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Sinthusek, Govit

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THE EFFECTS OF ZINC AND COPPER SUPPLEMENTATION ON BLOOD LIPIDS AND TRACE MINERALS DEPOSITION OF YOUNG MALE RATS FED EITHER COCONUT OIL OR CORN OIL

by

Govit Sinthusek

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Approved by

Alden C. Magee

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 Aden C. Magee

Committee Members Terry A. Bazzarre

William A. Powers

C. W. Seo

Mildred Johnson

March 18, 1983
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The purpose of this study was to investigate the effects of zinc and copper supplementation on plasma total and HDL-cholesterol and the availabilty of copper, iron, and zinc of young male rats fed either coconut oil or corn oil. Dietary factors included two levels of copper (1.5 and 6.0 ppm), two levels of zinc (7.5 and 30.0 ppm), and two fat sources (coconut oil and corn oil). Criteria for evaluating animal responses to various test diets included weight gain, plasma total and HDL-cholesterol levels, and copper, iron and zinc deposition in the liver.

Results indicated that rats fed diets containing corn oil had significantly higher ($p \leq 0.05$) weight gains than those fed coconut oil. The differences in weight gains, however, did not appear to be related to differences in food consumption. Plasma total and HDL-cholesterol levels were higher in animals fed the corn oil diets. Increased levels of zinc in the diets were associated with increases in total cholesterol in animals fed corn oil, but an increase in dietary zinc had no significant effect on total cholesterol of rats fed coconut oil. Copper supplements tended to lower total cholesterol levels, regardless of fat source. Zinc supplementation resulted in increases in HDL-cholesterol, while copper supplements were associated with decreased HDL-

cholesterol levels. The addition of zinc to the diets resulted in a highly significant ($p \leq 0.01$) plasma HDL/total cholesterol ratio in animals fed coconut oil.

Animals fed the coconut oil had higher liver copper levels than rats fed corn oil, while the level of zinc accumulation was greater in the animals fed corn oil. Copper supplementation resulted in significant decreases ($p \leq 0.05$) in liver iron deposition in animals fed both fat sources, but zinc supplementation had no apparent effect on liver iron deposition. As the length of the experimental period increased, animals on the coconut oil diets appeared to show excessive losses of hair over the entire body. Animals fed the corn oil diets, however, exhibited losses of hair locally around the forehead (baldness).

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CHAPTER I

INTRODUCTION

Current research suggests that an elevated plasma cholesterol level is a major identifiable risk factor in the development of coronary heart disease (CHD) (Gordon et al., 1977b). The elevation of plasma cholesterol is the result of a 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 et al., 1976; Watt et al., 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 CHD.

A high dietary zinc to copper ratio has been identified as a major factor associated with elevated cholesterol levels by Klevay (1973), while others believe that the zinc to copper ratio of the diet is not a major factor affecting cholesterol metabolism (Murthy & Petering, 1976; Lei, 1977; Fischer et al., 1980). The type of fat in the diet is another component that has been considered to have a major impact on cholesterol metabolism (Keys et al., 1960), and many health professionals recommend that the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) in the diet should be increased to protect against CHD (Keys et al., 1957; Hegsted et al., 1965). The bioavailability of some of the trace minerals apparently is influenced by the type of fat in the diet, and some researchers (Amine & Hegsted, 1975) have reported decreased bioavailability of certain trace minerals with diets containing polyunsaturated fatty acids sources.

The purpose of this study was to investigate the effects of zinc and copper supplementation on plasma total and high density lipoprotein (HDL) cholesterol and the availability of copper, iron, and zinc of young male rats fed either coconut oil or corn oil.

CHAPTER II

REVIEW OF LITERATURE

Coronary heart diease (CHD) is the leading cause of death in the United States (USDHEW, 1974) and accounts for about 35% of all deaths, twice the number caused by cancer. In the United States, the risk of CHD is higher in men than in women and in smokers than in nonsmokers. The possibility of CHD is also greater in diabetics and in individuals with electrocardiogram (EKG) abnormalities. The risk of CHD also increases with age, with high blood pressure, and with elevated concentrations of cholesterol in plasma (Insull, 1973).

Most research has been directed toward the elucidation of the various factors which promote elevated blood and tissue cholesterol levels in many animal species. One such factor studied is diet. Within the past decade several studies have suggested a relationship between several trace minerals and lipid metabolism, including the metabolism of cholesterol (Klevay, 1975; Murthy & Petering, 1976; Lei, 1977, 1978; Koo & Williams, 1981).

Amine and Hegsted (1971) found that dietary iron was inversely related to serum lipid levels. Schroder and coworkers (1971) reported that rats receiving chromium-deficient diets had elevated serum cholesterol levels. Doisy (1972) reported that manganese, a known cofactor in the biosynthesis of squalene, appeared to be directly related to serum cholesterol levels in man and chickens. In 1974, Iacano found that dietary calcium could alter cholesterol and lipid metabolism in the rabbit.

Klevay (1973) induced high cholesterol levels in rats by increasing the zinc to copper ratio in the diet and postulated that a high ratio of dietary zinc to copper was associated with high cholesterol levels. Klevay (1975) postulated that CHD was predominately a disease resulting from an imbalance of zinc to copper. The metabolic imbalance, according to Klevay, was due either to a relative or an absolute copper deficiency which was characteristic of a diet with a high zinc to copper ratio. Klevay further speculated that such an imbalance between these two trace minerals could occur in human diets. The imbalance may be due to excessive zinc, to a lack of protective substances in food or drinking water, or to alterations in physiological mechanisms which resulted in adverse changes in the distribution of zinc and copper in certain important organs.

Several studies, however, noted 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 (Petering, 1974; Murthy & Petering, 1976; Petering et al., 1977). Based on such studies, Petering and coworkers (1977) suggested that dietary copper levels governed the metabolic fate of cholesterol more than the dietary zinc to copper ratios.

Although the mechanisms responsible for the alteration of serum cholesterol by zinc and/or copper have not been fully elucidated, several possibilities exist. The elevation of serum cholesterol may be the result of the increased liver cholesterol synthesis and/or a decrease in cholesterol degradation. Elevation of serum cholesterol induced by a copper deficiency does not appear to be due to an increase in the rate of cholesterol biosynthesis or to a reduction of cholesterol degradation to biliary products (Lei, 1977, 1978). A second possibility is that a shift of cholesterol from the liver to the serum pool is responsible for the high levels of cholesterol observed in copper-deficient rats and the resulting high cholesterol levels is due to an alteration of lipoprotein cholesterol patterns.

There is evidence that any factor which alters the lipoprotein cholesterol patterns may cause the elevation of cholesterol. Allen and Klevay (1978) reported that a copper deficiency enhanced the transport of cholesterol from the liver to the serum which accounted for the rise in serum cholesterol levels and suggested that a copper deficiency affected lipoprotein metabolism.

Hooper et al. (1980) reported that HDL-cholesterol levels were lowered in men orally ingesting pharmacological doses of zinc and hypothesized that such a decrement in HDL-cholesterol was associated with the atherogenic process.

Koo and Williams, (1981) also reported a close association between the zinc status of adult male rats and HDL-cholesterol levels. It was found that acute zinc depletion resulted in a significant reduction in total serum cholesterol and that the reduction was due primarily to the decline in HDL-cholesterol. These findings, however, were contradictory to those of Miller and Miller (1975), who reported that the tissue cholesterol pool was unrelated to the plasma concentration of cholesterol but was inversely related to the plasma levels of HDL-cholesterol concentration. They postulated that HDL possibly facilitated the transport of cholesterol from peripheral tissues to the liver for subsequent catabolism and excretion. It was further proposed that the development of atheroscleroisis may be prevented more successfully by increasing plasma HDL-cholesterol concentration than by conventional attempts to lower the total plasma cholesterol and other lipoprotein cholesterols.

Epidemiological data suggest the development of CHD is enhanced when the diet is rich in saturated fats and cholesterol (Keys, 1970). Elevated levels of plasma lipids, particularly low density lipoprotein-cholesterol (LDL-cholesterol), are associated with increased risks of CHD, whereas HDL-cholesterol is thought to lower the risk of CHD (Gordon et al., 1977a). A major factor affecting plasma cholesterol and its distribution among the various lipoprotein fractions is diet. Plasma cholesterol levels in humans generally can be reduced by 15 mg percent if an individual adheres to diet that is low in cholesterol and saturated fat and high in polyunsaturated fat (Shorey et al., 1976; Watt et al., 1976). Some researchers, however, have suggested that the effect of such a diet is due primarily to the reduction in saturated fat and to a lesser extent to the replacement of the saturated fat with polyunsaturated fat (Hegsted et al., 1965; Keys et al., 1966).

The association between either zinc, copper, or zinc to copper ratio in the diets and the fluctuations of total and HDL-cholesterol is still unclear. Several studies have failed to confirm any relationship between zinc and total cholesterol, zinc/copper ratios to total cholesterol, or zinc/copper ratios to HDL-cholesterol in animals and humans (Hambidge, 1977; Caster & Doster. 1979; Geders, 1979; Fischer et al., 1980; Koo & Williams, 1981). Thus, further investigations of the relationship of the dietary zinc/copper ratios and plasma total and HDL-cholesterol is warranted.

There is little information about the association of dietary fat and trace minerals status in animals. Babatunde (1972) reported that the level of zinc required for optimum growth in pigs was increased as the level of dietary fat in the form of lard was increased. Amine and Hegsted (1975) reported that iron absorption was higher in animals fed diets containing coconut oil as the fat source than in diets with corn oil as the fat source. Bettger et al. (1979), however, reported that zinc deficiency resulted in severely depressed growth rates in rats, regardless of the source of fat. In addition, these researchers observed that rats fed hydrogenated coconut oil grew more slowly and had more severe skin lesions than did rats fed corn oil. It was postulated that zinc had a specific role in essential fatty acid metabolism. In 1980, Bettger et al. reported improved growth rate in zinc-deficient chicks fed low intakes of PUFA. These results were in contrast to similar findings the researchers had observed in rats, and suggested the difference in the apparent relationship of dietary zinc and essential fatty acid metabolism could be due to metabolic differences between chicks and rats.

Available information suggests that dietary fat can effect the availability of trace minerals known to be essential for maximum utilization by animals performance, but specific relationships between dietary fat and trace minerals such as copper, iron, and zinc are not clear. The effects of the dietary levels of certain trace minerals as influenced by the presence or absence of other trace minerals on lipoprotein components of the blood also remain somewhat an enigma and need further clarifications.

CHAPTER III

EXPERIMENTAL PROCEDURES

The purpose of this study was to determine the effects of copper and zinc supplements of lipid and mineral metabolism in young rats fed either coconut oil or corn oil. Criteria used to evaluate animal responses to various test diets included weight gain, plasma total and HDL-cholesterol levels, and copper, iron, and zinc deposition in the liver.

A 2x2x2 experimental design was used, and dietary factors included two levels of copper (1.5 and 6.0 ppm), two levels of zinc (7.5 and 30.0 ppm), and two fat sources (coconut oil and corn oil). In addition, control coconut oil and corn oil diets were included in the study for comparative purposes.

Animals

A total of 64 weanling male rats[1] were used for the experimental phases of the study. Eight additional weanling rats were sacrificed prior to the initial experimental period to provide baseline data on initial levels of plasma total and HDL-cholesterol and trace minerals in the liver. Two other groups of eight animals (controls) each were fed

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[1]Sprague-Dawley rats purchased from Holtzman Company, Madison, Wisconsin. These animals averaged 58 grams in weight initially.

non-supplemented coconut oil (0.5 ppm zinc and 1.5 ppm copper) and corn oil (0.6 ppm zinc and 1.5 ppm copper) diets for four weeks to provide information on levels of plasma total and HDL-cholesterol and liver trace minerals before changes in copper levels were made in the experimental animals

Eight replications were used, and animals were assigned to individual, stainless-steel, wire-bottom cages within each replication according to initial body weight in a randomized block fashion. The ten diets (eight experimental and two control) were also assigned at random to individual cages within each replication. All animals had free access to food and distilled water. During the first four weeks of the feeding period, the animals that were to receive an experimental diet were divided into four groups of 16 animals each. Two of the groups received diets containing coconut oil, and two groups received diets containing corn oil. One group of 16 animals fed each fat source received diets prepared to contain 1.5 ppm and 7.5 ppm of copper and zinc, respectively. The other two groups of 16 animals were fed diets prepared to contain 1.5 ppm and 7.5 ppm of copper and zinc. respectively. At the end of four weeks of feeding, each of the four subgroups was further divided into two groups of eight animals each to provide the final eight experimental diets used in the study. The animals from four of these eight subgroups were fed diets in which the copper was increased from 1.5 ppm to 6.0 ppm. The experimental feeding was continued for six more weeks.

The animals were weighed at weekly intervals, and the weight gains were calculated. At the end of ten weeks, animals were fasted for 12 hours and anesthetized with ether. Blood samples were taken from these animals via a heart puncture using 10 ml disposable syringes[2] and No. 20 disposable needles[3]. Prior to the drawing of blood, each syringe and needle was rinsed several times with a sodium heparin solution (10 USP units/ml). Plasma was obtained by spinning the blood samples at 2500 x g for 10 minutes at 10°C in a refrigerated centrifuge[4]. Livers of randomly selected animals were removed after the animals were sacrificed, weighed, dried in an oven at 60°C, and wet-ashed on a hot plate with nitric and perchloric acids.

Diets

Coconut oil and corn oil were selected for the study due to the contrast in the contents of polyunsaturated fatty acids, especially linoleic acid and saturated fatty acids. Coconut oil is also devoid of cholesterol and has a high content of lauric acid and myristic acid, the two

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

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

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

fatty acids previously shown to have the most potent hypercholesterolemic effect in man (Hegsted et al., 1965).

The level of fat[5] (coconut oil or corn oil) used for the diet was 13 percent (by weight). In addition to fat, each diet contained 51 percent starch[6], 28 percent spray dried egg white solids[7], 4 percent mineral mix[8], 2 percent cellulose[9] 2 percent vitamin mix, and 24 drops of oleum percomorphum[10] per kilogram of diet. The percentage of total calories supplied by the corn starch, egg white solids, and oil were 50, 20, and 30 percent respectively. The compositions of the vitamin and mineral mixtures are given in Appendix A, Tables 1 and 2.

Analyses of the diets revealed that the non-supplemented coconut oil or corn oil diets contained 1.5 ppm of copper. The non-supplemented coconut oil diet contained

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[5]Products of ICN Nutritional Biochemicals, Cleveland, Ohio.

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

[7]Teklad Test Diets, Madison, Wisconsin.

[8]Hawk-Oser Mineral Mix, Teklad Test Diets, Madison, Wisconsin.

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

[10]Mead Johnson and Company, Evansville, Indiana. The composition of this product is listed as 1250 units of vitamin A and 180 units of vitamin D per drop.

0.5 ppm of zinc, while the non-supplemented corn oil diet contained 0.6 ppm of zinc.

Appendix A, Table 3, shows the amounts of copper and zinc supplements used to provide the final concentrations of these minerals in the eight experimental diets. Sources for these two supplements were cupric sulfate and zinc carbonate.

Analytical Methods

In the separation of HDL-cholesterol fraction, a 175 µl aliquot of plasma was added to a Centrifi-Kit tube[11] containing a premeasured amount of sodium phosphotungstate and mixed. The mixture was centrifuged at 30 psig for 10 minutes in a Beckman Airfuge[11] with an A-100 fixed-angle rotor. The supernatant portion which contained only HDL-cholesterol was removed.

Plasma total and HDL-cholesterol were determined by a standard colorimetric method (Stanbio Laboratory Inc., 1973) with the Liebermann-Burchard cholesterol reagent[12]. For the cholesterol determination, 0.1 ml of plasma, 0.1 ml of distilled water (blank) and 0.1 ml of cholesterol standards were mixed with 6.0 ml of SR Direct Cholesterol Reagent, and placed in a 37°C water bath. After 20 minutes of incubation,

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[11]Beckman Instruments, Inc., Spinco Division, Palo Alto, California.

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

cholesterol determination was made by reading specimens and standard against the reagent blank at 625 nm on a spectrophotometer[13] within 30 minutes.

The liver residues obtained from wet-ashing were dissolved in 3 ml of 0.6 N HCl and diluted to 25 ml with redistilled water. Copper, iron, and zinc contents of appropriate aliquots were determined by means of atomic absorption spectrophotometry[14]. One-gram samples of each diet were also prepared for mineral analysis by the wet-ashing technique previously described. Copper and zinc determinations were made on appropriate aliquots of the ashed residues diluted to 25 ml with redistilled water by atomic absorption spectrophotometry.

Statistical Analyses

All data were subjected to standard analysis of variance techniques (Snedecor, 1962). The Duncan's Multiple Range test (Freud et al., 1960) was used to determine differences between individual means when significant differences were found between diets.

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

[14]Model 551, Instrumentation Laboratories, Wilmington, Massachusettes.

CHAPTER IV

RESULTS AND DISCUSSION

Detailed data obtained from this study are presented in Appendix B. Statistical analyses of all data are given in Appendix C.

Growth

The weight gains of animals fed corn oil and coconut oil with and without supplements are given in Table 1. Analysis of the data indicated that rats consuming the corn oil diets generally had significantly higher ($p \leq 0.05$) weight gains than did animals fed diets containing coconut oil (Appendix C, Table 1). The main effects of copper and zinc on weight gain were not statistically significant, but a highly significant ($p \leq 0.01$) zinc x copper interaction was observed. High copper, low zinc levels resulted in decreased weight gain, while high dietary copper and zinc levels were associated with increased weight gain. Separately, increasing copper or zinc levels of the diet were associated with slight but not statistically significant increases in weight gains, regardless of the source of fat.

Although the animals fed the corn oil diets appeared to consume more food during the experimental period than did rats consuming the coconut oil, adjusting of weight gains for food consumption by covariance analysis revealed that the

Table 1

Effects of Copper and Zinc Supplements on Growth of Young Rats Fed Coconut Oil and Corn Oil

Fat source	Dietary copper	Dietary zinc ppm: 7.5	30.0
	ppm	10 week weight gain (gm)[1]	
Coconut oil	1.5	294[2]ab ± 30	258a ± 42
	6.0	271ab ± 45	302bc ± 43
Corn oil	1.5	311bc ± 40	299ab ± 39
	6.0	282ab ± 45	344c ± 35

[1]Each value is the mean of 8 animals ± SEM

[2]Means not sharing common superscript letters are significantly different ($p \leq 0.05$).

effects of fat source on growth were still significant. Thus, it would appear that the differences in weight gains observed were not due to the differences in food consumption (Appendix C, Table 2).

Total Cholesterol

The analysis of variance (Appendix C, Table 3) revealed highly significant effects ($p \leq 0.01$) of fat source, dietary copper, and dietary zinc. Animals fed corn oil had higher plasma total cholesterol levels than did animals fed coconut

Table 1

Effects of Copper and Zinc Supplements on Food Consumption of Young Rats Fed Coconut Oil and Corn Oil

Fat source	Dietary copper	Dietary zinc ppm: 7.5	30.0
	ppm	10 week food consumption (gm)[1]	
Coconut oil	1.5	869[2a] ± 77	870[a] ± 103
	6.0	922[a] ± 89	944[a] ± 128
Corn oil	1.5	947[a] ± 42	955[a] ± 52
	6.0	894[a] ± 86	981[a] ± 47

[1]Each value is the mean of 8 animals ± SEM.

[2]Means not sharing common superscript letters are significantly different ($p \leq 0.05$).

oil (Table 2). Overall, copper supplementation tended to lower plasma total cholesterol levels, regardless of fat source. Zinc supplementation was associated with slight increases in plasma total cholesterol of animals fed coconut oil, but substantial increases in plasma total cholesterol levels of animals fed corn oil. The highest plasma total cholesterol was observed in rats fed corn oil supplemented with both copper and zinc. The effect of a high zinc level on plasma total cholesterol apparently depended upon the

Table 3

Effects of Copper and Zinc Supplements on Total Cholesterol Concentrations of Young Rats Fed Coconut Oil and Corn Oil

Fat source	Dietary copper	Dietary zinc ppm: 7.5	30.0
	ppm	mg/dl[1]	
Coconut oil	1.5	92.1[2b] ± 6.9	81.6[a] ± 4.6
	6.0	75.1[a] ± 3.5	80.9[a] ± 2.7
Corn oil	1.5	106.2[c] ± 8.0	125.1[d] ± 3.7
	6.0	95.1[b] ± 5.6	107.1[c] ±11.0

Baseline values:

Initial: 58.3 ± 7.6 mg/dl
Animals fed coconut oil after 4 weeks: 89.6 ± 12.9 mg/dl
Animals fed corn oil after 4 weeks: 86.8 ± 16.6 mg/dl

[1]Each value is the mean of 8 animals ± SEM

[2]Means not sharing common superscript letters are significantly different ($p \leq 0.05$).

type of fat and the level of copper in the diet.

Total cholesterol level was higher in animals fed corn oil for 4 weeks than those fed coconut oil. The level of total cholesterol, however, remained higher for animals maintained on the corn oil diets over the experimental period (Appendix B, Tables 9-10).

Table 4

Effects of Copper and Zinc Supplements on HDL-Cholesterol Concentrations of Young Rats Fed Coconut Oil and Corn Oil

Fat source	Dietary copper	Dietary zinc ppm: 7.5	Dietary zinc ppm: 30.0
	ppm	mg/dl[1]	
Coconut oil	1.5	44.7[2b] ± 6.5	53.3[c] ± 3.4
	6.0	32.3[a] ± 4.5	59.9[c] ±10.8
Corn oil	1.5	83.8[d] ± 4.3	98.8[e] ±13.5
	6.0	78.4[d] ± 5.7	82.9[d] ± 5.2

Baseline values:

Initial: 27.4 ± 3.9 mg/dl
Animals fed coconut oil for 4 weeks: 57.3 ± 14.3 mg/dl
Animals fed corn oil for 4 weeks: 59.6 ± 9.4 mg/dl

[1]Each value is the mean ± SEM

[2]Means not sharing common superscript letters are significantly different ($p \leq 0.05$).

HDL-Cholesterol

HDL-cholesterol levels of animals fed the various test diets are shown in Table 4. Analysis of the HDL-cholesterol data revealed highly significant ($p \leq 0.01$) differences in plasma HDL-cholesterol levels associated with fat source and the dietary level of copper or zinc (Appendix C, Table 4).

HDL-cholesterol levels were higher in animals fed corn oil than in the animals fed coconut oil. Increasing dietary copper resulted in lowered HDL-cholesterol levels, regardless of fat source and dietary zinc level. Increases in dietary zinc were generally associated with increases in HDL-cholesterol levels. These results would suggest that increases in HDL-cholesterol, a condition which has been suggested as favorable in preventing CHD, can be stimulated with zinc supplementation. High dietary copper levels were generally associated with lowered HDL-cholesterol levels in the plasma.

Animals fed with corn oil tended to have higher HDL-cholesterol both at the end of 4 weeks and at the end of the experimental period than did the animals fed coconut oil (Appendix B, Tables 9-10)

HDL-cholesterol/total cholesterol ratios of rats fed the various test diets are shown in Table 5. The rats fed the corn oil diets had significantly higher ($p \leq 0.05$) HDL-cholesterol/total cholesterol ratios than did animals fed the coconut oil diets (Appendix C, Table 5). Analysis of the data revealed that the main effect of zinc on HDL-choesterol/total cholesterol ratios was highly significant ($p \leq 0.01$) and that additional zinc was associated with increases in this ratio (Appendix C, Table 5). The effect of zinc, however, was only found to occur in those animals fed coconut oil. Copper supplementation generally had no significant effect on HDL-cholesterol/total cholesterol ratio, although the

Table 5

Effects of Copper and Zinc Supplements on HDL-Cholesterol to Total Cholesterol Ratios of Young Rats Fed Coconut Oil and Corn Oil

Fat source	Dietary copper	Dietary zinc ppm: 7.5	Dietary zinc 30.0
	ppm		
Coconut oil	1.5	0.49[1b] ± 0.07	0.68[c] ± 0.07
	6.0	0.43[a] ± 0.07	0.74[d] ± 0.13
Corn oil	1.5	0.79[e] ± 0.06	0.79[e] ± 0.12
	6.0	0.83[f] ± 0.06	0.78[e] ± 0.09

[1]Means not sharing common superscript letters are significantly different ($p \leq 0.05$)

highest ratio was observed in rats fed corn oil and supplemented with both copper and zinc.

Liver Copper

Liver copper data obtained in the study are presented in Table 6. Animals fed with corn oil had significantly lower ($p \leq 0.05$) liver copper levels than did animals fed coconut oil (Appendix C, Table 6). Increasing dietary copper resulted in highly significant ($p \leq 0.01$) liver copper deposition, regardless of fat source or dietary zinc

Table 6

Effects of Copper and Zinc Supplements on

Liver Copper Contents of Young Rats

Fed Coconut Oil and Corn Oil

Fat source	Dietary copper	Dietary zinc ppm: 7.5		Dietary zinc 30.0	
	ppm	µg/gm dry wt.[1]			
Coconut oil	1.5	9.3[2bc]	± 0.8	9.0[abc]	± 1.9
	6.0	14.6[d]	± 1.1	15.2[d]	± 0.5
Corn oil	1.5	6.3[a]	± 1.3	6.6[ab]	± 2.0
	6.0	15.9[d]	± 0.6	11.9[bc]	± 3.2

Baseline values:

Initial: 42.5 ± 8.1 µg/gm dry wt.
Animals fed coconut oil for 4 weeks: 12.8 ± 3.8 µg/gm dry wt.
Animals fed corn oil for 4 weeks: 9.7 ± 2.6 µg/gm dry wt.

[1]Each value is the mean ± SEM

[2]Means not sharing common superscript letters are significantly different ($p \leq 0.05$).

level. Increasing the dietary zinc level had no significant effect on liver copper level in this study.

A comparison of the average baseline copper value to the liver copper values over the experimental period indicated a decline in liver copper deposition. The decrease in liver copper deposition was more apparent in animals fed the corn oil diets(Appendix B, Tables 9-10).

Liver Iron

Increasing the dietary copper level from 1.5 ppm to 6.0 ppm was associated with a highly significant decrease ($p \leq 0.01$) in liver iron deposition, regardless of fat source (Table 7 and Appendix C, Table 7). Although the main effect

Table 7

Effects of Copper and Zinc Supplements on Liver Iron Contents of Young Rats Fed Coconut Oil and Corn Oil

Fat sources	Dietary copper	Dietary zinc ppm: 7.5	Dietary zinc ppm: 30.0
	ppm	µg/gm dry wt.[1]	
Coconut oil	1.5	199.4[2bc] ± 29.4	191.7[bc] ± 47.5
	6.0	153.3[ab] ± 45.0	134.3[ab] ± 31.5
Corn oil	1.5	273.7[c] ± 80.0	184.8[abc] ± 81.6
	6.0	135.5[ab] ± 34.6	99.4[a] ± 12.2

Baseline values:

Initial: 60.3 ± 13.3 µg/gm dry wt.
Animals fed coconut oil for 4 weeks: 93.8 ± 13.5 µg/gm dry wt.

Animals fed corn oil for 4 weeks: 97.7 ± 9.4 µg/gm dry wt.

[1]Each value is the mean ± SEM

[2]Means not sharing common superscript letters are significantly different ($p \leq 0.05$).

of zinc was not statistically significant, the lowest levels of liver iron were found in animals fed the corn oil diets containing 6.0 and 30.0 ppm of copper and zinc, respectively. Liver iron levels in animals increased with age. The type of fat in the diet did not appear to affect liver iron deposition (Appendix B, Tables 9-10).

Liver Zinc

Liver zinc levels of the animals fed the various test diets are given in Table 8. Significantly more liver zinc ($p \leq 0.05$) was observed in the animals fed the corn oil diets than in animals receiving coconut oil (Appendix C, Table 8).

Analysis of the data also revealed a significant fat x zinc interaction ($p \leq 0.05$). Interpretation of these results would suggest that increasing the level of diet zinc would increase liver zinc levels in animals fed a source of saturated fatty acids (such as coconut oil) and would decrease liver zinc levels in animals fed a source of polyunsaturated fatty acids (such as corn oil).

A comparison of the average baseline liver zinc values to the liver values over the experimental period showed that some increase in liver zinc deposition occurred in animals fed corn oil. The liver zinc levels of the animals fed coconut oil were essentially the same as the baseline levels (Appendix B, Tables 9-10).

Table 8

Effects of Copper and Zinc Supplements on
Liver Zinc Contents of Young Rats
Fed Coconut Oil and Corn Oil

Fat source	Dietary copper	Dietary zinc ppm: 7.5	30.0
	ppm	µg/gm dry wt.[1]	
Coconut oil	1.5	74.5[2ab] ± 3.4	75.6[ab] ± 6.4
	6.0	72.5[a] ± 4.9	78.3[ab] ± 4.6
Corn oil	1.5	90.8[c] ±14.1	83.6[bc] ± 6.4
	6.0	91.1[c] ± 5.9	84.2[bc] ± 4.3

Baseline values:

Initial: 169.2 ± 59.7 µg/gm dry wt.
Animals fed coconut oil for 4 weeks: 73.2 ± 5.2 µg/gm dry wt.
Animals fed corn oil for 4 weeks: 77.2 ± 9.1 µg/gm dry wt.

[1]Each value is the mean ± SEM

[2]Means not sharing common superscript letters are significantly different ($p \leq 0.05$).

Hair Coat Appearance

As the length of the experimental period increased, excessive losses of hair over the entire body or primarliy around the forehead only (baldness) were observed in some of the animals fed the test diets (Appendix B, Tables 12-13). Of the 22 animals which exhibited baldness, 15 of these were on the corn oil diets, while 7 of the animals received the coconut oils. Ten of the animals receiving coconut oil exhibited excessive hair loss of the entire body, while only 3 of the rats receiving the corn oil diets had excessive hair losses from the entire body.

CHAPTER V

GENERAL DISCUSSION

Results of this study indicated that corn oil supports growth better than coconut oil. These results were similar to those reported by Bettger et al. (1979) who reported that rats fed coconut oil grew less rapidly than those fed corn oil when zinc was limited. Dietary levels of 1.5 and 7.5 ppm of copper and zinc, respectively, appear to be marginal for optimum growth since additional growth was observed when the dietary levels of both minerals were increased in the diet. 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 of zinc. There is also an indication that a dietary Zn/Cu ratio of 5 may be required for optimum growth of young rats because when the ratio was either 1.25 or 20 the growth of young rats was not as good as when the ratio was 5. There is also the possibility that the level of copper and zinc required for maximum growth may be higher if the fat source contains a high degree of saturated fatty acids, while increasing the polyunsaturated fatty acid content of the diet may have a sparing effect of these two minerals.

The decreases in total and HDL-cholesterol levels observed in rats fed corn oil diets low in dietary zinc were similar to the previous findings of Koo and Williams (1981). Increasing dietary copper was associated with lower total and HDL-cholesterol levels in young rats fed either coconut oil or corn oil. The elevation of total cholesterol level associated with a decrease in dietary copper in corn oil diet was in agreement with previous reports (Lei, 1977, 1978).

Recent information, however, suggests that gross measurements of serum or plasma cholesterol alone may not be as good predictors of the risk of CHD as is a measurement of HDL-cholesterol. Miller and Miller (1975) have suggested that the tissue cholesterol pool is unrelated to the plasma concentration of cholesterol and is inversely related to the plasma level of HDL-cholesterol. The HDL-cholesterol/total cholesterol ratio has been suggested as a more accurate indicator for predicting the risk associated with CHD. A low HDL/total cholesterol ratio would suggest a high risk possibility of CHD. In this study animals fed the corn oil diets had higher HDL-cholesterol/total cholesterol ratios than did animals fed the coconut oil diets. Increasing the level of dietary zinc in rats fed coconut oil resulted in a significant increase in this ratio, but an increase in dietary zinc had little effect on the ratio in animals fed corn oil. An increase in dietary copper had little effect on the HDL-cholesterol/total cholesterol ratio, regardless of the type of fat in the diet. These data would support the hypothesis that zinc plays a more important role in the elevation of this ratio than does copper, especially if saturated fatty acids are predominated in the diet.

Liver copper levels were observed to be significantly higher in young rats fed coconut oil than in animals fed corn oil. Increasing the level of dietary copper resulted in marked increases in liver copper deposition in animals fed either fat source. Increases in dietary zinc had no apparent effect on liver copper deposition on rats fed diets high in either saturated or polyunsaturated fatty acids. Based on these data, it would appear that the availability of copper is enhanced if the diet contains a high percentage of saturated fatty acids.

The availability of dietary iron for liver deposition did not appear to be influenced by the different types of dietary fat since liver iron levels were similar in animals fed either coconut oil or corn oil. These data do not support previous findings of Amine and Hegsted (1975), who reported that the presence of a saturated fat enhanced the absorption and utilization of iron in rats. The level of copper in the diet appeared to have a significant effect on liver iron deposition, because increased levels of dietary copper were associated with marked reductions in liver iron levels. Increasing levels of dietary zinc were associated with decreases in liver iron levels which did not appear to be statistically significant.

In contrast to the copper findings, the availability of zinc for liver deposition appeared to be greater when the diet contained a high percentage of polyunsaturated fatty acids. Increases in dietary copper had no significant effect on liver zinc deposition in this study. Increases in dietary zinc were associated with slight increases in liver zinc levels in rats fed coconut oil and slight decreases in liver zinc levels in animals fed corn oil.

Although the exact causes for the baldness and/or excessive loss of body hair observed in some of the animals fed the experimental diets are not known, some speculations can be made. The type of skin lesion which was described as baldness could be associated with diets composed of a high percentage of PUFA since this condition was more prevalent in animals fed corn oil. There is the possibility that lipid peroxidation of the unsaturated bonds occurred in the corn oil diets with time, and the presence of excess lipid peroxides may have caused this particular type of skin lesion. Bettger et al. (1979) suggested that skin lesions and reduced weight gains occurring in young rats fed coconut oil diets low in zinc were due to a precipitated essential fatty acid deficiency. Thus, there is a possibility that a similar situation occurred in this study since approximately 31 percent of the animals receiving coconut oil had excessive losses of body hair.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Summary

Young male rats were fed diets containing two levels of zinc, two levels of copper, and two fat sources (coconut oil and corn oil). Criteria used to evaluate the responses of animals fed the different diets included weight gain, plasma total and HDL-cholesterol levels, and copper, iron, and zinc deposition in the liver.

Rats fed diets containing corn oil had significantly higher ($p \leq 0.05$) weight gains than rats fed diets containing coconut oil. The differences in weight gains appeared to be associated with differences in fatty acid composition of the diets and not due to differences in food consumption. The data also suggested that at least levels of 6 ppm of copper and 30 ppm of zinc were needed for optimal growth for young rats. There is also the possibility that these levels may be higher if the diet is composed of primarily saturated fatty acids.

Animals fed corn oil diets had higher plasma total and HDL-cholesterol levels than did animals fed coconut oil. Zinc supplementation was associated with elevated plasma total cholesterol levels in animals fed corn oil, whereas copper supplementation was associated with lower plasma total cholesterol levels, regardless of fat source. An increase in dietary zinc was associated with an increase in HDL-cholesterol, while lower HDL-cholesterol levels were generally associated with an increase in copper supplementation. Zinc supplementation was associated with an increase in HDL-cholesterol/total cholesterol ratio, particularly in animals fed coconut oil.

Liver copper deposition was significantly higher ($p \leq 0.05$) in rats fed coconut oil containing a low level of copper (1.5 ppm) than in rats fed corn oil diets low in copper. Copper supplementation resulted in highly significant increases in liver copper levels in rats fed either fat source. The presence of unsaturated fatty acids in the diet appeared to favor the deposition of iron in livers of young rats. Increasing both copper and zinc in the diet resulted in decreases in liver iron deposition of animals fed both fat sources, although the effect of zinc was not statistically significant.

As the length of the experimental period progressed, rats fed the coconut oil diets appeared to exhibit excessive losses of hair from the entire body. Rats fed the corn oil, however, showed a tendency to lose hair locally around the forehead (baldness) as the length of the experimental period increased.

Recommendations

In order to demonstrate the effect of fat source on young rats, the amount of fat included in the test diets was modified to provide 30% of the total calories. This is a level that is approximately three times the amount usually found in a typical rat diet. Thus, it would be interesting to evaluate the response of young rats with regard to the criteria used for this study with dietary fat levels lower than those used for this study. There is also the possibility that the diets containing corn oil, which is composed of a high percentage of PUFA, may undergo excessive lipid peroxidation in an experiment that is continued at least 10 weeks. Perhaps different effects of fat source would be noted if an antioxidant was used to protect the unsaturated fatty acids.

There were two kinds of skin lesions developed in the course of the study. These lesions appeared to be distinct and related to a particular type of dietary fat. Animals fed the coconut oil diet may have been marginal in essential fatty acids, while the type of lesions observed in animals fed corn oil may have been related to the oxidation of the unsaturated bonds which predominated in these particular diets. Studies involving the supplementation of essential fatty acids and/or antioxidants to saturated and unsaturated fat diets could be beneficial in clarifying this situation.

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APPENDIX A

Table 1

Composition of Vitamin Mixture[a]

Constituents	Amount per 2 kg mix
	mg
Biotin	20
Folic acid	100
Vitamin B_{12}	2
Thiamin HCl	500
Pyridoxine HCl	500
	gm
Riboflavin	1
Nicotinic acid	1
Menadione (2-methyl-napthaquinone)	1
Ca pantothenate	3
p-aminobenzoic acid	100
Inositol	100
Choline chloride	150
DL-methionine	600
Corn starch	1040

[a]All vitamin and methionine purchased from ICN Pharmaceutical, Inc., Cleveland, Ohio.

Table 2

Composition of Mineral Mix, Hawk-Oser[a]

Constituents	g/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)	0.09246
Potassium iodide	0.0414

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

Table 3

Zinc and Copper Supplementation Data

	Dietary treatments[a]							
	1	2	3	4	5	6	7	8
Total zinc required	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
Zinc in basal diet	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.6
Difference in zinc	7.0	7.0	29.5	29.5	6.9	6.9	29.4	29.4
Zinc supplementation[b]	13.5	13.5	56.6	56.6	13.2	13.2	56.4	56.4
Total copper required	1.5	6.0	1.5	6.0	1.5	6.0	1.5	6.0
Copper in basal diet	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Difference in copper	-	4.5	-	4.5	-	4.5	-	4.5
Copper supplementation[c]	-	17.7	-	17.7	-	17.7	-	17.7

[a]Quantity expressed in ppm.

[b]Supplemented in form of $ZnCO_3$.

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

APPENDIX B

Table 1

Weight Gains of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

Rep.		Coconut oil				Corn oil			
	Cu (ppm)	1.5	6.0	1.5	6.0	1.5	6.0	1.5	6.0
	Zn (ppm)	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
		Weight gains at 10 weeks (grams)							
1		278	337	273	246	362	288	340	393
2		345	254	315	347	311	302	352	391
3		315	329	287	302	367	327	308	348
4		302	242	220	305	282	210	278	320
5		287	262	253	341	320	213	306	322
6		232	308	243	222	251	307	282	332
7		299	234	294	314	264	339	309	362
8		296	202	175	340	328	267	218	283
Total		2354	2168	2060	2417	2485	2253	2393	2751
Mean		294	271	258	302	311	282	299	344

Table 2

Food Consumption of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

		Coconut oil				Corn oil			
Rep.	Cu (ppm)	1.5	6.0	1.5	6.0	1.5	6.0	1.5	6.0
	Zn (ppm)	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
		Food consumption at 10 weeks (grams)							
1		739	958	923	665	950	866	983	1067
2		905	879	954	980	972	929	993	1005
3		891	1076	913	978	1012	972	912	970
4		880	793	732	954	868	761	866	895
5		820	874	917	1020	959	775	903	961
6		788	965	757	823	895	956	979	1005
7		936	1002	1022	1065	952	1017	1036	995
8		994	828	742	1065	964	876	967	946
Total		6953	7375	6960	7550	7572	7152	7639	7844
Mean		869	922	870	944	947	894	955	981

Table 3

Total Cholesterol Concentration of Young Rats Red Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

	Coconut oil				Corn oil			
Rep. Cu (ppm)	1.5	6.0	1.5	6.0	1.5	6.0	1.6	6.0
Zn (ppm)	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
	Total cholesterol concentration (mg/dl)							
1	87.5	72.5	78.8	79.5	102.5	91.5	120.2	100.9
2	105.3	73.7	80.5	78.2	98.0	102.5	126.5	110.5
3	90.6	79.2	83.6	81.8	117.5	88.5	128.8	109.6
4	96.0	70.8	82.0	83.9	100.8	92.0	123.3	105.5
5	91.3	77.6	84.0	82.0	95.8	87.0	131.5	114.3
6	82.9	79.0	72.1	78.2	107.5	99.4	124.0	107.1
7	98.3	78.3	89.2	77.9	117.6	100.8	126.2	83.9
8	85.1	70.0	82.3	85.5	109.1	99.1	120.0	124.8
Total	737.0	601.1	652.5	647.0	848.8	760.8	1000.5	856.6
Mean	92.1	75.1	81.6	80.9	106.2	95.1	125.1	107.1

Table 4

HDL-Cholesterol Concentration of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

Rep.		Coconut oil				Corn oil			
	Cu (ppm)	1.5	6.0	1.5	6.0	1.5	6.0	1.5	6.0
	Zn (ppm)	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
		HDL-cholesterol concentration (mg/dl)							
1		48.1	40.5	53.5	62.9	85.3	79.4	92.5	82.0
2		50.2	28.0	57.1	60.3	80.5	82.2	95.1	90.5
3		37.3	29.8	54.5	60.8	85.3	75.1	100.7	86.4
4		56.5	30.2	48.9	79.0	88.0	75.8	109.3	88.3
5		46.0	35.5	52.7	58.9	79.0	77.7	104.7	80.9
6		42.8	33.9	59.5	37.1	91.0	68.0	70.0	84.1
7		35.7	25.6	59.6	64.8	83.8	89.1	98.6	77.6
8		40.7	34.5	56.6	55.5	77.5	80.0	119.4	73.6
Total		357.3	258.0	442.4	479.3	670.4	627.3	790.3	663.4
Mean		44.7	32.3	55.3	59.9	83.8	78.4	98.8	82.9

Table 5

HDL-Cholesterol/Total Cholesterol Ratios of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

	Coconut oil				Corn Oil			
Rep. Cu (ppm)	1.5	6.0	1.5	6.0	1.5	6.0	1.5	6.0
Zn (ppm)	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
1	0.55	0.56	0.68	0.79	0.83	0.87	0.77	0.81
2	0.48	0.38	0.71	0.77	0.82	0.80	0.75	0.82
3	0.41	0.38	0.65	0.74	0.73	0.85	0.78	0.79
4	0.59	0.43	0.60	0.94	0.87	0.82	0.89	0.84
5	0.50	0.46	0.63	0.72	0.82	0.89	0.80	0.71
6	0.52	0.43	0.83	0.47	0.85	0.68	0.56	0.79
7	0.36	0.33	0.67	0.83	0.71	0.88	0.78	0.92
8	0.48	0.49	0.69	0.65	0.71	0.81	0.99	0.59
Total	3.89	3.46	5.46	5.91	6.34	6.60	6.32	6.27
Mean	0.49	0.43	0.68	0.74	0.79	0.83	0.79	0.78

Table 6

Liver Copper Contents of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

	Coconut oil				Corn oil			
Rep. Cu (ppm)	1.5	6.0	1.5	6.0	1.5	6.0	1.5	6.0
Zn (ppm)	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
				µg/gm dry wt.				
1	8.9	14.8	6.3	15.3	4.5	15.0	8.6	14.9
2	9.5	16.3	11.6	15.9	5.7	16.6	7.8	15.4
3	10.4	13.9	9.8	14.6	7.9	16.0	3.4	9.1
4	8.4	13.3	8.3	15.1	6.9	16.1	6.5	8.3
Total	37.2	58.3	36.0	60.0	25.0	63.7	26.3	47.7
Mean	9.3	14.6	9.0	15.2	6.3	15.9	6.6	11.9

Table 7

Liver Iron Contents of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

	Coconut oil				Corn oil			
Rep. Cu (ppm) Zn (ppm)	1.5 7.5	6.0 7.5	1.5 30.0	6.0 30.0	1.5 7.5	6.0 7.5	1.5 30.0	6.0 30.0
	μm/gm dry wt.							
1	222.4	181.6	232.0	136.5	326.7	172.1	116.0	101.0
2	158.9	91.0	162.1	125.7	255.0	158.8	106.9	118.2
3	184.4	207.9	159.1	93.5	151.8	82.1	308.6	92.8
4	232.0	132.6	213.6	181.4	361.3	128.8	207.8	85.5
Total	797.7	613.1	766.8	537.1	1094.8	541.8	739.3	397.5
Mean	199.4	153.3	191.7	134.3	273.7	135.5	184.8	99.4

Table 8

Liver Zinc Contents of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

	Coconut oil				Corn oil			
Rep. Cu (ppm)	1.5	6.0	1.5	6.0	1.5	6.0	1.5	6.0
Zn (ppm)	7.5	7.5	30.0	30.0	7.5	7.5	30.0	30.0
	μg/gm dry wt.							
1	71.1	72.1	78.2	76.9	85.4	81.9	86.8	86.6
2	71.1	65.1	73.5	74.8	85.5	90.1	72.6	89.1
3	77.5	74.4	66.7	75.3	77.7	95.2	86.8	77.7
4	78.1	78.5	84.1	86.2	114.7	97.0	88.1	83.3
Total	297.8	290.1	302.5	313.2	363.3	364.2	334.3	336.7
Mean	74.5	72.5	75.6	78.3	90.8	91.1	83.6	84.2

Table 9

Coconut Oil Control Group Data

Animal	Total cholesterol	HDL-cholesterol	Liver microminerals Zinc	Copper	Iron
	mg/dl	mg/dl	μg/gm dry wt.		
1	83.3	49.0	75.7	14.6	115.3
2	100.0	50.0	66.2	10.4	89.1
3	83.3	45.0	76.3	16.9	87.2
4	83.3	60.0	65.8	9.8	99.1
5	111.1	85.0	70.8	12.2	96.5
6	66.7	40.0	82.4	17.8	108.1
7	100.0	55.0	72.7	5.8	68.5
8	88.9	74.0	75.9	14.8	86.4
Total	716.6	458.0	585.8	102.3	750.2
Mean	89.6	57.3	73.2	12.8	93.8

Table 10

Corn Oil Control Group Data

Animal	Total cholesterol	HDL-cholesterol	Liver microminerals Zinc	Copper	Iron
	mg/dl	mg/dl	μg/gm dry wt.		
1	94.4	60.0	70.3	11.8	86.8
2	83.3	57.0	68.8	5.0	85.5
3	116.7	73.0	70.5	6.8	89.8
4	66.7	40.0	74.3	8.0	106.0
5	88.9	60.0	77.7	12.8	93.5
6	100.0	65.0	81.6	12.2	107.8
7	83.3	68.0	75.6	11.0	87.8
8	61.1	54.0	99.0	9.6	108.7
Total	694.4	477.0	617.8	77.2	765.9
Mean	86.8	59.6	77.2	9.7	97.7

Table 11

Baseline Data

Animal	Total cholesterol	HDL-cholesterol	Liver microminerals Zinc	Copper	Iron
	mg/dl	mg/dl	µg/gm dry wt.		
1	48.9	33.0	178.1	38.8	60.5
2	52.1	25.0	128.4	26.3	78.7
3	54.3	28.0	259.1	41.1	65.4
4	58.5	23.0	136.1	56.5	35.2
5	64.9	26.0	100.7	46.9	44.0
6	74.5	28.0	100.0	46.3	69.3
7	54.3	30.0	195.6	37.3	60.5
8	58.5	27.0	255.5	46.8	68.7
Total	466.0	219.2	1353.5	340.0	482.3
Mean	58.3	27.4	169.2	42.5	60.3

Table 12

Extensive Losses of Hair over the Entire Body of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

	Coconut oil				Corn oil				
Rep. Cu (ppm) Zn (ppm)	1.5 7.5	6.0 7.5	1.5 30.0	6.0 30.0	1.5 7.5	6.0 7.5	1.5 30.0	6.0 30.0	Total
1	1	0	0	1	0	0	0	0	2
2	0	1	0	0	0	0	0	0	1
3	0	0	0	1	0	0	0	0	1
4	0	0	1	0	0	1	0	0	2
5	0	0	0	0	0	1	0	0	1
6	0	0	1	1	0	0	0	0	2
7	0	0	0	0	1	0	0	0	1
8	1	1	1	0	0	0	0	0	3
Total	2	2	3	3	1	2	0	0	13

Table 13

Losses of Hair Around the Forehead of Young Rats Fed Coconut Oil and Corn Oil Diets Supplemented with Copper and Zinc

	Coconut oil				Corn oil				
Rep. Cu (ppm) Zn (ppm)	1.5 7.5	6.0 7.5	1.5 30.0	6.0 30.0	1.5 7.5	6.0 7.5	1.5 30.0	6.0 30.0	Total
1	0	1	0	0	1	0	0	0	2
2	0	0	0	1	0	1	1	1	4
3	0	1	0	0	1	1	0	0	3
4	1	0	0	0	1	0	0	1	3
5	0	0	0	0	1	0	1	0	2
6	0	0	0	0	1	1	0	0	2
7	1	0	1	0	0	0	0	0	2
8	0	0	0	1	0	1	1	1	4
Total	2	2	1	2	5	4	3	3	22

APPENDIX C

Table 1

Analysis of Variance of Weight Gain Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	63	163400	
Replication	7	32196	4599
Treatments	7	40917	5845 *
Fat	1	16002	16002 **
Zinc	1	3752	3752
Copper	1	2836	2836
Fat x zinc	1	1620	1620
Fat x copper	1	473	473
Zinc x copper	1	15749	15749 **
Fat x zinc x copper	1	485	485
Error	49		1842

*Significant (≤ 0.05)

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

Table 2

Covariance Analysis of Weight Gain Data

Source of Variation	d.f.	x^2	xy	y^2	Deviation From Regression		
					d.f.	d_{yx}^2	Mean Square
Total	63	534310	102151	163400			
Replication	7	138610	42543	32196			
Treatments	7	94860	54093	40917			
Error	49	300840	5515	90287	48	90186	1879
Treatment plus error	56	395700	59608	131204	55	122225	
For testing adjusted means,					7	32039	4577 *

*Significant ($p \leq 0.05$)

Table 3

Analysis of Variance of Total Cholesterol Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	63	18168.2	
Replication	7	243.0	34.7
Treatments	7	15654.9	2236.4 **
Fat	1	10759.8	10759.8 **
Zinc	1	700.9	700.0 **
Copper	1	2196.4	2196.4 **
Fat x zinc	1	1259.9	1259.9 **
Fat x copper	1	108.9	108.9
Zinc x copper	1	67.7	67.7
Fat x zinc x copper	1	561.3	561.3 **
Error	49	2270.3	46.3

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

Table 4

Analysis of Variance of HDL-Cholesterol Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	63	32170.1	
Replication	7	521.0	74.4
Treatments	7	28559.1	4079.9 **
Fat	1	23040.2	23040.2 **
Zinc	1	3335.8	3335.8 **
Copper	1	838.7	838.7 **
Fat x zinc	1	358.7	358.7 *
Fat x copper	1	186.3	186.3
Zinc x copper	1	48.2	48.2
Fat x zinc x copper	1	751.3	751.3 **
Error	49	3090.0	63.1

*Significant ($p \leq 0.05$)

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

Table 5

Analysis of Variance of HDL-Cholesterol/Total Cholesterol Ratios Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	63	1.73	
Replication	7	0.00	0.00
Treatments	7	1.29	0.18 **
Fat	1	0.72	0.72 **
Zinc	1	0.21	0.21 **
Copper	1	0.00	0.00
Fat x zinc	1	0.30	0.30 **
Fat x copper	1	0.00	0.00
Zinc x copper	1	0.01	0.01
Fat x zinc x copper	1	0.05	0.05 **
Error	49	0.44	0.01

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

Table 6

Analysis of Variance of Liver Copper Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	31	507.8	
Replication	3	18.6	6.2
Treatments	7	418.8	59.8 **
Fat	1	27.6	27.6 **
Zinc	1	5.5	5.5
Copper	1	351.8	351.8 **
Fat x zinc	1	8.1	8.1
Fat x copper	1	6.2	6.2
Zinc x copper	1	5.7	5.7
Fat x zinc x copper	1	13.9	13.9
Error	21	70.4	3.4

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

Table 7

Analysis of Variance of Liver Iron Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	31	157415.7	
Replication	3	11173.3	3724.4
Treatments	7	80137.3	11448.2 *
Fat	1	106.9	106.9
Zinc	1	11502.7	11502.7
Copper	1	53570.8	53570.8 **
Fat x zinc	1	4829.0	4829.9
Fat x copper	1	7215.0	7215.0
Zinc x copper	1	860.1	860.1
Fat x zinc x copper	1	2052.8	2052.8
Error	21	66105.1	3147.9

*Significant ($p \leq 0.05$)

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

Table 8

Analysis of Variance of Liver Zinc Data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	31	3015.4	
Replication	3	608.8	202.9 **
Treatments	7	1455.5	207.9 **
Fat	1	1185.8	1185.8 **
Zinc	1	25.6	25.6
Copper	1	1.2	1.2
Fat x zinc	1	221.6	221.6 *
Fat x copper	1	0.1	0.1
Zinc x copper	1	12.5	12.5
Fat x zinc x copper	1	8.8	8.8
Error	21	951.1	45.3

*Significant ($p \leq 0.05$)

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