Zinc	9.6±5.7 (2)	9.5±4.5 (2)	9.3±4 (8)	13.7 (1)	8.9±3.7 (2)	9.0±4.1 (9)
Serum Zinc	100±30 (2)	120±40 (2)	240±330 (8)	80 (1)	90±0 (2)	240±320 (9)
Dietary Copper	2.1±1.6 (2)	4.1±0.7 (2)	1.8±1.6 (8)	3.2 (1)	2.9±2.3 (2)	2.0±1.7 (9)
Serum Copper	130±40 (2)	120±20 (2)	110±17 (8)	160 (1)	140±7 (2)	110±10 (9)
Zn/Cu ratio	0.8±0.5 (2)	1.0±0.5 (2)	2.3±3.7 (8)	0.5 (1)	0.67±0.03 (2)	2.3±3.4 (9)
FEMALES						
Dietary Zinc	9.3±4.0 (12)	8.6±2.5 (12)	9.4±3.2 (11)		8.5±1.6 (2)	9.2±3.3 (33)
Serum Zinc	170±210 (12)	130±80 (12)	140±70 (11)		110±10 (2)	150±140 (33)
Dietary Copper	2.2±1.5 (12)	2.2±2.1 (12)	2.0±1.1 (11)		2.5±2.2 (2)	2.1±1.6 (33)
Serum Copper	150±40 (12)	110±20 (12)	130±0.2 (11)		140±30 (2)	130±40 (33)
Zn/Cu ratio	1.1±1.2 (12)	1.2±0.6 (12)	1±0.7 (11)		0.8±0.2 (2)	1.2±0.9 (33)

1. BMI= $\frac{\text{weight (kg)}}{\text{height (m)}^2}$ Overweight is 25-29.9 $kg\text{-}m^{-2}$ weight for height. Obesity is greater than or equal to 30 $kg\text{-}m^{-2}$ weight for height.

2. Percent body fat estimated from sum of four skinfolds (Durnin and Womersley, 1973). Overweight is 20-28 percent and 22-30 percent for males and females, respectively. Obesity is greater than 28 percent and greater than 30 percent for males and females, respectively.

Table A-13

Wilcoxon Two Sample Test of Minerals by Zinc Supplementation of Males

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
Initial Serum Zinc	117	110	14.00	0.00	1.00
(n)	3	(5)			
Final Serum Zinc	70	51		-0.53	0.59
(n)	(3)	(5)	10.00		
Initial Serum Copper	110	120	33.00	0.00	1.00
(n)	(5)	(7)			
Final Serum Copper	80	55	16.00	1.24	0.21
(n)	(3)	(5)			
Initial Dietary Zinc	9	10	8.00	0.19	0.85
(n)	(3)	(5)			
Initial Dietary Copper	2	2	28.00	-0.64	0.51
(n)	(5)	(7)			

Table A-14

Wilcoxon Two Sample Test of Minerals by Zinc Supplementation of Females

Variable	Medians for		Wilcoxon	Approximate	
	Zinc	Placebo	S Value	Z Score	P value
Initial Serum Zinc (n)	122 (17)	107 (9)	84.5	-1.01	0.98
Final Serum Zinc (n)	85 (12)	89 (9)	58.5	0.09	0.92
Initial Serum Copper (n)	124 (17)	146 (17)	290.0	-0.51	0.60
Final Serum Copper (n)	56 (12)	90 (12)	75.5	0.42	0.67
Initial Dietary Zinc (n)	9 (12)	9 (9)	96.0	-0.14	0.88
Initial Dietary Copper (n)	2 (5)	2 (7)	346.0	1.30	0.19

Table A-15

Wilcoxon Two Sample Test of Walk Time by Subject Zinc Supplementation and Gender

Variable	Medians for Zinc	Medians for Placebo	Wilcoxon S Value	Approximate Z Score	Approximate P value
		MALES			
Initial Walk Time	16	15	12.0	-0.30	0.7
(n)	(3)	(5)			
Final Walk Time	15	15	8.0	0.19	0.8
(n)	(3)	(5)			
		FEMALES			
Initial Walk Time	16	15	90.0	-0.60	0.5
(n)	(12)	(9)			
Final Walk Time	14	14	65.0	-0.4	0.70
(n)	(12)	(7)			