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THE EFFECTS OF ZINC AND COPPER SUPPLEMENTATION ON GROWTH, LIPID PROFILES, AND TRACE MINERAL STATUS IN YOUNG MALE RATS

The University of North Carolina at Greensboro PH.D. 1985

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THE EFFECTS OF ZINC AND COPPER SUPPLEMENTATION ON GROWTH, LIPID PROFILES, AND TRACE MINERAL STATUS IN YOUNG MALE RATS

by

Nesba Ama Frimpong

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Approved by

Allen C. Mayee
Dissertation Adviser

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of the Graduate School at The University of North Carolina at Greensboro.

Dissertation Adviser Allen C. Magee

Committee Members Elizabeth L. Schiller
Michael Liebman
Mildred Johnson

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The purposes of this study were to investigate the effects of low and high levels of zinc and copper supplements, and the effects of zinc/copper ratios on growth, lipid profiles, and trace mineral status of young male rats. The study was conducted in two phases to afford data collection to evaluate the objectives. Dietary variables used in Experiment 1 (low level supplementation) included four levels of zinc (0, 5, 10, and 20 ppm), and four levels of copper (0, 0.56, 1.68, and 5.04 ppm). Dietary variables used in Experiment 2 (high level supplementation) included four levels of zinc (0, 50, 100, and 200 ppm), and four levels of copper (0, 5.6, 16.8, and 50.4 ppm). Criteria used for evaluating animal responses to various test diets included weight gain, hemoglobin level, copper, iron and zinc deposition in the liver, and serum levels of triglycerides, total cholesterol, and HDL-cholesterol. The effects of zinc/copper ratios on the parameters were evaluated by comparing data from Experiment 1 and Experiment 2 treatments with the same zinc/copper ratios but different levels of zinc and copper.

Results indicated that rats fed diets containing high levels of zinc and copper had significantly ($p \leq 0.01$) higher weight gains than rats fed low levels of zinc and copper. A dietary zinc/copper ratio of four was associated with maximal growth. Increasing levels of dietary zinc were associated with highly significant ($p \leq 0.01$) decreases in hemoglobin levels, while increases in dietary copper resulted in highly significant ($p \leq 0.01$) increases in hemoglobin levels. Maximum hemoglobin level was observed when the dietary zinc/copper ratio was one.

Zinc supplementation significantly ($p \leq 0.01$) increased liver zinc deposition but was associated with significant ($p \leq 0.01$) decreases in the deposition of both copper and iron in the liver. Copper supplementation was associated with significant ($p \leq 0.01$) increases in liver copper deposition, trend in increase in liver zinc deposition and significant ($p \leq 0.01$) decreases in liver iron deposition. A dietary zinc/copper ratio of four enhanced liver zinc deposition, a ratio of one enhanced liver copper deposition, while a ratio of six was associated with increased liver iron deposition.

An increase in dietary zinc was associated with significant ($p \leq 0.01$) increase in serum triglyceride levels, while an increase in dietary copper was associated with significant ($p \leq 0.01$) decrease in serum total cholesterol and HDL-cholesterol levels. Dietary zinc/copper ratios had no significant effect on the serum lipids. As the experimental period progressed, animals fed the nonsupplemented zinc diets developed skin lesions on the legs, around the mouth, and the eyes resulting in blindness to some of the rats. Animals fed the high zinc diets exhibited extensive hair losses.

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TABLE OF CONTENTS

Page

APPROVAL PAGE ii

ACKNOWLEDGMENTS iii

LIST OF TABLES. vi

CHAPTER

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 4

III. EXPERIMENTAL PROCEDURES. 9

Animals. 10
Diets. 11
Analytical Methods 12
Statistical Analyses 15

IV. RESULTS AND DISCUSSION 17

Growth 17
Hemoglobin 19
Liver Copper 21
Liver Iron 23
Liver Zinc 23
Serum Triglyceride Levels. 26
Serum Total Cholesterol. 28
Serum HDL-Cholesterol. 28
Zinc/Copper Ratios 28
Hair Coat and Skin Appearance. 36

V. GENERAL DISCUSSION 37

VI. SUMMARY AND RECOMMENDATIONS. 42

Summary. 42
Recommendations. 44

BIBLIOGRAPHY. 46

Page

APPENDICES

Appendix A. Composition of Experimental Diets 50

Appendix B. Raw Data. 55

Appendix C. Statistical Analyses. 67

LIST OF TABLES

Table		Page
1	Effects of Zinc and Copper Supplements on Weight Gain of Young Male Rats	18
2	Effects of Zinc and Copper Supplements on Hemoglobin Levels of Young Male Rats	20
3	Effects of Zinc and Copper Supplements on Liver Copper Levels of Young Male Rats	22
4	Effects of Zinc and Copper Supplements on Liver Iron Levels of Young Male Rats	24
5	Effects of Zinc and Copper Supplements on Liver Zinc Levels of Young Male Rats	25
6	Effects of Zinc and Copper Supplements on Serum Triglyceride Levels of Young Male Rats	27
7	Effects of Zinc and Copper Supplements on Serum Total Cholesterol Levels of Young Male Rats	29
8	Effects of Zinc and Copper Supplements on Serum HDL-Cholesterol Levels of Young Male Rats	30
9	Effect of Zinc/Copper Ratios and Level of Zinc and Copper Supplementation on Parameters	31
A- 1	Composition of Basal Diet	51
A- 2	Zinc and Copper Supplementation Data	52
A- 3	Composition of Mineral Mix, Hawk Oser	53
A- 4	Composition of Vitamin Mixture	54
B- 1	Total Weight Gain of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2	56
B- 2	Hemoglobin Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2	57

CHAPTER I

INTRODUCTION

The leading cause of death in the United States is coronary heart disease (CHD) (Anon, 1969; Williams & Caliendo, 1984), and hypercholesterolemia is one of the principal indicators of the risk of the disease. Studies suggest that the distribution of cholesterol among the plasma lipoproteins may have more predictive value for the incidence of the disease than the total concentration of blood cholesterol. High density lipoprotein cholesterol (HDL-C) is suggested to have predictive positive effect against CHD, while low density lipoprotein cholesterol (LDL-C) is suggested to increase the risk of developing CHD (Gordon, Castelli, Hjortland, Kannel, & Dawber, 1977; Kannel, 1971).

The elevation of plasma cholesterol levels can result from series of complex interactions of individuals with their environments. Diet has frequently been cited as one environmental factor which affects the predisposition of an individual to CHD (Shorey, Sewall, & O'Brien, 1976; Watt, Wiley, & Fletcher, 1976). Diet, however, is a component which may have a multifactorial impact on the development of this disease, and several components have been associated with it.

The quality and quantity of dietary fat has been shown to have an effect on plasma cholesterol levels. Dietary saturated fatty acids (SFA) have been associated with increased blood cholesterol levels, while dietary intakes of polyunsaturated fatty acids (PUFA) have been

associated with a lowering of blood levels of cholesterol and other lipids (McGandy & Hegsted, 1975). Epidemiological studies of various population groups have long suggested to some investigators (Keys, 1957) that high fat diets are associated with increased blood cholesterol levels, and thus with increased mortality from heart disease. Americans as a group have been observed to consume 50% or more of total calories as fat (Friend, 1967). Recently, health professionals have recommended that the ratio of PUFA to SFA in the American diet be increased while the total fat content be decreased to about 30% of caloric intake to protect against the development of CHD (Senate Select Committee, 1977).

In addition to its effect on blood cholesterol levels, dietary fat has been shown to affect trace mineral status. Lukaski, Klevay, and Bolonchuk (1982) have suggested that the amount and type of dietary fat can influence zinc, iron, and copper status. Other studies have shown an interrelationship between dietary fat and iron (Amine & Hegsted, 1975) and also zinc (Bettger, Reeves, Moscatelli, Reynolds, & O'Dell, 1979; Bettger, Reeves, Moscatelli, Savage, & O'Dell, 1980).

Other dietary components that some studies have reported to be associated with CHD include trace minerals. A high dietary zinc to copper ratio was identified as a major factor associated with elevated cholesterol levels by Klevay (1973). Other researchers, however, contended that the zinc to copper ratio of the diet was not a major factor affecting cholesterol metabolism (Fischer, Giroux, Belonje, & Shah, 1980; Koo & Ramlet, 1983; Murthy & Petering, 1976). Hooper (1980) reported

that oral ingestion of pharmacological doses of zinc (160 mg) lowered HDL-cholesterol in normal men, and that high doses of the mineral might be atherogenic.

Since lipid metabolism and dietary lipid quality and quantity are considered to be factors in the etiology of atherosclerosis in man, the possibility that dietary minerals play a role in lipid metabolism must be viewed as important. Thus, a study to investigate the possible interrelationship between dietary fat and trace minerals, as well as the effect of zinc and copper supplement on growth, trace mineral status, and lipid profiles in young male rats seemed warranted.

The objectives of the study were the following:

1. To investigate the effects of low and high zinc and copper supplements on growth, lipid profiles, and trace mineral status of young male rats.
2. To investigate the effects of zinc/copper ratios on growth, lipid profiles, and trace mineral status of young male rats.

CHAPTER II

REVIEW OF LITERATURE

The role of essential trace minerals in lipid metabolism and thus their possible role in the etiology of CHD has been a subject of many nutritional investigations. Doisy (1972) showed that manganese was directly related to serum cholesterol levels in man and chickens, while Schroeder, Mitchener, and Nason (1971) showed that chromium deficiency was associated with elevated cholesterol levels in rats. Amine and Hegsted (1975) reported that dietary iron was inversely related to serum lipid levels in rats, and Iacano (1974) observed that dietary calcium could alter cholesterol and lipid metabolism in the rat.

Two other trace minerals which have been found to have a possible role in lipid metabolism are zinc and copper. Klevay (1973) hypothesized that an alteration in the ratios of metallic elements ingested by rats would alter the concentration of plasma cholesterol and collected data showing that an increased ratio of zinc to copper (40:1 as compared with a ratio of 5:1) caused an increased concentration of plasma cholesterol. In 1975, Klevay postulated that dietary copper deficiency either alone or associated with an increased intake of zinc was a key factor in the etiology of cardiovascular disease. This postulate was supported by several reports by Allen and Klevay (1978a, 1978b, 1978c). Recently, they reported that copper deficiency in rats produced a hypercholesterolemia with a significant reduction in the percentage of plasma

cholesterol associated with HDL and an increase in plasma cholesterol associated with the LDL fraction (Allen & Kelvay, 1980).

In contrast, other studies failed to show such relationships between dietary zinc/copper ratios and plasma cholesterol levels in experimental animals (Caster & Doster, 1979; Fischer et al., 1980; Helwid, Mulnix, & Regenstein, 1978; Woo & Gibbs, 1981), as well as in humans (Geders, 1979; Hambidge, 1977). Some studies, however, reported that serum cholesterol levels were inversely related to dietary copper and serum copper levels in rats, while there appeared to be no relationship between serum zinc and cholesterol levels (Murthy & Petering, 1976; Petering, Murthy, & O'Flaherty, 1977). Based on such studies, Petering and co-workers (1977) suggested that dietary copper levels governed the metabolic fate of cholesterol more than the dietary zinc to copper ratios.

The results of two studies (Koo & Williams, 1981; Koo & Ramlet, 1983), however, revealed a close association between zinc status of adult male rats and serum cholesterol level. It was found that acute zinc depletion (0.37 ppm zinc in basal diet) was associated with a significant reduction in total serum cholesterol as compared to zinc supplemented controls (41 ppm zinc). This relationship was, however, solely dependent upon serum zinc status rather than serum copper.

The quality and quantity of dietary fat has been shown to have an effect on plasma cholesterol levels. The ingestion of fats containing PUFA as a replacement for fats high in SFA has been found by some investigators to be associated with a lowering of the blood levels of

cholesterol and other lipids in rats (Dumaswala, 1970; Pawar & Tidwell, 1967) as well as in humans (Keys, Anderson, & Grande, 1960; Vega, 1982). High fat diets have been found to be associated with increased blood cholesterol levels. In a study where rats were fed 20% fat (by weight), the mean serum total cholesterol level was 103 mg/dl (Dumaswala, 1970); while in another study, a dietary fat intake of 13% was associated with mean total cholesterol and HDL-cholesterol levels of 87 mg/dl and 60 mg/dl, respectively (Sinthusek & Magee, 1984).

The literature on the interrelationships between dietary fat and trace mineral status in animals is limited. Babatunde (1972) found that the level of zinc required for optimum growth and feed utilization apparently increased as the level of dietary fat was increased, suggesting that increasing fat levels decreased zinc availability. Amine and Hegsted (1975) reported that iron absorption was greater in rats fed diets containing coconut oil than in rats fed diets containing corn oil. Amine and Hegsted (1975) also reported that high fat diets enhanced the absorption and utilization of iron. These individuals further suggested that diets which are high in animal products, saturated fats, and sugar and low in fiber and phytates combine most of the factors which are known to favor iron absorption.

Contemporary analyses of copper and zinc content of mixed Western type diets suggest that many such diets provide less than the recommended

daily intakes of 2 mg and 15 mg per day for copper and zinc, respectively (Guthrie & Robinson, 1977; Holden, Wolf, & Mertz, 1979; Hunt, Murphy, Gomez, & Smith, 1979; Klevay, Reck, & Barcome, 1979; White, 1969). The results of a survey of 22 subjects conducted by Holden et al. (1979) showed that the mean daily intake of copper in self-selected diets in America was 1 mg/day and the range was 0.24-2.5 mg. This observation would indicate that Americans consumed 12-125% of the Recommended Dietary Allowances (RDA) for copper. Similar results were obtained for zinc. It was found that the mean daily zinc intake was 8.6 mg and the range was 5.9-12.4 mg. These reflect zinc intake of 39-83% of the RDA.

Mills and Murray (1960) reported that 3 ppm of copper was the minimum requirement for growth of rats, while Forbes and Yohe (1960) reported growing rats fed casein or egg white diets require a minimum of 12 ppm zinc. Dietary zinc/copper ratio of five has been suggested by some investigators to be essential for optimum growth in rats (Sinthusek & Magee, 1984).

Murthy, Klevay, and Petering (1974) reported that the zinc levels of aorta, liver, and heart of rats did not change with variations in either dietary zinc or copper, but the copper contents of these same tissues responded to variations of dietary zinc or copper. Several investigators have reported a possible antagonistic interrelationship between zinc and copper and between zinc and iron when levels of dietary zinc, not generally considered toxic, were fed to young rats (Magee & Grainger, 1979; Motsinger & Magee, 1980; Son, 1984).

In light of the available data, there appears to be an important interrelationship between dietary fat, trace minerals and lipid profiles.

High fat diets are associated with increased blood cholesterol and lipid levels. Some types of dietary fat have been observed to be associated with increased or decreased absorption of certain trace minerals. Some studies have shown that relative or absolute high intakes or deficiency of certain trace minerals such as zinc and copper are associated with hypercholesterolemia and hence with the possible involvement in the etiology of CHD. This study was designed to investigate the effects of feeding deficient, normal, and high intakes of zinc and copper on growth, lipid profiles, and liver status of trace minerals.

CHAPTER III

EXPERIMENTAL PROCEDURES

The purposes of this study were to investigate the effects of low and high zinc and copper supplements, and the effects of zinc/copper ratios on growth, lipid profiles, and trace minerals status in young male rats.

The experiment was conducted in two phases to afford data collection to evaluate each objective. In Experiment 1, the animals were fed a high fat diet with zinc and copper at levels likely to occur in a normal North American mixed diet. At the end of six weeks, the animals were sacrificed and samples collected for analyses. In Experiment 2, animals were fed the same level of fat as well as similar ratios of zinc and copper but the absolute levels of zinc and copper were increased tenfold. At the end of six weeks the animals were sacrificed and samples collected for analyses.

The criteria used to evaluate animal response to various test diets included weight gain; hemoglobin levels; serum levels of total cholesterol, high density lipoprotein cholesterol, and triglycerides; and liver levels of copper, zinc, and iron.

A 4 x 4 factorial design was used for both experiments. For Experiment 1, dietary factors included one level of fat (21% corn oil), four levels of zinc supplements (0, 5, 10, and 20 ppm), and four levels of copper supplements (0, 0.56, 1.68, and 5.04 ppm). For Experiment 2,

dietary factors included the same level of fat (21% corn oil), four levels of zinc supplements (0, 50, 100, and 200 ppm), and four levels of copper (0, 5.6, 16.8, and 50.4 ppm). The basal diet contained 0.30 ppm copper, 0.34 ppm zinc, and 73.39 ppm iron on a dry weight basis.

Animals

A total of 96 weanling male albino rats[1] were used in Experiment 1, and 112 were used in Experiment 2. The rats were assigned to the experimental group with six rats per treatment group in Experiment 1 and seven rats per treatment group in Experiment 2.

A randomized block design involving six and seven replications for Experiments 1 and 2, respectively, were used for the study. The animals were assigned to individual, stainless steel, wire bottom cages within each replication according to initial body weight. The experimental diets were assigned at random to individual cages within each replication. All animals had free access to food and distilled water.

The animals were weighed at the end of six weeks, and total weight gain was calculated. They were fasted for 12 hours and anesthetized with ether. Blood samples were taken from the animals via a heart puncture using 10 ml disposable syringes[2] and 20 gauge disposable

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[1]Sprague-Dawley rats purchased from Holtzman Company, Madison, Wisconsin. These animals averaged 53 and 55 grams in weight initially in Experiments 1 and 2, respectively.

[2]B-D Plastipak, Becton, Dickinson, and Company, Rutherford, New Jersey.

needles.[3] Serum was obtained by spinning the blood samples at 2500 xg for 10 minutes at 10°C in a refrigerated centrifuge[4]. Livers from the animals were removed, weighed, and dried at 60°C in an oven. The dried livers were then ashed with hot nitrate, sulphuric acid, and 50% hydrogen peroxide. Two gram samples from each diet were dried at 60°C and wet-ashed by the procedure used for the dried livers.

Diets

Twenty-one percent (by weight) of corn oil[5] was used for the study to reflect the 40% caloric consumption as fat by the American public. Zinc and copper levels used in Experiment 1 were based on the minerals requirements for rats (12 mg Zn/kg diet, and 5 mg Cu/kg diet), and also on the results of the survey conducted by Holden (1979) which suggested that Americans consume 12-125% of the RDA for copper and 39-83% of the RDA for zinc. Thus the zinc and copper levels were selected to reflect levels likely to occur in a normal North American mixed diet. Zinc and copper levels used for Experiment 2 were selected to test Klevay's (1973) hypothesis that the zinc/copper ratio is an important factor affecting cholesterol metabolism. If Klevay's hypothesis is true, then it would be expected that maintaining similar ratios in both experiments should evoke the same response regardless of the absolute amounts of the minerals used.

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[3]Stylex, Pharmaseal Laboratories, Glendale, CA.

[4]Model J-6B, Beckman Instrument Company, Irvine, DA.

[5]Mazola corn oil, Best Foods, CPC International, Englewood Cliffs, NJ.

In addition to fat, each diet contained 51% cornstarch[6], 20% egg white solids[7], 4% mineral mix[8], 2% cellulose[9], 2% vitamin mix[10], and 24 drops of oleum percomorphum[11]. The percentage of total calories supplied by fat, cornstarch, and egg white solids were 40, 43, and 17, respectively. Zinc and copper supplements were provided by zinc carbonate and cupric sulfate, respectively. The composition of all diets, mineral mix, and vitamin mix are given in Appendix A, Tables A-1 through A-4.

Analytical Methods

Hemoglobin was determined by the method of Shenk, Hall, and King (1934). A sharp razor blade was used to remove a small portion of the tip of the tail of the animal. Blood was drawn to the first mark on a graduated diluting pipette. The pipette containing the blood was placed in a freshly prepared 0.1% sodium carbonate solution, and the pipette was filled quickly to the second mark with the solution. The resulting mixture was drained freely into a culture tube. The optical density of the sample was read against a water reference in a

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[6]Corn starch, Teklad Test Diets, Madison, Wisconsin.

[7]Egg white solids, Teklad Test Diets, Madison, Wisconsin.

[8]Hawk-Oser mineral mix, Teklad Test Diets, Madison, Wisconsin.

[9]Alphacel, ICN Nutritional Biocehmicals, Cleveland, Ohio.

[10]Vitamin mix, ICN Nutritional Biochemicals, Cleveland, Ohio.

[11]Mead Johnson and Company, Evansville, Indiana. The composition of this product is listed as 1250 USP units for vitamin D per drop.

spectrophotometer[12] with the wavelength set at 542 nm. The grams of hemoglobin per 100 ml of blood were calculated using the equation: Optical density (OD) x 28.575 gm Hb per 100 ml blood.

Serum triglyceride concentration was determined enzymatically by the method of Geigel, Ham, and Glema (1975). The serum triglycerides were first extracted by adding 0.5 ml 40 mmol/liter sulfuric acid, and 2.5 ml extraction reagent (n-nonane/isopropanol: 2.0/3.5 by vol.) to 0.1 ml of sample, 0.1 ml distilled water (blank), and to 0.05, 0.1, and 0.2 ml triolein standard[13] in test tubes. The content of all tubes were vortexed for 4-5 seconds and centrifuged at 2500 x g for 12 minutes at 25°C. Then, 0.25 ml of the upper phase was pipetted into other tubes, and 0.25 ml of transesterifying reagent (NaOH in isopropanol, 100 mmol/liter) was added to each tube. The tubes were vortexed, and allowed to stand for five minutes at room temperature. Then 0.25 ml of oxidizing reagent (sodium periodate, 18 mol/liter, in 2.0 mol/liter acetic acid) was added to each tube, vortexed, and allowed to stand for two minutes at room temperature. Three ml of working color reagent (4 ml acetylacetone in 100 ml amonium acetate, 6.0 mol/liter, pH 6.0 at 25°C) was added to each tube and vortexed. All tubes were then placed in a water bath at 57°C for 10 minutes. Triglyceride concentration was determined by reading the samples and standards against the reagent blank at 415 nm.

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[12]Spectronic 20, Bausch & Lomb, Rochester, New York.

[13]Sigma Chemical Company, St. Louis, Missouri.

In the separation of HDL-cholesterol fraction, 0.175 ml aliquot of serum was added to a Centrifi-Kit tube[14] containing a premeasured amount of sodium phototungstate and mixed. The mixture was centrifuged at 30 psi for 10 minutes in a Beckman Airfuge[15] with an A-100 fixed-angle rotor. The supernatant portion contained only HDL-cholesterol and 0.05 ml of the supernatant was pipetted into a test tube. Into other tubes, 0.05 ml of distilled water (blank), 0.025 ml, and 0.05 ml cholesterol standards were pipetted.

For total cholesterol determination, 0.05 ml of serum, 0.05 ml of distilled water (blank), 0.05 ml, and 0.1 ml of cholesterol standards were pipetted into test tubes. Serum total and HDL-cholesterol were determined by a standard colorimetric method (Stanbio Laboratory Inc., 1973) with the Liebermann-Burchard cholesterol reagent.[15] Three ml of SR Direct Cholesterol Reagent was pipetted into all tubes prepared for HDL-cholesterol and total cholesterol analysis. The tubes were vortexed and incubated in a water bath at 37°C for 20 minutes. The samples and standards were read against the reagent blank at 625 nm on the Spectronic 20 spectrophotometer within 30 minutes of incubation.

The liver and diet residues obtained from wet-ashing were dissolved in 3 ml of 0.6 N HCl and diluted to 25 ml with redistilled water. Copper and zinc contents of appropriate aliquots were determined with an atomic absorption spectrophotometer.[16]

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[14]Beckman Instruments, Inc., Spinco Division, Palo Alto, CA.

[15]Stanbio Laboratory, Inc., San Antonio, Texas.

[16]Model 551, Instrumentation Laboratories, Wilmington, MA.

The concentration of iron in the diets and liver was determined by the method of Parks, Hood, Hurwitz, and Ellis (1943) as modified by Matrone, Peterson, Baxley, and Grinnels (1947). One ml aliquots from liver, diets and iron standards (5 mcg and 10 mcg) was pipetted into 25 ml capacity graduated iron tubes. One ml of 10% hydroxylamine hydrochloride solution and 0.5 ml of O-phenanthroline (1.5% in 95% ethanol) were added to each tube. A small square of congo red indicator paper was added to the mixture, and the pH was adjusted to the alkaline end point with concentrated 50% amonium hydroxide. The solution was made to the 20 ml mark on the iron tube, mixed, and read against a redistilled water blank at 490 nm in a Spectronic 20 spectrophotometer.

Statistical Analyses

Some animals died before the end of the experimental period, and some mineral samples were lost in the ashing process. For the purpose of analysis, an estimated parameter value was used for the missing values. The estimator used the mean of the animals receiving the diet common to the missing animals.

In order to evaluate the first objective, all data collected in Experiments 1 and 2 were subjected to standard analysis of variance techniques (Snedecor & Cochran, 1980). The Least Significant Difference (LSD) and Range Test (Freund, Livermore, & Miller, 1960) were used to determine differences between individual means when significant differences were found between diets. To evaluate the second objective, dietary zinc/copper ratios were calculated by adding the baseline zinc and copper levels to the supplementation levels in each experiment. Out of

the 16 possible dietary zinc/copper ratios for each experiment, there were seven ratios which were common to both experiments. The data on the seven ratios were subjected to trend analysis techniques described by Keppel (1982).

CHAPTER IV

RESULTS AND DISCUSSION

The raw data obtained from this study are presented in Appendix B. Statistical analyses of all data are given in Appendix C. Partial correlation matrix among the variables revealed that the variables had independent effects.

Growth

Weight gains of animals from Experiments 1 and 2 are given in Table 1. Analysis of the data for Experiment 1 (low zinc and copper supplementation) revealed a highly significant ($p \leq 0.01$) zinc effect, but the effect of copper on growth was not statistically significant (Appendix C, Table C-1). Weight gains of the animals increased as supplemental zinc was increased up to 10 ppm. Interpretation of a highly significant ($p \leq 0.01$) zinc x copper interaction indicated that increased levels of supplemental copper were associated with decreased weight gains in young rats fed a zinc supplement of 5 ppm or less. When the diets contained supplemental levels of 10 and 20 ppm of zinc, increased levels of dietary copper had no effect on weight gains.

When the levels of zinc and copper supplements were high (Experiment 2), analysis of the data revealed highly significant ($p \leq 0.01$) effects of zinc and copper on growth (Appendix C, Table C-1). Increasing levels of supplemental zinc or copper were associated with marked increases in weight gains with the maximum weight gains associated with

Table 1

Effects of Zinc and Copper Supplements on Weight Gain of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm) 0	0.56	1.68	5.04	Mean
Experiment 1	Weight Gain (gm)[a]				
0	92 ± 11[b]	84 ± 15	62 ± 5	64 ± 8	76
5	203 ± 14	131 ± 9[b]	137 ± 6	122 ± 2[b]	148
10	206 ± 19[b]	199 ± 12	204 ± 14	218 ± 25[b]	207
20	187 ± 18	204 ± 18	204 ± 19	225 ± 15	205
Mean	172	155	152	157	
	$LSD^{c}_{0.05}$:40				
	$LSD_{0.01}$:53				

	Dietary Copper Supplements (ppm) 0	5.6	16.8	50.4	Mean
Experiment 2	Weight Gain (gm)[d]				
0	100 ± 22[b]	60 ± 11[e]	80 ± 13[e]	66 ± 17[e]	77
50	161 ± 27[f]	207 ± 14	247 ± 9	220 ± 15	209
100	193 ± 17	242 ± 8	228 ± 16	250 ± 16[e]	228
200	195 ± 12	221 ± 13	242 ± 8	235 ± 11	223
Mean	162	183	199	193	
	$LSD^{3}_{0.05}$:38				
	$LSD_{0.01}$:50				

[a]Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b]Mean of 5 animals ± SEM.
[c]Least significant differences at specified probability levels.
[d]Each value is the mean of 7 animals ± SEM unless otherwise indicated.
[e]Mean of 6 animals ± SEM.
[f]Mean of 4 aniamls ± SEM.

100 ppm level of zinc or 16.8 ppm level of copper. A highly significant ($p \leq 0.01$) zinc x copper interaction was also observed in Experiment 2, and it would appear that when no zinc supplement was added to the diets, increasing levels of copper supplementation were associated with decreases in weight gains. When the level of zinc supplementation was increased to at least 50 ppm, increased levels of dietary copper were associated with increases in weight gains.

Hemoglobin

Hemoglobin concentrations obtained from rats in Experiments 1 and 2 are shown in Table 2. Analysis of the data (Appendix C, Table C-2) for Experiment 1 (low zinc and copper supplementation) revealed that increasing levels of supplemental zinc were associated with highly significant decreases ($p \leq 0.01$) in hemoglobin levels. Copper supplementation apparently had no effect on hemoglobin levels of rats in Experiment 1.

When high levels of zinc and copper supplements were added to the diets of young rats (Experiment 2), increasing levels of zinc were associated with highly significant decreases ($p \leq 0.01$) in hemoglobin levels, while increasing levels of copper were generally associated with highly significant increases ($p \leq 0.01$) in hemoglobin levels (Appendix C, Table C-2). Interpretation of a highly significant ($p \leq 0.01$) zinc x copper interaction indicated that the adverse effect of supplemental zinc on hemoglobin levels could be counteracted by increases in dietary copper levels.

Table 2

Effects of Zinc and Copper Supplements on Hemoglobin Levels of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm) 0	0.56	1.68	5.04	Mean
Experiment 1	Hemoglobin (gm/100 ml)[a]				
0	15.0 ± 0.5[b]	14.8 ± 1.4	14.7 ± 0.6	15.0 ± 0.9	14.9
5	13.7 ± 0.6	14.8 ± 1.0[b]	14.0 ± 0.6	15.0 ± 0.3[b]	14.4
10	12.3 ± 0.7[b]	12.7 ± 0.6	13.0 ± 0.4	14.3 ± 0.2[b]	13.1
20	12.8 ± 0.7	11.3 ± 1.1	12.7 ± 0.5	14.0 ± 0.4	12.7
Mean	13.5	13.4	13.6	14.6	
$LSD^{c}_{0.05}$: 2.0					
$LSD_{0.01}$: 2.7					
	Dietary Copper Supplements (ppm) 0	5.6	16.8	50.4	Mean
Experiment 2	Hemoglobin (gm/100 ml)[d]				
0	12.6 ± 1.0[b]	14.8 ± 0.7[e]	13.0 ± 0.4[e]	14.3 ± 0.7[e]	13.7
50	12.0 ± 1.1[f]	13.3 ± 0.6	11.6 ± 0.6	13.0 ± 0.5	12.5
100	10.1 ± 0.6	12.3 ± 0.3	12.4 ± 0.6	12.5 ± 0.4[e]	11.8
200	7.9 ± 0.8	12.7 ± 0.5	12.6 ± 0.2	13.3 ± 0.5	11.6
Mean	10.7	13.2	12.4	13.3	
$LSD^{c}_{0.05}$: 1.5					
$LSD_{0.01}$: 1.9					

[a] Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b] Mean of 5 animals ± SEM.
[c] Least significant differences at specified probability levels.
[d] Each value is the mean of 7 animals ± SEM unless otherwise indicated.
[e] Mean of 6 animsal ± SEM.
[f] Mean of 4 animals ± SEM.

Liver Copper

The effects of zinc and copper supplementation on liver copper deposition in young rats in Experiments 1 and 2 are presented in Table 3. Analysis of the data from Experiment 1 revealed highly significant ($p \leq 0.01$) effects due to zinc and copper supplementations (Appendix C, Table C-3). Apparently, when the zinc supplement was increased up to 10 ppm, a progressive decrease in liver copper deposition was observed. A supplemental level of 20 ppm of zinc, however, resulted in a level of liver copper deposition which was fairly close to the level of copper deposition observed in young rats fed no zinc supplement. As the level of copper supplement of the diet increased, the level of copper deposited in the liver tended to increase.

In Experiment 2, highly significant ($p \leq 0.01$) effects of zinc and copper were noted on liver copper deposition. Although increases in zinc supplements were generally associated with decreases in liver copper deposition, it would appear that the adverse effect of zinc on liver copper was influenced to some degree by the amount of copper supplement in the diet. Increases in liver copper deposition were generally associated with increases in dietary copper levels, although there was some indication that the effect of copper was influenced by the level of zinc in the diet. A highly significant ($p \leq 0.01$) zinc x copper interaction was observed (Appendix C, Table C-3). Low dietary zinc and high dietary copper levels were associated with increased liver copper deposition, while high zinc and low copper levels were associated with decreased liver copper deposition.

Table 3

Effects of Zinc and Copper Supplements on Liver Copper Levels of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm)				
	0	0.56	1.68	5.04	Mean
Experiment 1	Liver Copper (mcg Cu/gm dry weight)[a]				
0	1.11 ± 0.2[b]	1.41 ± 0.3[b]	1.37 ± 0.1	2.07 ± 0.3[b]	1.49
5	0.71 ± 0.1	0.83 ± 0.1[c]	0.91 ± 0.2[b]	1.26 ± 0.2[b]	0.93
10	0.60 ± 0.1[b]	0.67 ± 0.1[b]	0.70 ± 0.1[b]	1.12 ± 0.2[c]	0.77
20	1.01 ± 0.1[b]	0.74 ± 0.1[b]	0.87 ± 0.2[b]	1.06 ± 0.2	0.92
Mean	0.86	0.91	0.96	1.38	
$LSD^{d}_{0.05}$: 0.45					
$LSD_{0.01}$: 0.60					

	Dietary Copper Supplements (ppm)				
	0	5.6	16.8	50.4	Mean
Experiment 2	Liver Copper (mcg Cu/gm dry weight)[e]				
0	0.88 ± 0.2[b]	2.05 ± 0.2[f]	1.35 ± 0.2[f]	2.45 ± 0.2[b]	1.68
50	0.76 ± 0.2[c]	1.14 ± 0.1	1.73 ± 0.1	1.73 ± 0.2	1.34
100	0.50 ± 0.1	1.08 ± 0.1	1.71 ± 0.1	1.42 ± 0.1[f]	1.63
200	0.50 ± 0.1	1.17 ± 0.1	1.52 ± 0.1	1.70 ± 0.1	1.22
Mean	0.66	1.36	1.58	1.83	
$LSD^{d}_{0.05}$: 0.37					
$LSD_{0.01}$: 0.49					

[a] Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b] Mean of 5 animals ± SEM.
[c] Mean of 4 animals ± SEM.
[d] Least significant differences at specified probability levels.
[e] Each value is the mean of 7 animals ± SEM unless otherwise indicated.
[f] Mean of 6 animals ± SEM.

Liver Iron

Liver iron deposition in young rats fed the various dietary regimens used in this study is given in Table 4. Increases in low levels of dietary zinc supplements (Experiment 1) resulted in highly significant decreases ($p \leq 0.01$) in liver iron levels of young rats, while increases in low levels of copper supplements had no apparent effect on liver iron deposition in this experiment (Appendix C, Table C-4).

Increasing levels of high zinc or copper supplements (Experiment 2) were associated with highly significant ($p \leq 0.01$) decreases in liver iron deposition (Appendix C, Table C-4). A highly significant ($p \leq 0.01$) zinc x copper interaction was also observed from the analysis of the data from Experiment 2. Zinc apparently had a greater effect on liver iron deposition than did copper, but a combination of both minerals at increasing level in the diet appeared to have a synergistic effect on liver iron deposition which resulted in more marked reductions in liver iron levels.

Liver Zinc

Liver zinc levels of the animals fed the various dietary regimens used in this study are given in Table 5. Analysis of the data from Experiment 1 revealed a highly significant ($p \leq 0.01$) increase in liver zinc deposition associated with increases in dietary zinc (Appendix C, Table C-5). This effect of dietary zinc, however, appeared to occur only with the 20 ppm zinc supplement and when the diet also contained at least a copper supplement of 0.56 ppm. The copper effect was not significant in this experiment.

Table 4

Effects of Zinc and Copper Supplements on Liver Iron Levels of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm)				
	0	0.56	1.68	5.04	Mean
Experiment 1	Liver iron (mcg Fe/gm dry weight)[a]				
0	1297 ± 206[b]	1435 ± 354[b]	1316 ± 333	1048 ± 283[b]	1274
5	1010 ± 76	1487 ± 135[c]	1179 ± 106[b]	964 ± 243[b]	1160
10	1039 ± 96[b]	771 ± 137[b]	880 ± 177[b]	807 ± 212[c]	874
20	1006 ± 110[b]	896 ± 173[b]	950 ± 163[b]	698 ± 56	887
Mean	1088	1147	1081	879	
$LSD^{d}_{0.05}$	484				
$LSD_{0.01}$	644				

	Dietary Copper Supplements (ppm)				
	0	5.6	16.8	50.4	Mean
Experiment 2	Liver Iron (mcg Fe/gm dry weight)[e]				
0	1329 97[b]	1087 ± 80[f]	1144 ± 193[f]	1021 ± 143[b]	1145
50	1140 ± 320[c]	514 ± 32	429 ± 34	427 ± 24	628
100	905 ± 152	602 ± 50	459 ± 33	398 ± 476	591
200	689 ± 45	496 ± 45	343 ± 28	377 ± 25	476
Mean	1016	675	594	556	
$LSD^{d}_{0.05}$	251				
$LSD_{0.01}$	333				

[a]Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b]Mean of 5 animals ± SEM.
[c]Mean of 4 animals ± SEM.
[d]Least significant differences at specified probability levels.
[e]Each value is the mean of 7 animals ± SEM unless otherwise indicated.
[f]Mean of 6 animals ± SEM.

Table 5

Effects of Zinc and Copper Supplements on Liver Zinc Levels of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm)				
	0	0.56	1.68	5.04	Mean
Experiment 1	Liver Zinc (mcg Zn/gm dry weight)[a]				
0	10.1 ± 0.5[b]	11.6 ± 2.9[b]	9.8 ± 0.9	11.0 ± 1.3[b]	10.6
5	8.7 ± 0.8	9.7 ± 1.0[c]	9.3 ± 0.6[b]	8.1 ± 0.9[b]	11.9
10	9.6 ± 0.7[b]	8.6 ± 0.8[b]	9.1 ± 0.5[b]	9.9 ± 0.9[c]	9.3
20	10.4 ± 0.3[b]	29.4 ± 12.8[b]	17.2 ± 7.6[b]	29.3 ± 13.7	21.6
Mean	9.7	14.8	11.4	14.6	
$LSD^{d}_{0.05}$	14.48				
$LSD_{0.01}$	19.26				

	Dietary Copper Supplements (ppm)				
	0	5.6	16.8	50.4	Mean
Experiment 2	Liver Zinc (mcg Zn/gm dry weight)[e]				
0	9.8 ± 0.9[b]	11.5 ± 0.6[f]	11.3 ± 1.5[f]	11.3 ± 1.1[b]	11.0
50	35.0 ± 19.7[c]	17.3 ± 8.3	36.8 ± 10.8	38.6 ± 10.1	31.9
100	24.3 ± 8.8	15.2 ± 5.5	41.1 ± 11.6	15.4 ± 6.1[f]	24.0
200	26.1 ± 10.6	34.5 ± 11.6	45.2 ± 10.4	50.4 ± 11.5	39.1
Mean	23.8	19.6	33.6	28.9	
$LSD^{d}_{0.05}$	22.75				
$LSD_{0.01}$	30.17				

[a]Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b]Mean of 5 animals ± SEM.
[c]Mean of 4 animals ± SEM.
[d]Least significant differences at specified probability levels.
[e]Each value is the mean of 7 animals ± SEM unless otherwise indicated
[f]Mean of 6 animals ± SEM.

Analysis of the data from Experiment 2 revealed that the overall effects of zinc and copper on liver zinc deposition were highly significant ($p \leq 0.01$) (Appendix C, Table C-5). In the rats fed diets containing 0 or 5.6 ppm copper supplements, increases in dietary zinc from 0 to 200 ppm had no marked effect on liver zinc levels, but when supplemental levels of 16.8 or 50.4 ppm of copper were included in the diet, increasing levels of supplemental zinc were associated with marked increases in liver zinc deposition. The increases in liver zinc deposition associated with increases in dietary copper also appeared to occur only when the level of dietary zinc supplement was at least 200 ppm.

Serum Triglyceride Levels

Serum triglyceride values of young male rats fed the various dietary regimens used in Experiments 1 and 2 are given in Table 6. When low zinc and copper supplements were used in diets, increasing the zinc supplements from 0 to 10 ppm, resulted in highly significant ($p \leq 0.01$) increases in serum triglyceride levels in young rats which tended to level off or even decrease as the level of zinc supplement was increased to 20 ppm. Increases in dietary copper apparently had no significant effect on serum triglycerides in young rats in Experiment 1. Serum triglyceride levels of young rats did not appear to be affected when high levels of zinc or copper supplements were added to the diets, again suggesting that levels of dietary zinc higher than 20 ppm had no effect on this parameter (Appendix C, Table C-6).

Table 6

Effects of Zinc and Copper Supplements on Serum Triglyceride Levels of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm)				
	0	0.56	1.68	5.04	Mean
Experiment 1	Serum Triglyceride (mg/100 ml)[a]				
0	29 ± 6[b]	24 ± 4	26 ± 4	16 ± 3	24
5	44 ± 5	33 ± 7[b]	42 ± 8	24 ± 5[b]	36
10	52 ± 8[b]	41 ± 6	44 ± 7	60 ± 9[b]	49
20	33 ± 9	46 ± 9	48 ± 9	44 ± 7	43
Mean	40	36	40	36	
$LSD^{c}_{0.05}$: 17					
$LSD_{0.01}$: 23					

	Dietary Copper Supplements (ppm)				
	0	5.6	16.8	50.4	Mean
Experiment 2	Serum Triglyceride (mg/100 ml)[d]				
0	41 ± 13[b]	26 ± 6[e]	31 ± 3[e]	31 ± 16[b]	30
50	21 ± 6[f]	37 ± 9	52 ± 12	50 ± 7	40
100	41 ± 6	45 ± 7	55 ± 15	47 ± 12[e]	47
200	42 ± 6	58 ± 13	58 ± 13	42 ± 8	50
Mean	37	42	49	43	
$LSD^{c}_{0.05}$: 25					
$LSD_{0.01}$: 33					

[a] Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b] Mean of 5 animals ± SEM.
[c] Least significant differences at specified probability levels.
[d] Each value is the mean of 7 animals ± SEM unless otherwise indicated.
[e] Mean of 6 animals ± SEM.
[f] Mean of 4 animals ± SEM.

Serum Total Cholesterol

The effects of zinc and copper supplements on serum total cholesterol levels of young rats from Experiments 1 and 2 are shown in Table 7. Increases in low levels of supplemental zinc (5 to 20 ppm) or low levels of supplemental copper (0.56 to 5.04 ppm) had no significant effect on serum total cholesterol in this study. High levels of zinc supplementation (50 to 200 ppm) also had no significant effect on serum total cholesterol levels of young rats, but high levels of copper supplements (5.6 to 50.4 ppm) were associated with a highly significant ($p \leq 0.01$) decrease in serum total cholesterol levels in this study (Appendix C, Table C-7).

Serum HDL-Cholesterol

Table 8 contains serum HDL-cholesterol values obtained from rats in Experiments 1 and 2. As was observed with total serum cholesterol, the only effect of dietary supplements on HDL-cholesterol was observed when dietary copper supplements of 5.6 to 50.4 ppm were used. In this instance, increasing the level of copper in the diets of rats in Experiment 2 resulted in highly significant ($p \leq 0.01$) decreases in HDL-cholesterol levels in the serum. Thus, it would appear that copper had a greater effect on HDL-cholesterol than did zinc (Appendix C, Table C-8).

Zinc/Copper Ratios

The effects of zinc/copper ratios as provided by low and high levels of zinc and copper supplementation on all parameters tested in the study are given in Table 9. Analysis of the combined data revealed that the weight gains of animals fed the high absolute zinc and copper

Table 7

Effects of Zinc and Copper Supplements on Serum Total Cholesterol Levels of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm)				
	0	0.56	1.68	5.04	Mean
Experiment 1	Serum Total Cholesterol (mg/100 ml)[a]				
0	70 ± 11[b]	73 ± 9	79 ± 5	74 ± 6	74
5	91 ± 6	85 ± 7[b]	80 ± 5	92 ± 9[b]	87
10	88 ± 8[b]	84 ± 6	92 ± 11	87 ± 10[b]	88
20	64 ± 6	91 ± 5	90 ± 6	82 ± 5	82
Mean	78	83	85	84	
$LSD^{c}_{0.05}$	20				
$LSD_{0.01}$	27				

	Dietary Copper Supplements (ppm)				
	0	5.6	16.8	50.4	Mean
Experiment 2	Serum Total Cholesterol (mg/100 ml)[d]				
0	81 ± 4[b]	71 ± 7[e]	78 ± 8[e]	82 ± 9[b]	78
50	74 ± 8[f]	86 ± 7	74 ± 3	76 ± 6	78
100	103 ± 5	68 ± 4	75 ± 10	66 ± 6[e]	78
200	86 ± 6	81 ± 6	75 ± 5	72 ± 6	79
Mean	86	77	76	74	
$LSD^{c}_{0.05}$	16				
$LSD_{0.01}$	21				

[a]Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b]Mean of 5 animals ± SEM.
[c]Least significant differences at specified probability levels.
[d]Each value is the mean of 7 animals ± SEM unless otherwise indicated.
[e]Mean of 6 animals ± SEM.
[f]Mean of 4 animals ± SEM.

Table 8

Effects of Zinc and Copper Supplements on Serum HDL-Cholesterol Levels of Young Male Rats

Dietary Zinc Supplements (ppm)	Dietary Copper Supplements (ppm)				
	0	0.56	1.68	5.04	Mean
Experiment 1	Serum HDL-Cholesterol (mg/100 ml)[a]				
0	58 ± 8[b]	60 ± 9[b]	65 ± 5	54 ± 4[b]	59
5	59 ± 10[b]	71 ± 4[b]	65 ± 4	64 ± 6[b]	65
10	71 ± 8[b]	65 ± 7	69 ± 12	74 ± 10[b]	69
20	55 ± 6	70 ± 5	72 ± 7	65 ± 5	65
Mean	61	67	68	67	
$LSD^{c}_{0.05}$: 20					
$LSD_{0.01}$: 27					

	Dietary Copper Supplements (ppm)				
	0	5.6	16.8	50.4	Mean
Experiment 2	Serum HDL-Cholesterol (mg/100 ml)[d]				
0	67 ± 4[b]	42 ± 5[e]	56 ± 7[e]	54 ± 9[b]	55
50	55 ± 10[f]	67 ± 7	59 ± 4	53 ± 4	59
100	84 ± 5	59 ± 5	50 ± 4	57 ± 4	63
200	72 ± 7	62 ± 4	56 ± 4	53 ± 5	61
Mean	70	58	55	54	
$LSD^{c}_{0.05}$: 15					
$LSD_{0.01}$: 19					

[a] Each value is the mean of 6 animals ± SEM unless otherwise indicated.
[b] Mean of 5 animals ± SEM.
[c] Least significant differences at specified probability levels.
[d] Each value is the mean of 7 animals ± SEM unless otherwise indicated.
[e] Mean of 6 animals ± SEM.
[f] Mean of 4 animals ± SEM.

Table 9

Effect of Zinc/Copper Ratios and Level of Zinc and Copper Supplementation on Parameters.

Level of Supplementation	Zinc/Copper Ratio							
	1	2	3	4	6	12	35	Mean
	Weight Gain (gm)							
Low zinc and copper supplement[a]	122 ± 2	218 ± 25[b]	137 ± 6	225 ± 15[c]	131 ± 9[b]	199 ± 12	206 ± 19	178
High zinc and copper supplement[b]	220 ± 15	250 ± 16[c]	247 ± 9	235 ± 11	228 ± 16	242 ± 9	221 ± 13	235
Mean	171	235	192	230	180	221	213	
	Hemoglobin (gm/100 ml)							
Low zinc and copper supplement[a]	15.0 ± 0.3	14.4 ± 0.2[b]	14.0 ± 0.6	14.0 ± 0.4[c]	14.8 ± 1.0[b]	12.7 ± 0.6	12.4 ± 0.7	13.9
High zinc and copper supplement[b]	13.0 ± 0.5	12.5 ± 0.4[c]	11.6 ± 0.5	13.3 ± 0.5	12.4 ± 0.6	12.6 ± 0.2	12.7 ± 0.5	12.6
Mean	14.0	13.5	12.8	13.7	13.6	12.7	12.6	
	Liver Copper (mcg Cu/gm Dry Weight							
Low zinc and copper supplement[a]	1.3 ± 0.2	1.1 ± 0.2[b]	0.9 ± 0.2	1.1 ± 0.2[c]	0.8 ± 0.1[b]	0.7 ± 0.1	0.6 ± 0.1	0.9
High zinc and copper supplement[b]	1.7 ± 0.2	1.4 ± 0.1[c]	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	1.6
Mean	1.49	1.27	1.32	1.38	1.27	1.09	0.89	

Level of Supplementation	Zinc/Copper Ratio														Mean
	1		2		3		4		6		12		35		
	Liver Iron (mcg Fe/gm Dry Weight)														
Low zinc and copper supplement[a]	964	± 243	807	± 212[b]	1179	± 106	698	± 56[c]	1487	± 135[b]	771	± 137	1039	± 96	992
High zinc and copper supplement[d]	427	± 24	398	± 47[c]	429	± 34	377	± 26	459	± 33	343	± 28	496	± 45	419
Mean	696		603		804		537		973		557		767		
	Liver Zinc (mcg Zn/gm Dry Weight)														
Low zinc and copper supplement[a]	8.1	± 0.9	9.9	± 0.9[b]	9.3	± 0.6	29.2	± 13[c]	9.7	± 1[b]	8.6	± 0.8	9.6	± 0.7	12.7
High zinc anc copper supplement[d]	38.6	± 10	15.4	± 6.1[c]	36.8	± 11	50.4	± 12	41.1	± 12	45.2	± 10	34.5	± 12	37.9
Mean	23.4		12.7		23.1		39.8		25.4		26.9		22.1		
	Serum Triglyceride (mg/100 ml)														
Low zinc and copper supplement[a]	24	± 5	61	± 9[b]	42	± 8	44	± 7[c]	33	± 5[b]	40	± 7	52	± 8	42
High zinc and copper supplement[d]	50	± 6	45	± 13[c]	52	± 12	42	± 8	55	± 16	56	± 13	58	± 13	51
Mean	37		53		47		43		44		48		55		

Level of Supplementation	Zinc/Copper Ratio							
	1	2	3	4	6	12	35	Mean
	Serum Total Cholesterol (mg/100 ml)							
Low zinc and copper supplement[a]	92 ± 9	87 ± 10[b]	80 ± 5	82 ± 8[c]	85 ± 7[b]	84 ± 6	88 ± 8	85
High zinc and copper supplement[d]	76 ± 4	66 ± 6[c]	74 ± 3	72 ± 6	75 ± 10	75 ± 5	81 ± 7	74
Mean	84	77	77	77	80	80	85	
	Serum HDL-Cholesterol (mg/100 ml)							
Low zinc and copper supplement[a]	64 ± 6	74 ± 10[b]	65 ± 4	65 ± 5	71 ± 4[b]	65 ± 7	71 ± 8	68
High zinc and copper supplement[d]	53 ± 4	57 ± 4[c]	59 ± 4	53 ± 5	50 ± 4	56 ± 4	62 ± 4	56
Mean	59	66	62	59	61	61	67	

[a]Each value is the mean of 5 animals ± SEM unless otherwise indicated.

[b]Mean of 4 animals ± SEM.

[c]Mean of 6 animals ± SEM.

[d]Each value is the mean of 7 animals ± SEM unless otherwise indicated.

supplements were significantly higher ($p \leq 0.01$) than those fed the low zinc and copper supplements (Appendix C, Table C-9). The zinc/copper ratio had a highly significant ($p \leq 0.01$) effect on weight gains. When low levels of zinc and copper supplements were used in the diets, marked improvements in weight gains were associated with rats receiving zinc/copper ratios of 2, 4, 12, and 35. With the high levels of zinc and copper supplementation, the influence of zinc/copper ratio did not appear to have much effect on growth (Appendix C, Table C-10).

Hemoglobin levels of young rats fed low levels of zinc and copper supplements were significantly ($p \leq 0.01$) higher than the levels of young rats fed high zinc and copper supplements (Appendix C, Table C-9). Increasing the zinc/copper ratio from 1 to 35 was associated with progressive decreases in hemoglobin levels of rats fed low levels of zinc and copper supplements. The highest level of hemoglobin was observed in rats fed the low zinc and copper supplements with a zinc copper ratio of one. Zinc/copper ratio had no significant effect on hemoglobin levels of rats fed the high zinc and copper supplements (Appendix C, Table C-10).

Higher levels of copper were found in the livers of rats fed the high zinc and copper supplements than were found in rats fed the lower supplemental levels of these two minerals (Table 9). Such an increase in liver copper deposition in rats from Experiment 2 would be expected since more dietary copper would have been available for tissue incorporation. Increases in the zinc/copper ratios were associated with decreases in liver copper deposition. The maximum liver copper deposition was observed when the ratio of zinc/copper was one. It would appear that

zinc/copper ratios were more critical when low supplemental levels of zinc and copper were used than when high levels of these supplements were used in the diets.

Higher liver iron levels were observed in animals fed low supplemental levels of zinc and copper than were found in animals fed diets containing high supplemental levels of these two minerals (Table 9). The zinc/copper ratio appeared to be more critical when low supplemental levels of zinc and copper were in the diets than when the diets contained fairly high concentrations of these supplements. The highest levels of iron deposition were observed in rats fed low zinc and copper supplements maintained on a ratio of six while the lowest liver iron levels were noted in animals in Experiment 1 when the zinc/copper ratio was four. Liver iron deposition in rats fed the high zinc and copper supplements were essentially the same magnitude, regardless of zinc/copper ratio.

Liver zinc levels of animals fed high zinc supplements were significantly ($p \leq 0.01$) higher than liver zinc levels found in rats fed the low zinc supplements (Appendix C, Table C-9). With the exception of a zinc/copper ratio of four, increasing this ratio in the diet had no marked effects on liver zinc levels of animals fed either the low or high supplemental levels of zinc and copper (Appendix C, Tables C-9 and C-10). A zinc/copper ratio of four was associated with an approximately three-fold increase in liver zinc levels in animals fed the low mineral supplements and approximately a 33% increase in liver zinc level in animals fed the high mineral supplements.

Increases in zinc/copper ratios generally appeared to have little influence on serum triglyceride levels, total cholesterol levels, or

HDL-cholesterol levels in young rats fed either the low or high supplemental levels of zinc and copper (Table 9). Serum triglyceride levels of animals from each experiment were approximately the same magnitude. Total cholesterol and HDL-cholesterol levels of animals fed the low supplemental levels of zinc or copper tended to be higher than corresponding values observed in animals fed high supplemental levels of zinc or copper.

Hair Coat and Skin Appearance

Hair loss and dermatitis over the body were noticed in some rats by the third week of the experimental period in both Experiments 1 and 2. As the length of the experimental period increased, hair loss became extensive, and the dermatitis developed into lesions on the legs, around the mouth, and the eyes and resulted in blindness in some of the rats. In both Experiments 1 and 2, rats that suffered skin lesions exhibited little or no hair loss, while those that exhibited extensive hair loss developed little or no skin lesions. Generally, rats fed the 0 ppm zinc supplemented diet regardless of the level of dietary copper developed skin lesions, while those fed high levels of zinc exhibited excessive hair losses (Appendix B, Tables B-9 and B-10).

CHAPTER V

GENERAL DISCUSSION

Results of this study indicated that when young rats were fed levels of zinc and copper likely to occur in a North American mixed diet, the levels supported growth in the animals. Growth, however, was significantly ($p \leq 0.01$) increased when the dietary levels of both minerals were increased in the diet. Based on the results of this, it would appear that a minimum zinc level of 10.34 ppm and a copper level of 0.30 ppm (the amount of copper in the nonsupplemented basal diet) were adequate to support growth in the rats. This observation supports the findings of Forbes and Yohe (1960) who reported that growing rats fed casein or egg white diets require a minimum of 12 ppm zinc. There is also an indication from this study that considering the zinc/copper ratios tested in this study, a ratio of four may be required for optimum growth of young rats when levels of dietary zinc and copper were less than 20 ppm and 5.04 ppm, respectively. Sinthusek and Magee (1984) reported that optimum growth was observed in young rats when the zinc/copper ratio was five and the levels of dietary zinc and copper were 7.5 ppm and 1.5 ppm, respectively. This result is similar to the zinc/copper ratio of four obtained in this study. The dietary zinc/copper ratio does not appear to affect weight gain at higher dietary levels of the minerals.

Decreases in hemoglobin levels associated with increased dietary zinc levels observed in this study were similar to previous findings

(Magee & Grainger, 1979; Motsinger & Magee, 1980) and was probably being mediated through an effect on copper and/or iron metabolism. The adverse effect of zinc on hemoglobin formation could be the result of the effect of this mineral on copper and iron status of the system. Settlemire and Matrone (1967) also reported that zinc interferes with the incorporation of iron into ferritin and the release of iron from ferritin for hemoglobin formation. The maximum hemoglobin formation was observed when dietary zinc/copper ratio was one.

Zinc supplementation was associated with decreases in the deposition of liver copper which is in agreement with previous findings (Fisher et al., 1980; Motsinger & Magee, 1980). The adverse effect of dietary zinc on liver copper deposition was offset when dietary copper levels were increased. Increasing the level of dietary copper resulted in marked increases in liver copper deposition in the animals. The maximum liver copper deposition was observed when the ratio of dietary zinc to dietary copper was one.

The availability of dietary iron for liver deposition appeared to be adversely affected by increases in dietary zinc alone when the animals were fed low zinc and copper levels. Similar results were obtained by Son (1984). At higher dietary zinc and copper levels, both minerals adversely affected liver iron deposition. Liver iron deposition in the rats was most enhanced when dietary zinc/copper ratio was six.

Zinc supplementation was associated with increased liver zinc deposition. Addition of copper to the diet appeared to increase liver zinc deposition. This is contrary to the results obtained by Underwood

(1971) who reported that high dietary intakes of copper aggravated signs of zinc deficiency in pigs. The contradition in the results obtained in this study and those reported by Underwood (1977) may be explained by the different dietary copper levels used in each study. Dietary copper levels used in this study ranged from 0.56 ppm to 50.4 ppm while the levels reported by Underwood (1971) ranged from 125 ppm to 250 ppm. Although dietary zinc/copper ratio had no significant effect on liver zinc deposition probably due to the large intragroup variability, it would appear that liver zinc deposition was enhanced when the dietary zinc/copper ratio was four.

The lowest serum triglyceride level was observed when the diet was not supplemented with zinc. Adding 5 ppm zinc to the basal diet was associated with a highly significant ($p \leq 0.01$) increase in serum triglyceride levels. Dietary copper had no significant effect on triglyceride levels in this study. These results are similar to previous findings of Koo and Williams (1981) but contradict the findings of others (Petering et al., 1977; Woo & Gibbs, 1981). Koo and Williams (1981) reported that a dietary zinc level of 0.37 ppm was associated with decreased serum triglyceride level. Increasing dietary zinc level to 41 ppm resulted in a significant ($p \leq 0.05$) increase in serum triglyceride level. Petering and co-workers (1977), on the other hand, reported that increasing dietary zinc and copper levels resulted in decreased triglyceride values while Woo and Gibbs (1981) did not find any significant effect of dietary zinc levels (ranging from 10 ppm to 500 ppm) on serum triglyceride, total cholesterol, or HDL-cholesterol levels in rats.

Dietary zinc/copper ratios had no effect on triglyceride levels in this study. This result is in agreement with the previous report of Fisher et al. (1980).

Dietary zinc and copper levels likely to occur in a normal mixed diet had no significant effect on serum total cholesterol and HDL-cholesterol levels of young rats. When the animals were fed higher levels of zinc and copper, dietary zinc had no significant effect on total cholesterol and HDL-cholesterol levels, but the addition of 5.6 ppm copper to the baseline diet resulted in a significant ($p \leq 0.05$) decrease in total cholesterol and HDL-cholesterol levels. Further increases in dietary copper did not result in significant changes in total cholesterol and HDL-cholesterol levels. These results support previous findings (Murthy & Petering, 1976; Petering et al., 1977), but contradicts the findings of others (Koo & Ramlet, 1983; Koo & Williams, 1981). Dietary zinc/copper ratios had no significant effects on serum total cholesterol and HDL-cholesterol levels in this study. This is in agreement with previous findings (Caster & Doster, 1979; Fisher et al., 1981; Helwig et al., 1978; Woo & Gibbs, 1981), but contradicts the findings of others (Allen & Klevay, 1978; Klevay, 1973).

This study does not reveal the exact causes leading to extensive hair loss and the development of skin lesions in some of the animals fed the experimental diets. Skin lesions developed in the rats fed the 0 ppm supplemented zinc diets regardless of the level of dietary copper. According to Underwood (1977), zinc deficiency in rats is characterized by coarseness, dermatitis, scaling, and cracking of the paws. In severe

zinc deficiency, the legs become tender, easily injured and often raw and bleeding. These symptoms were observed in only the rats fed the non-zinc supplemented diets. The development of skin lesions may be a manifestation of the possible role of zinc in tissue synthesis since zinc is essential for protein and nucleic acids metabolism. Many of the animals with extensive hair loss were from the high zinc supplemented group. The mechanism by which high zinc levels may be associated with extensive hair loss is not apparent in this study.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

Young male rats were fed diets consisting of four levels of zinc and four levels of copper. The study was conducted in two phases. In Experiment 1 animals were fed low dietary zinc and copper levels, while in Experiment 2 animals were fed high dietary zinc and copper levels. Criteria used to evaluate the responses of the animals to the various test diets included weight gain, hemoglobin level, liver copper, iron and zinc deposition, and triglyceride, total cholesterol and HDL-cholesterol levels in the serum. The effects of zinc/copper ratios on the parameters were evaluated by analyzing treatments from Experiments 1 and 2 with the same ratios but different zinc and copper levels.

Rats fed diets containing high levels of zinc and copper had significantly ($p \leq 0.01$) higher weight gains than rats fed low levels of zinc and copper. The data suggested that a minimum zinc level of 10 ppm and a copper level of 0.30 ppm were adequate for optimal growth of young rats. When the levels of dietary zinc and copper were less than 20 ppm and 5.04 ppm, respectively, zinc/copper ratio of four was associated with optimal growth in this study. At higher levels of supplementation, zinc/copper ratios did not affect growth.

Increasing levels of dietary zinc were associated with highly significant ($p \leq 0.01$) decreases in hemoglobin levels, while increases

in dietary copper resulted in highly significant ($p \leq 0.01$) increases in hemoglobin levels. Maximum hemoglobin levels were observed when the zinc/copper ratio was one.

Zinc supplementation significantly ($p \leq 0.01$) increased liver zinc deposition but was associated with significant ($p \leq 0.01$) decreases in the deposition of either copper or iron in the liver. Copper supplementation was associated with significant ($p \leq 0.01$) increases in liver copper deposition, a trend in increase in liver zinc deposition and significant ($p \leq 0.01$) decreases in liver iron deposition. The maximum deposition of liver zinc was observed in the rats when dietary zinc level was 100 ppm or 200 ppm and dietary copper level was 16.8 ppm. Maximum liver copper deposition was observed when the dietary copper level was 50.4 ppm and zinc supplement was 0 ppm. Liver iron deposition was enhanced when the levels of both zinc and copper were low. A zinc/copper ratio of four appeared to enhance liver zinc deposition while a ratio of one enhanced liver copper deposition.

Lowest serum triglyceride levels were observed in rats when diets were not supplemented with zinc. The addition of 5 ppm zinc to the basal diet was associated with a highly significant ($p \leq 0.01$) increase in serum triglyceride level. Further increases in dietary zinc did not affect serum triglyceride levels. Dietary copper had no significant effect on triglyceride levels.

Serum total cholesterol and HDL-cholesterol levels were not affected by dietary zinc levels. The addition of 5.6 ppm copper to the baseline diet resulted in a significant ($p \leq 0.01$) decrease in total

cholesterol and HDL-cholesterol levels. Further increases in dietary copper did not result in significant changes in total cholesterol and HDL-cholesterol levels. Dietary zinc/copper ratios had no significant effect on serum lipids.

As the experimental period progressed, extensive hair loss or skin lesions were observed in some of the rats. Animals fed the non-supplemented zinc diet developed skin lesions on the legs, around the mouth and the eyes resulting in blindness in some of the rats. Animals fed the high zinc diets exhibited extensive hair losses.

It would appear from this study that although zinc/copper ratios, as well as the absolute levels of zinc and copper may be important in growth, hemoglobin formation, and in liver copper, iron and zinc deposition, the ratios do not affect serum lipid levels. There is the indication that the absolute levels of the minerals in the diet may be more important in lipid metabolism.

Recommendations

The present study used a "nonatherogenic" diet with no cholesterol. Future studies should include cholesterol in all of the experimental diets since it may be a better approximation of the Western diet than diets which are totally devoid of cholesterol.

The biochemical explanation for the close association between the development of skin lesions and consumption of zinc-deficient diet is not readily available from the information obtained in this study. In view of the suggested role of zinc in protein synthesis, and the role of vitamin B_{12} and folic acid in cellular reproduction, future studies

should investigate the possible interrelationship of these nutrients in growth and wound healing.

BIBLIOGRAPHY

Allen, K., & Klevay, L. (1978a). Cholesterolemia and cardiovascular abnormalities in rats caused by copper deficiency. Atherosclerosis, 29, 81-93.

Allen, K., & Klevay, L. (1978b). Cholesterol metabolism in copper deficient rats. Life Science, 22, 1691-1698.

Allen, K., & Klevay, L. (1978c). Copper deficiency and cholesterol metabolism in the rat. Atherosclerosis, 31, 259-261.

Allen, K., & Klevay, L. (1980). Hyperlipoproteinemia in rats due to copper deficiency. Nutrition Reports International, 22, 295-299.

American Psychological Association. (1983). Publication manual of the American psychological association (3rd ed.). Washington, DC: Author.

Amine, E. K., & Hegsted, D. M. (1975). Effect of dietary carbohydrates and fats on inorganic iron absorption. Journal of Agricultural Food Chemistry, 23, 205-208.

Anon, A. (1969). Vital statistics of the United States: 1967 mortality. Washington, DC: U.S. Department of Health, Education and Welfare.

Babatunde, G. M. (1972). Optimum levels of zinc in the diets of pigs in the tropics as influenced by the addition of graded levels of land. Journal of Science of Food and Agriculture, 23, 113-120.

Bettger, W. J., Reeves, P. G., Moscatelli, E. A., Reynolds, G., & O'Dell, B. L. (1979). Interaction of zinc and essential fatty acids in the rat. Journal of Nutrition, 109, 480-488.

Bettger, W. J., Reeves, P. G., Moscatelli, E. A., Savage, J. E., & O'Dell, B. L. (1980). Interaction of zinc and polyunsaturated fatty acids in the chick. Journal of Nutrition, 110, 50-58.

Caster, W. O., & Doster, J. M. (1979). Effect of the dietary zinc/copper ratio on plasma cholesterol level. Nutrition Reports International, 19, 773-775.

Doisy, E. A. (1972). Trace Elements in environmental health. Columbia: University of Missouri Press.

Dumaswala, U. J. (1970). Effect of dietary fats and carbohydrates on serum lipids in the rat. Lancet, 2, 723-725.

Fisher, P. W. F., Giroux, A., Belonje, B., & Shah, B. G. (1980). The effect of dietary copper and zinc on cholesterol metabolism. American Journal of Clinical Nutrition, 33, 1019-1025.

Forbes, R. M., & Yohe, M. (1960). Zinc requirement and balance studies with the rats. Journal of Nutrition, 70, 53-57.

Freund, J. E., Livermo-e, P. E., & Miller, I. (1960). Manual of experimental statistics. Englewood Cliffs, NJ: Prentice-Hall.

Friend, B. (1967). Nutrients in United States food supply: A review of trends, 1909-1913 to 1965. American Journal of Clinical Nutrition, 20, 907-914.

Geders, J. M. (1979). Relationship between zinc and copper and nutritional status and risk factors associated with cardiovascular disease. Federation Proceedings, 38, 552a.

Gielgel, J. L., Ham, A. B., & Glema, W. (1975). Manual and semi-automated procedures for measurement of triglycerides in serum. Clinical Chemistry, 21, 1575-1581.

Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B., & Dawber, T. R. (1977). High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. American Journal of Medicine, 62, 707-714.

Guthrie, B., & Robinson, M. F. (1977). Daily intake of manganese, copper, zinc, and cadmium by New Zealand women. British Journal of Nutrition, 38, 55-63.

Hambidge, K. M. (1977). The role of zinc and other trace metals in pediatric nutrition and health. Pediatric Clinic of North America, 24, 95-106.

Helwig, L. R., Mulnix, E. J., & Regenstein, J. M. (1978). Effects of varied zinc/copper ratios on egg and plasma cholesterol level in white leghorn hens. Journal of Food Science, 43, 666-669.

Holden, J. M., Wolf, W. R., & Mertz, W. (1979). Zinc and copper in self-selected diets. Journal of the American Dietetic Association, 75, 23-28.

Hooper, P. L. (1980). Zinc lowers high-density lipoprotein cholesterol levels. Journal of the American Medical Association, 244, 1960-1961.

Hunt, I. F., Murphy, N. J., Gomez, J., & Smith, J. C. (1979). Dietary zinc intake of low-income pregnant women of Mexican decent. American Journal of Clinical Nutrition, 32, 1511-1517.

Iacono, J. M. (1974). Effect of varying the dietary level of calcium on plasma and tissue lipids of rabbits. Journal of Nurition, 104, 1165-1171.

Kannel, W. B. (1971). The disease of living. Nutrition Today, 6(3), 2-5.

Keppel, G. (1982). Design and analysis: A researchers handbook (2nd ed.). Englewood Cliffs, NJ: Prentice-Hall.

Keys, A. (1957). Diet and the epidemiology of coronary heart disease. Journal of the American Medical Association, 164, 1912-1919.

Keys, A., Anderson, J. T., & Grande, F. (1960). Diet-type (fat constant) and blood lipids in man. Journal of Nutrition, 70, 257-266.

Klevay, L. M. (1973). Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. American Journal of Clinical Nutrition, 26, 1060-1068.

Klevay, L. M. (1975). Coronary heart disease: The zinc/copper hypothesis. American Journal of Clinical Nutrition, 28, 764-774.

Klevay, L. M., Reck, S. J., & Barcome, D. F. (1979). Evidence of dietary copper and zinc deficiency. Journal of the American Medical Association, 241, 1916-1918.

Koo, S. I., & Ramlet, J. S. (1983). Dietary cholesterol decreases the serum level of zinc: Further evidence for the positive relationship between serum zinc and high-density lipoproteins. American Journal of Clinical Nutrition, 37, 918-923.

Koo, S. I, & Williams, D. A. (1981). Relationship between the nutritional status of zinc and cholesterol concentration of serum lipoproteins in adult male rats. American Journal of Clinical Nutrition, 34, 2376-2381.

Lukaski, H. C., Klevay, L. M., & Bolonchuk, W. W. (1982). Influence of dietary lipids on iron, zinc, and copper retention in trained athletes. Federation Proceedings, 41, 275a.

Magee, A. C., & Grainger, F. P. (1979). Zinc-protein interrelationships in young rats. Nutrition Reports International, 20, 771-776.

Matrone, G., Peterson, W. J., Braxley, H. M., & Grinnels, C. D. (1947). Copper and iron in the blood serum of dairy cows. Journal of Dairy Science, 30, 131-124.

McGandy, R. B, & Hegsted, D. M. (1975). Quantitative effects of dietary fat and cholesterol in man. In A. J. Vergroesen (Ed.), The role of fat in human nutrition. New York: Academic Press.

Mills, C. F., & Murray, G. (1960). Preparation of a semisynthetic diet low in copper for copper-deficiency studies with the rat. Journal of the Science of Food and Agriculture, 11, 547-552.

Motsinger, B. M., & Magee, A. C. (1980). Zinc supplementation of optimal and suboptimal protein diets. 1. Responses of young rats fed different quality proteins. Nutrition Reports International, 22, 581-588.

Murthy, L., Klevay, M., & Petering, H. G. (1974). Interrelationships of zinc and copper nutriture in the rat. Journal of Nutrition, 104, 1458-1465.

Murthy, L., & Petering, H. G. (1976). Effect of dietary zinc and copper interrelationships on blood parameters of the rat. Journal of Agricultural Food Chemistry, 24, 808-811.

Parks, R. Q., Hood, S. L., Hurwitz, C., & Ellis, G. H. (1943). Quantitative chemical microdetermination of twelve elements in plant tissue. Industrial Engineering Chemistry (Analytical Edition), 15, 527-533.

Pawar, S. S., & Tidwell, H. C. (1967). Cholesterol and lipid levels in blood and livers of rats fed unsaturated fats. American Journal of Physiology, 213(6), 1350-1352.

Petering, H. G., Murthy, L., & O'Flaherty, E. (1977). Influence of dietary copper and zinc on rat lipid metabolism. Journal of Agricultural Food Chemistry, 25, 1105-1109.

Senate Select Committee. (1977). U.S. dietary goals. Washington, DC: Government Printing Office.

Settlemire, C. T., & Matrone, G. (1967). In vivo interference of zinc with ferritin iron in the rat. Journal of Nutrition, 92, 153-158.

Schroeder, H. A., Mitchener, M., & Nason, A. P. (1971). Influence of various sugars, chromium and other trace metals on serum cholesterol and glucose of rats. Journal of Nutrition, 101, 247-258.

Shenk, J. H., Hall, J. L., & King, H. H. (1934). Spectrophotometric characteristics of hemoglobins. Journal of Biological Chemistry, 105, 741-747.

Shorey, R. T., Sewall, B., & O'Brien, M. (1976). Efficacy of diet and exercise in the reduction of serum cholesterol and triglycerides in free-living adult males. American Journal of Clinical Nutrition, 29, 512-521.

Sinthusek, G., & Magee, A. C. (1984). Relationships of dietary zinc/copper ratios to plasma cholesterol and liver trace mineral deposition in young rats fed saturated and unsaturated fats. Nutrition Research, 4, 841-851.

Snedecor, G. W., & Cochran, W. G. (1980). Statistical methods (7th ed.). Ames: Iowa State University Press.

Son, S. M. (1984). Effects of zinc and vitamin B-6 supplementation on growth, and mineral deposition of young rats fed various levels of protein. Unpublished doctoral dissertation, University of North Carolina at Greensboro, Greensboro, NC.

Underwood, E. J. (1977). Trace elements in human and animal nutrition (4th ed.). New York: Academic Press.

Vega, G. L. (1982). Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. Journal of Lipid Research, 23(6), 811-822.

Watt, E. W., Wiley, J., & Fletcher, G. F. (1976). Effect of dietary control and exercise training on daily food intake and serum lipids. American Journal of Clinical Nutrition, 29, 900-904.

White, H. S. (1969). Inorganic elements in weighed diets of girls and young women. Journal of the American Dietetic Association, 55, 38-43.

Williams, E. R., & Caliendo, M. A. (1984). Nutrition: Principles, issues, and applications. New York: McGraw-Hill.

Woo, W., & Gibbs, D. L. (1981). Lipid metabolism. American Journal of Clinical Nutrition, 34, 120-121.

APPENDIX A

COMPOSITION OF THE EXPERIMENTAL DIETS

Table A-1

Composition of Basal Diet

Constituents	gm/kg	% Composition	% Kcal Distribution
Fat	210	21	40
Cornstarch	510	51	43
Egg white solids	200	20	17
Mineral mix	40	4	-
Vitamin mix	20	2	-
Cellulose	20	2	-
Oleum percomophum	24 drops		

Table A-2

Zinc[a] and Copper[b] Supplementation Data[c]

Experimental Diets	Experiment 1[d]		Experiment 2[e]	
	Zinc Supplementation	Copper Supplementation	Zinc Supplementation	Copper Supplementation
1	0	0	0	0
2	5	0	50	0
3	10	0	100	0
4	20	0	200	0
5	0	0.56	0	5.6
6	5	0.56	50	5.6
7	10	0.56	100	5.6
8	20	0.56	200	5.6
9	0	1.68	0	16.8
10	5	1.68	50	16.8
11	10	1.68	100	16.8
12	20	1.68	200	16.8
13	0	5.04	0	50.4
14	5	5.04	50	50.4
15	10	5.04	100	50.4
16	20	5.04	200	50.4

[a]Supplemented in form of $ZnCO_3$.

[b]Supplemented in form of $CuSO_4 \cdot 5H_2O$.

[c]Quantity expressed in ppm.

[d]Baseline zinc and copper were 0.36 ppm and 0.31 ppm, respectively.

[e]Baseline zinc and copper were 0.31 ppm and 0.30 ppm, respectively.

Table A-3

Composition of Mineral Mix, Hawk-Oser[a]

Constituents	gm/kg
Calcium citrate	309.6733
Calcium phosphate, monobasic	113.251215
Potassium phosphate, dibasic	219.7224
Potassium chloride	125.291315
Sodium chloride	77.410815
Calcium carbonate	68.900715
Magnesium carbonate	33.4304
Magnesium sulfate	38.500415
Ferric citrate (USP 16.7% Fe)	12.997
Sodium fluoride	0.507845
Manganese sulfate (H_2O)	0.18072
Aluminum potassium sulfate (12 H_2O)	0.09245
Potassium iodide	0.0414

[a]Product of Teklad Test Diets, Madison, Wisconsin.

Table A-4

Composition of Vitamin Mixture[a]

Constituents	Amount per 2 kg Mix
	mg
Vitamin B_{12}[b]	2
Biotin	20
Folic Acid	100
Thiamin HCL	500
Pyridoxine HCL	500
	gm
Menadione (2-methyl-napthaquinone)	1
Riboflavin	1
Nicotinic acid	1
Calcium pantothenate	3
p-Aminobenzoic acid	91
Inositol	100
Choline chloride	150
DL-methionine	600
Cornstarch[c]	1649

[a]All vitamins and methionine purchased from ICN Nutritional Biochemicals, Cleveland, Ohio.

[b]Vitamin B_{12} compound is a 0.1% trituration of B_{12} in mannitol. Therefore, 2 gm of this material will give 2 mg of vitamin B_{12} for the mixture.

[c]Teklad Test Diets, Madison, Wisconsin.

APPENDIX B

RAW DATA

Table B-1

Total Weight Gain of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Total Weight Gain at 6 Weeks (grams)							
				Experiment 1				
1	80	62	88	(92)[a]	107	123		92
2	182	221	245	175	229	164		203
3	(207)	196	210	143	254	231		207
4	160	260	209	159	141	191		187
5	117	96	133	61	52	46		84
6	104	146	117	(131)	148	142		131
7	156	185	186	213	243	211		199
8	255	214	139	170	206	241		204
9	64	61	55	55	84	52		62
10	133	134	148	120	127	162		137
11	176	212	183	257	171	223		204
12	173	197	151	272	250	179		204
13	95	65	53	72	58	40		64
14	126	(122)	118	119	119	128		122
15	180	276	279	(218)	185	169		218
16	224	219	289	240	181	198		225
				Experiment 2				
1	(100)	76	154	58	62	(100)	151	100
2	(161)	85	(161)	168	175	(161)	216	161
3	236	167	236	168	212	215	114	193
4	228	167	236	159	186	217	174	195
5	(60)	112	63	40	43	60	44	60
6	164	216	275	199	183	180	233	207
7	212	278	248	254	246	225	230	242
8	270	212	226	238	183	246	172	221
9	74	50	134	(80)	63	55	103	80
10	268	277	262	252	224	218	229	247
11	291	216	175	198	273	236	209	228
12	232	240	217	245	251	286	226	242
13	99	48	136	(66)	38	25	52	66
14	149	256	248	224	246	239	181	220
15	211	(250)	275	309	209	231	264	250
16	228	236	207	195	265	271	246	235

[a]( ) Estimated missing plot value.

Table B-2

Hemoglobin Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Hemoglobin (gm/100 ml blood)							
				Experiment 1				
1	15	15	16	(15)[a]	16	13		15
2	12	14	13	14	16	13		14
3	(12)	13	14	10	13	12		12
4	14	12	10	14	15	12		13
5	9	18	14	18	16	14		15
6	17	15	11	(15)	15	16		15
7	10	12	14	13	13	14		13
8	13	7	13	13	9	13		11
9	15	15	13	15	17	13		15
10	12	13	15	16	15	13		14
11	12	13	14	14	12	13		13
12	13	15	12	12	13	11		13
13	17	16	15	17	14	11		15
14	15	(15)	14	15	15	16		15
15	14	14	15	(14)	15	14		14
16	14	15	13	13	15	14		14
				Experiment 2				
1	(13)	15	13	14	12	(13)	9	13
2	(12)	15	(12)	11	10	(12)	12	12
3	11	12	11	10	11	9	7	10
4	11	7	7	5	7	10	8	8
5	(15)	13	16	14	17	13	16	15
6	14	16	13	11	13	12	14	13
7	12	13	13	11	12	12	13	12
8	14	14	12	12	12	11	14	13
9	13	14	13	(13)	14	11	13	13
10	12	12	12	12	11	9	13	12
11	15	12	14	11	12	12	11	12
12	13	13	13	12	12	13	12	13
13	15	15	15	(14)	16	11	14	14
14	15	12	13	14	13	11	13	13
15	14	(13)	13	12	11	13	12	13
16	14	14	12	14	12	12	15	13

[a]( ) Estimated missing plot value.

Table B-3

Liver Copper Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Liver Copper Concentration (mcg Cu/gm dry wt.)							
				Experiment 1				
1	1.74	0.52	1.30	(1.11)[a]	1.43	0.54		1.11
2	0.64	0.58	(0.71)	0.82	0.89	0.60		0.71
3	(0.60)	0.40	0.45	0.83	0.73	0.59		0.60
4	(1.01)	1.14	0.65	1.16	1.14	0.94		1.01
5	(1.42)	1.04	0.69	1.23	2.08	2.05		1.42
6	(0.83)	0.56	0.64	(0.83)	1.04	1.08		0.83
7	(0.67)	0.42	0.57	0.56	1.04	0.75		0.67
8	(0.74)	0.41	0.85	0.71	0.66	1.08		0.74
9	1.52	0.94	1.77	1.39	1.59	1.01		1.37
10	(0.91)	0.74	0.71	1.00	1.60	0.52		0.91
11	(0.70)	0.41	1.21	0.74	0.56	0.56		0.70
12	(0.87)	1.82	0.76	0.73	0.55	0.50		0.87
13	(2.07)	1.90	2.02	1.25	2.66	2.53		2.07
14	1.25	(1.26)	0.51	1.10	1.74	1.68		1.26
15	(1.12)	1.65	0.92	(1.12)	0.84	1.05		1.12
16	0.69	0.72	0.85	0.69	1.92	1.49		1.06
				Experiment 2				
1	(0.88)	1.29	0.65	1.00	1.08	(0.88)	0.36	0.88
2	(0.76)	1.34	(0.76)	0.65	0.75	(0.76	0.31	0.76
3	0.40	0.61	0.71	0.52	0.61	0.33	0.32	0.50
4	0.58	0.48	0.64	0.55	0.62	0.28	0.28	0.49
5	(2.06)	1.25	1.88	2.42	2.87	1.82	2.09	2.06
6	1.63	1.00	1.57	0.83	0.67	1.26	1.02	1.14
7	1.30	1.32	0.96	1.24	0.91	0.79	1.01	1.08
8	1.17	1.70	1.07	1.43	1.18	0.65	1.01	1.17
9	2.16	1.02	1.34	(1.35)	1.25	1.08	1.25	1.35
10	1.61	1.62	1.14	1.74	1.88	2.22	1.89	1.73
11	1.48	1.43	2.19	1.55	1.44	1.53	2.34	1.71
12	1.43	1.49	1.55	2.12	1.56	1.32	1.15	1.52
13	2.22	2.40	1.79	(2.45)	3.29	(2.45)	2.56	2.45
14	2.58	1.74	1.72	1.62	1.72	1.42	1.28	1.73
15	1.49	(1.42)	1.69	1.42	1.76	1.18	0.96	1.42
16	2.27	1.36	1.87	1.90	1.60	1.36	1.52	1.70

[a]( ) Estimated missing plot value.

Table B-4

Liver Iron Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Liver Iron Concentration (mcg Fe/gm dry wt.)							
	Experiment 1							
1	1175.94	856.40	1920.41	(1296.95)[a]	913.23	1618.76		1296.95
2	797.97	853.03	(1009.66)	1322.75	988.09	1086.45		1009.66
3	(1038.56)	735.32	933.75	1302.33	1091.94	1129.45		1038.56
4	(1005.89)	702.08	1357.85	1107.26	966.36	895.92		1005.89
5	(1435.39)	1006.80	1015.03	933.79	2813.36	1407.98		1435.39
6	(1486.71)	1380.98	1884.63	(1486.71)	1398.07	1283.14		1486.71
7	(770.85)	577.97	479.14	1113.08	591.83	1092.22		770.85
8	(895.45)	711.83	616.46	835.60	1572.66	740.70		895.45
9	746.86	1024.68	570.82	1564.04	2825.79	1163.98		1229.36
10	(1178.49)	953.32	888.54	1344.18	1367.38	1339.05		1178.49
11	(897.77)	749.38	779.94	856.55	1538.92	474.04		879.77
12	(949.45)	541.00	1526.09	788.34	882.12	1009.68		949.45
13	(1215.46)	965.01	762.48	1120.13	1570.41	1659.27		1215.46
14	392.19	(964.19)	494.17	1213.37	1718.26	1002.95		964.19
15	(807.24)	368.38	559.05	(807.24)	1311.09	990.42		807.24
16	633.93	847.37	474.35	762.91	657.36	810.81		697.79
	Experiment 2							
1	(1329.00)	1113.19	1257.84	1159.88	1607.82	(1329.00)	1506.29	1329.00
2	(1140.33)	2082.30	(1140.33)	993.01	799.00	(1140.33)	687.01	1140.33
3	731.83	1047.41	819.16	1725.04	802.29	754.60	453.46	904.83
4	562.50	537.67	867.60	718.71	798.96	684.35	654.29	689.15

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
5	(1086.90)	1431.85	1190.44	1032.51	989.11	991.92	885.59	1086.90
6	555.81	536.61	445.72	633.82	370.64	551.96	506.27	514.40
7	593.82	444.14	653.24	621.03	584.48	847.11	468.91	601.82
8	441.45	496.07	553.98	315.32	492.65	468.17	704.82	496.07
9	2016.91	681.09	1183.61	(1143.95)	771.73	1143.95	1066.41	1143.95
10	295.14	552.88	389.47	516.41	482.57	381.21	386.72	429.20
11	357.26	411.99	584.74	534.79	524.73	416.16	384.85	459.22
12	412.79	426.29	311.61	351.78	269.76	235.58	394.31	343.16
13	1038.41	827.52	1058.05	(1021.37)	1516.60	(1021.37)	666.27	1021.37
14	485.38	542.06	383.36	380.97	370.34	396.07	432.10	427.18
15	592.15	(398.14)	464.13	301.24	400.04	299.04	332.24	398.14
16	344.06	497.98	350.97	396.24	380.58	275.37	390.73	376.56

[a]( ) Estimated missing plot.

Table B-5

Liver Zinc Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Liver Zinc Concentration (mcg Zn/gm dry wt.)							
				Experiment 1				
1	10.19	8.79	9.51	(10.11)[a]	10.18	11.88		10.11
2	9.68	8.64	(8.70)	9.64	4.83	10.70		8.70
3	(9.62)	8.60	7.74	11.78	9.43	10.57		9.62
4	(10.49)	9.70	9.91	10.62	10.93	11.29		10.49
5	(11.54)	8.88	8.46	8.31	23.19	8.87		11.54
6	(9.72)	8.81	7.83	(9.72)	12.36	9.86		9.72
7	(8.65)	9.84	5.60	8.54	9.90	9.39		8.65
8	(29.39)	7.93	57.33	9.66	8.03	63.98		29.39
9	8.44	7.27	10.04	12.50	12.46	8.11		9.80
10	(9.28)	8.33	8.65	8.37	11.37	9.67		9.28
11	(9.14)	8.02	10.62	7.77	9.71	9.50		9.14
12	(17.24)	9.93	9.38	9.01	47.71	10.18		17.24
13	(10.93)	9.00	8.85	8.97	12.70	15.15		10.93
14	6.86	(8.07)	4.97	9.90	9.85	8.76		8.07
15	(9.87)	9.98	7.37	(9.87)	10.67	11.45		9.87
16	8.50	9.56	47.38	8.69	90.18	10.95		29.21
				Experiment 2				
1	(9.83)	10.30	8.27	7.76	13.20	(9.83)	9.62	9.83
2	(34.97)	23.65	(34.97)	93.48	12.37	(34.97)	10.38	34.97
3	51.94	12.01	64.26	12.22	10.95	10.01	8.84	24.32
4	53.32	8.64	13.64	78.25	10.17	9.93	8.91	11.49
5	(11.49)	10.18	12.90	13.81	10.32	10.68	11.05	11.49
6	7.95	9.23	9.67	67.31	8.67	9.03	9.32	17.31
7	10.02	47.96	9.42	10.47	9.15	10.91	8.54	15.21
8	45.85	9.42	12.88	79.09	10.99	11.46	71.65	34.48
9	10.33	4.47	15.74	(11.24)	11.78	12.15	12.95	11.24
10	43.33	43.08	7.51	64.94	78.32	8.61	11.58	36.77
11	45.79	9.37	70.64	77.55	63.99	10.81	9.78	41.13
12	58.02	44.65	52.53	12.16	91.71	44.75	12.52	45.19
13	9.76	10.38	9.78	(11.28)	15.15	(11.28)	11.33	11.28
14	14.74	8.23	68.95	64.69	51.73	52.27	9.33	38.56
15	9.61	(15.38)	9.08	46.03	10.54	8.80	8.21	15.38
16	11.80	45.41	69.11	91.39	74.30	47.86	12.57	50.35

[a]( ) Estimated missing plot value.

Table B-6

Serum Triglyceride Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Serum Triglyceride Concentration (mg/100 ml)							
				Experiment 1				
1	47	18	23	(29)[a]	18	38		29
2	64	45	53	27	37	39		44
3	(52)	71	64	28	60	37		52
4	65	44	17	10	16	47		33
5	37	32	15	22	10	25		24
6	28	43	44	(33)	20	28		33
7	45	38	32	16	49	63		41
8	79	40	62	26	23	46		46
9	25	31	12	19	33	34		26
10	54	25	72	20	38	42		42
11	73	47	30	32	32	51		44
12	54	26	27	49	48	85		48
13	20	18	20	9	7	24		16
14	17	(24)	28	12	40	22		24
15	57	83	42	(60)	81	39		60
16	50	41	25	50	26	72		44
				Experiment 2				
1	(44)	72	77	37	15	(44)	20	44
2	(21)	8	(21)	38	17	(21)	21	21
3	51	36	41	34	72	26	24	41
4	54	69	28	21	44	31	47	42
5	(26)	40	37	9	41	17	12	26
6	26	48	84	25	24	35	17	37
7	48	61	60	42	44	23	38	45
8	37	121	51	55	87	38	15	58
9	25	31	39	(31)	31	20	40	31
10	48	119	43	34	35	61	24	52
11	82	128	21	12	43	71	29	55
12	49	83	107	35	21	78	17	56
13	26	9	92	(31)	15	(31)	13	31
14	20	71	62	26	56	45	67	50
15	55	(45)	85	69	4	36	19	45
16	69	27	26	31	71	56	17	42

[a]( ) Estimated missing plot value.

Table B-7

Serum Total Cholesterol Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Serum Total Cholesterol Concentration (mg/100 ml)							
				Experiment 1				
1	64	70	100	(70)[a]	35	82		70
2	76	73	106	89	94	105		91
3	(88)	65	102	109	85	80		88
4	67	52	70	53	52	91		64
5	91	68	100	52	81	48		73
6	101	75	190	(85)	68	81		85
7	83	64	84	108	75	88		84
8	102	87	76	111	84	83		91
9	89	95	74	79	62	76		79
10	88	70	66	82	72	100		80
11	105	104	70	134	60	76		92
12	75	76	110	99	101	77		90
13	81	95	72	66	76	51		74
14	65	(92)	108	84	89	115		92
15	78	62	78	(87)	115	103		87
16	100	50	79	96	67	101		82
				Experiment 2				
1	(81)	81	81	84	92	(81)	68	81
2	(74)	66	(74)	81	56	(74)	93	74
3	113	117	80	114	89	108	97	103
4	81	119	71	73	96	74	90	86
5	(71)	90	77	44	85	73	56	71
6	66	97	98	110	89	58	82	86
7	66	66	83	46	75	76	65	68
8	111	93	78	71	84	71	58	81
9	79	101	79	(78)	98	60	50	78
10	73	84	78	60	73	76	74	74
11	73	136	60	58	58	70	67	75
12	78	99	76	69	75	60	67	75
13	68	93	111	(82)	77	(82)	63	82
14	74	89	72	70	87	58	79	76
15	69	(66)	93	64	58	51	59	66
16	73	99	58	69	53	82	73	72

[a]( ) Estimated missing plot value.

Table B-8

Serum HDL-Cholesterol Concentration of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Mean
	Serum HDL-Cholesterol Concentration (mg/100 ml)							
				Experiment 1				
1	56	53	84	(58)[a]	33	62		58
2	65	62	9	62	79	78		59
3	(71)	57	88	92	49	69		71
4	59	44	53	41	48	82		55
5	54	44	98	45	74	43		60
6	80	61	81	(71)	63	72		71
7	76	47	56	90	47	73		65
8	62	72	74	88	52	73		70
9	75	70	72	69	40	62		65
10	72	56	49	72	70	69		65
11	65	79	41	124	51	56		69
12	47	64	93	88	74	67		72
13	60	52	52	45	70	45		54
14	52	(64)	77	70	73	46		64
15	50	53	75	(64)	97	95		64
16	77	48	56	73	62	72		65
				Experiment 2				
1	(67)	57	72	66	80	(67)	58	67
2	(55)	32	(55)	66	47	(55)	75	55
3	99	87	59	95	70	91	85	84
4	70	99	57	49	82	62	84	72
5	(42)	51	56	27	45	43	32	42
6	42	72	72	94	70	47	73	67
7	43	58	78	43	58	71	63	59
8	73	57	69	60	68	64	44	62
9	68	69	71	(56)	60	32	36	56
10	56	74	63	46	51	52	72	59
11	55	55	44	51	42	35	68	50
12	58	78	56	51	52	55	40	56
13	47	28	80	(54)	66	(54)	47	54
14	55	57	63	38	60	42	53	53
15	59	(57)	72	59	57	45	49	57
16	70	51	46	60	31	62	50	53

[a]( ) Estimated missing plot value.

Table B-9

Extensive Hair Loss Over the Body of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Total
	Extensive Hair Loss							
	Experiment 1							
1	0	0	0	-	0	0		0
2	0	0	1	0	0	0		1
3	-	0	0	0	0	0		0
4	0	1	0	0	0	0		1
5	0	0	0	0	0	0		0
6	0	0	0	-	1	0		1
7	1	0	1	1	0	0		3
8	0	0	0	0	0	0		0
9	0	0	0	0	0	0		0
10	0	1	0	0	0	1		2
11	0	1	1	0	0	1		3
12	0	0	0	1	0	0		1
13	0	0	0	0	0	0		0
14	-	-	0	1	0	0		2
15	1	0	0	-	1	0		2
16	0	0	1	0	0	1		2
	Experiment 2							
1	-	0	0	0	0	-	0	0
2	-	0	-	1	1	-	0	2
3	0	0	1	0	0	1	0	2
4	0	0	0	0	1	1	0	2
5	-	0	0	0	0	0	0	0
6	1	0	0	1	0	1	0	3
7	1	1	1	0	1	0	1	5
8	0	0	0	0	0	1	1	2
9	0	0	0	-	0	0	0	0
10	0	0	0	0	1	1	1	3
11	1	1	1	1	1	0	1	6
12	1	0	0	1	0	0	0	2
13	0	0	0	-	0	0	0	0
14	1	0	1	1	0	1	0	4
15	1	-	1	1	1	1	1	6
16	1	0	1	0	1	1	1	5

0 = Absence of extensive hair loss; 1 = Presence of extensive hair loss;
- = Missing plot

Table B-10

Skin Lesions Over the Body of Young Male Rats Fed Diets Supplemented with Zinc and Copper for Experiments 1 and 2

Experimental Diets	Replicates							
	1	2	3	4	5	6	7	Total
	Skin Lesions							
	Experiment 1							
1	0	1	1	-	0	0		2
2	0	0	0	0	0	0		0
3	-	0	0	0	0	0		0
4	0	0	0	0	0	0		0
5	1	1	0	1	1	1		5
6	0	0	0	-	0	0		0
7	0	0	0	0	0	0		0
8	0	0	0	0	0	0		0
9	1	1	1	1	1	1		6
10	0	0	0	0	0	0		0
11	0	0	0	0	0	0		0
12	0	0	0	0	0	0		0
13	1	1	1	1	1	1		6
14	0	-	0	0	1	0		1
15	0	0	0	-	0	0		0
16	0	0	0	0	0	0		0
	Experiment 2							
1	-	1	0	1	1	-	0	3
2	-	1	-	0	0	-	0	1
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	-	0	0	1	1	1	1	5
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	1	0	0	1
9	1	1	1	-	1	1	0	5
10	0	0	0	0	0	0	0	0
11	0	0	1	0	0	0	0	1
12	0	0	0	0	0	0	0	0
13	1	1	1	1	1	1	1	7
14	0	0	0	0	0	0	0	0
15	0	-	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0

0 = Absence of skin lesions; 1 = Presence of skin lesions;
- = Missing plot.

APPENDIX C

STATISTICAL ANALYSES

Table C-1

Analysis of Variance of Weight Gain Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Experiment 1	
Total	90	395546	
Replicates	5	2197	439
Zinc	3	275743	91914**
Linear	1	199170	199170**
Quadratic	1	75443	75443**
Cubic	1	1130	1130
Copper	3	5914	1971
Zinc & copper	9	28067	3119**
Error	70	83625	1195
		Experiment 2	
Total	101	595216	
Replicates	6	8056	1343
Zinc	3	429648	143216**
Linear	1	229408	229408**
Quadratic	1	179739	179739**
Cubic	1	20501	20501**
Copper	3	22983	7661**
Linear	1	10198	10198**
Quadratic	1	12399	12399**
Cubic	1	386	386
Zinc & copper	9	32755	3639**
Error	80	101776	1272

**Highly significant ($p \leq 0.01$)

Table C-2

Analysis of Variance of Hemoglobin Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	
		Experiment 1		
Total	90	355.50		
Replicates	5	16.60	3.30	
Zinc	3	76.40	25.50**	
Linear	1	67.40		67.50**
Quadratic	1	4.80		4.80
Cubic	1	4.20		4.20
Copper	3	22.00	7.30	
Zinc & copper	9	21.20	2.40	
Error	70	219.30	3.13	
		Experiment 2		
Total	102	463.02		
Replicates	6	40.98	6.83**	
Zinc	3	74.32	24.67**	
Linear	1	59.26		59.26**
Quadratic	1	14.06		14.06**
Cubic	1	1.00		1.00
Copper	3	126.89	42.33**	
Linear	1	73.94		73.94**
Quadratic	1	14.99		14.99**
Cubic	1	37.97		37.97**
Zinc & copper	9	69.89	7.78**	
Error	81	151.02	1.86	

**Highly significant ($p \leq 0.01$)

Table C-3

Analysis of Variance of Liver Copper Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	
		Experiment 1		
Total	79	23.34		
Replicates	5	1.57	0.31	
Zinc	3	7.27	2.42**	
Linear	1	3.02		3.02**
Quadratic	1	4.18		4.18**
Cubic	1	0.07		0.07
Copper	3	4.04	1.35**	
Linear	1	3.96		3.96**
Quadratic	1	0.06		0.06
Cubic	1	0.02		0.02
Zinc & copper	9	1.35	0.15	
Error	59	9.12	0.15	
		Experiment 2		
Total	101	42.17		
Replicates	6	1.25	0.21	
Zinc	3	4.45	1.48**	
Linear	1	2.59		2.59**
Quadratic	1	1.70		1.70**
Cubic	1	0.16		0.16
Copper	3	21.13	7.04**	
Linear	1	14.40		14.40**
Quadratic	1	5.40		5.40**
Cubic	1	1.33		1.33*
Zinc & copper	9	5.63	0.62**	
Error	80	9.70	0.12	

**Highly significant ($p \leq 0.01$)

Table C-4

Analysis of Variance of Liver Iron Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
		Experiment 1	
Total	79	18251571	
Replicates	5	2938052	587610*
Zinc	3	2871910	957303**
Linear	1	2149488	2149488**
Quadratic	1	464055	464055
Cubic	1	258367	258367
Copper	3	983662	327887
Zinc & copper	9	1079147	119905
Error	59	10378798	175912
		Experiment 2	
Total	101	16536895	
Replicates	6	215551	35925
Zinc	3	7421611	2473870**
Linear	1	5048115	5048115**
Quadratic	1	1819725	1819725**
Cubic	1	553771	553771**
Copper	3	3696678	1232226**
Linear	1	1793557	1793557**
Quadratic	1	1367534	1367534**
Cubic	1	535887	535887**
Zinc & copper	9	743240	82582
Error	80	4459817	55748

*Significant ($p \leq 0.05$)

**Highly significant ($p \leq 0.01$)

Table C-5

Analysis of Variance of Liver Zinc Data

Source of Variation	Degrees of Freedom	Sum of Square	Mean Square
		Experiment 1	
Total	79	14175.98	
Replicates	5	1116.05	223.21
Zinc	3	2304.76	768.25**
Linear	1	1339.02	1339.02**
Quadratic	1	960.73	960.73*
Cubic	1	5.01	5.01
Copper	3	311.30	103.77
Zinc & copper	9	1093.56	121.51
Error	59	9276.21	157.22
		Experiment 2	
Total	101	66646.38	
Replicates	6	12116.49	2019.42**
Zinc	3	9360.43	3120.14**
Linear	1	6017.28	6017.28**
Quadratic	1	334.01	334.01
Cubic	1	3009.14	3009.14**
Copper	3	3920.33	1306.78*
Linear	1	1093.09	1093.09
Quadratic	1	995.08	995.08
Cubic	1	1832.15	1832.15*
Zinc & copper	9	3856.65	428.52
Error	80	36616.37	457.70

*Significant ($p \leq 0.05$)

**Highly significant ($p \leq 0.01$)

Table C-6

Analysis of Variance of Serum Triglyceride Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	
		Experiment 1		
Total	90	32752		
Replicates	5	4177	835**	
Zinc	3	8719	2906**	
Linear	1	4541		4541**
Quadratic	1	3865		3865**
Cubic	1	313		313
Copper	3	361	120	
Zinc & copper	9	3898	433	
Error	70	15597	223	
		Experiment 2		
Total	101	70759		
Replicates	6	13631	1272	
Zinc	3	4496	1499	
Copper	3	1893	631	
Zinc & copper	9	5896	655	
Error	80	44845	561	

**Highly significant ($p \leq 0.01$)

Table C-7

Analysis of Variance of Serum Total Cholesterol Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	
		Experiment 1		
Total	90	30454		
Replicates	5	2552	510	
Zinc	3	2216	739	
Copper	3	630	210	
Zinc & copper	9	3080	342	
Error	70	21975	314	
		Experiment 2		
Total	101	30981		
Replicates	6	5491	915	
Zinc	3	24	8	
Copper	3	2513	838*	
Linear	1	1092		1092*
Quadratic	1	945		945*
Cubic	1	476		476
Zinc & copper	9	5569	619	
Error	80	17385	217	

*Significant ($p \leq 0.01$)

Table C-8

Analysis of Variance of Serum HDL-Cholesterol Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	
		Experiment 1		
Total	90	26755		
Replicates	5	1871	374	
Zinc	3	1315	438	
Copper	3	727	242	
Zinc & copper	9	1403	156	
Error	70	21440	306	
		Experiment 2		
Total	101	25770		
Replicates	6	750	125	
Zinc	3	935	312	
Copper	3	4133	1378**	
Linear	1	1969		1969**
Quadratic	1	1505		1505**
Cubic	1	659		659
Zinc & copper	9	4869	541	
Error	80	15083	189	

**Highly significant ($p \leq 0.05$)

Table C-9

Analysis of Variance of Data From Experiments 1 and 2 with the Same Ratio but Different Levels of Zinc and Copper

Source of Variation	Degrees of Freedom		Sum of Squares		Mean Square	
			Weight Gain			
Total	81		233628			
Level of Supplement	1		75731		75731**	
Ratio	6		48207		8035**	
Linear		1		18486		18486**
Quadratic		1		2758		2758
Cubic		4		26963		13482*
Level & ratio	6		35291		5882**	
Error	68		79488		1169	
			Hemoglobin			
Total	81		207.82			
Level of Supplement	1		37.58		37.58**	
Ratio	6		26.40		4.40*	
Linear		1		12.25		12.25*
Quadratic		1		3.07		3.07
Cubic		4		11.08		2.77
Level & ratio	6		25.25		4.20*	
Error	68		120.55		1.77	
			Liver Zinc			
Total	81		56708.82			
Level of Supplement	1		12631.12		12597.83**	
Ratio	6		4514.35		752.39	
Level & ratio	6		1554.36		259.06	
Error	68		37438.22		550.56	
			Liver Copper			
Total	81		22.35			
Level of Supplement	1		9.44		9.44**	
Ratio	6		3.06		0.51**	
Linear		1		2.57		2.57**
Quadratic		1		0.20		0.20
Cubic		4		0.29		0.07
Level & ratio	6		0.90		0.15	
Error	68		8.96		0.13	

Source of Variation	Degrees of Freedom		Sum of Squares		Mean Square	
			Liver Copper			
Total	81		22.35			
Level of Supplement	1		9.44		9.44**	
Ratio	6		3.06		0.51**	
Linear		1		2.57		2.57**
Quadratic		1		0.20		0.20
Cubic		4		0.29		0.07
Level & ratio	6		0.90		0.15	
Error	68		8.96		0.13	
			Liver Iron			
Total	81		13406311			
Level of Supplement	1		7437307		7437307**	
Ratio	6		1887896		314649**	
Linear		1		29187		29187
Quadratic		1		26042		26042
Cubic		4		1832667		458167**
Level & ratio	6		1134741		189124**	
Error	68		3146553		46273	
			Serum Triglyceride			
Total	81		57944			
Level of Supplement	1		1806		1806	
Ratio	6		2907		485	
Level & ratio	6		3907		651	
Error	68		49616		730	
			Serum Total Cholesterol			
Total	81		24790			
Level of Supplement	1		2954		2954**	
Ratio	6		845		141	
Level & ratio	6		608		101	
Error	68		20366		300	
			Serum HDL-Cholesterol			
Total	81		16363			
Level of Supplement	1		3196		3196**	
Ratio	6		731		122	
Level & ratio	6		517		86	
Error	68		11902		175	

*Significant ($p \leq 0.05$)
**Highly significant ($p \leq 0.01$)

Table C-10

Analysis of Variance of Zinc/Copper Ratio Effect Alone on Parameter Level

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Weight Gain		Experiment 1[a]	
Total	37	105619	
Ratio	6	72272	12045**
Error	31	33347	1076
		Experiment 2[b]	
Total	47	52279	
Ratio	6	6139	1023
Error	41	46140	1125
Hemoglobin		Experiment 1[a]	
Total	37	94.41	
Ratio	6	37.57	6.26**
Error	31	60.83	1.96
		Experiment 2[b]	
Total	47	71.84	
Ratio	6	12.12	2.02
Error	41	59.72	1.46
Liver Copper		Experiment 1[a]	
Total	37	6.11	
Ratio	6	2.07	0.35*
Error	31	4.04	0.13
		Experiment 2[b]	
Total	47	6.80	
Ratio	6	1.88	0.31*
Error	41	4.92	0.12
Liver Iron		Experiment 1[a]	
Total	37	5530310	
Ratio	6	2712435	452073**
Error	31	2817875	90892
		Experiment 2[b]	
Total	47	438694	
Ratio	6	110016	18336
Error	41	328678	8017

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Liver Zinc		Experiment 1[a]	
Total	37	7761.46	
Ratio	6	2075.86	345.98
Error	31	5685.61	183.41
		Experiment 2[b]	
Total	47	36919.71	
Ratio	6	5167.09	861.18
Error	41	31752.62	774.45
Triglyceride		Experiment 1[a]	
Total	37	13838	
Ratio	6	5156	859
Error	31	8682	380
		Experiment 2[b]	
Total	47	42301	
Ratio	6	1367	228
Error	41	40934	998
Total Cholesterol		Experiment 1[a]	
Total	37	10196	
Ratio	6	618	103
Error	31	9578	309
		Experiment 2[b]	
Total	47	11640	
Ratio	6	852	142
Error	41	10788	263
HDL-Cholesterol		Experiment 1[a]	
Total	37	7763	
Ratio	6	529	88
Error	31	7234	313
		Experiment 2[b]	
Total	47	5404	
Ratio	6	735	123
Error	41	4669	114

[a]Low zinc and copper supplementation.
[b]High zinc and copper supplementation.
*Significant ($p \leq 0.05$).
**Highly significant ($p \leq 0.01$).