EFFECT OF DIETARY ZINC AND COPPER ON
PLASMA ZINC, COPPER, TOTAL CHOLESTEROL, AND
HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN YOUNG
ADULT MALES

by

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(ABSTRACT)

An eight week controlled feeding study was conducted to determine the effects of dietary zinc and copper on plasma zinc, copper, total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) levels in young adult males receiving two levels of zinc. Source of dietary calcium was also varied, however possible interactions or effects of calcium were not determined in this study. Subjects consumed zinc supplements of 10 mg/day, in combination with 1 of 3 diets, resulting in zinc:copper ratios of 30:1 (Dairy Calcium, or DC group), 20:1 (Control or CO group), and 20:1 (Calcium Carbonate or CC group). Copper content of all diets was approximately 1 mg/day. Plasma levels of zinc, copper, TC, and HDL-C did not differ significantly between the groups. However, plasma levels of copper, zinc, and HDL-C, were found to be significantly affected by the specific week of controlled feeding across all 3 groups (p<.05). Plasma copper at baseline was significantly lower than at weeks 2, 4, 6, 8, and post treatment. Plasma zinc at baseline, and weeks 2 and 4, was found to be significantly lower than at weeks 6, 8, and post treatment. At week 6, plasma HDL-C was noted to be significantly higher than at baseline, weeks 2, 4, 8, and post treatment. Spearman correlation coefficients determined negative correlations between plasma copper and TC (r=-0.39, p<0.04), and plasma copper and zinc (r=-0.43, p<0.02) in the DC group. A positive correlation was also noted between plasma zinc and TC (r=0.32, p<0.10) in the DC group. Plasma copper and HDL-C were determined to be negatively correlated in the CO group (r=-0.48, p<0.005). Plasma zinc and HDL-C were found to be negatively correlated in the CC group (r=-0.58, p<0.001).
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INTRODUCTION

Cardiovascular disease (CVD) has been determined to be the leading cause of death in the United States, as well as in other industrialized nations (Castelli, 1983). This major cause of mortality causes approximately 54% of all deaths, twice as many as are caused by malignant neoplasms (National Center for Health Statistics, 1986).

CVD has been found to affect the brain, kidneys, and heart. When the brain is affected, a cerebrovascular accident (CVA), or stroke, may occur. Renal failure may be the result when the kidneys are involved. Coronary heart disease (CHD), which includes angina, coronary insufficiency, myocardial infarction, and congestive heart failure, has been determined to be another manifestation of CVD.

Various factors have been found to be associated with CVD, or specifically atherosclerosis. Diabetics, and those with certain electrocardiogram abnormalities have a greater incidence of CVD (Miller et al, 1975). Risk of developing some form of the disease is greater among men than women (Gordon et al, 1977), and among smokers of cigarettes than non-smokers (Nesje et al, 1985). Also identified as risk factors are physical inactivity (Miller et al, 1975), obesity (Gordon et al, 1977), hypertension (Klevay, 1987), and increasing age (Miller et al, 1977). However, the factor which has probably received the most attention, as well as undergone intensive research, has been serum cholesterol level.

Serum cholesterol can accumulate in the walls of arteries, and be a prominent part of bulky plaques which inhibit the flow of blood, and contribute to clot formation. Alterations in aortal morphology have also been observed in the presence of elevated cholesterol levels (Hunsaker et al, 1983). Arterial obstructions have been found to lead to heart attack, stroke, or renal failure (Miller et al, 1975).

The cholesterol of atherosclerotic plaques is derived mainly from low-density lipoproteins (LDL) that circulate in the blood stream (Brown and Goldstein, 1977).
Low density lipoprotein cholesterol (LDL-C) has been determined to account for approximately 70% of serum total cholesterol (TC) (Miller et al, 1977). With an increased LDL-C level, the risk of clot formation is elevated, therefore increasing the risk of a CVA. While evidence has been found that the infiltration of LDL-C from the serum or plasma occurs (Brown and Goldstein, 1977), the possible contribution of local cholesterol synthesis or impaired clearance of cholesterol from the arterial wall have not been definitely established.

It has been suggested that transport of cholesterol from extra-hepatic tissues to the liver for subsequent catabolism and excretion, may be a function of high density lipoproteins (HDL) (Hammett et al, 1979). In tissue culture studies, HDL enhanced removal of free cholesterol from fibroplasts, smooth muscle cells, macrophages, and lipid accumulated arterial walls (Ballantyne et al, 1982). In epidemiological studies, patients with CHD had lower levels of high density lipoprotein cholesterol (HDL-C) than normal patients (Miller et al, 1977, Ballantyne et al, 1982). The ongoing Framingham study has demonstrated an inverse relationship between HDL-C and incidence of CHD (Castelli et al, 1986). An inverse correlation between LDL-C and HDL-C has also been identified (Gordon et al, 1977).

Alterations in lipoprotein and cholesterol concentrations have been linked to certain dietary components, or minerals. Sodium, magnesium, sulfur, calcium, and manganese have all been noted to be associated with metabolism of cholesterol or lipoproteins (Klevay, 1976). Copper and zinc have also been studied to determine the role(s) or effect(s) these minerals may have on the etiology of CVD.

In 1975, Klevay developed the zinc/copper hypothesis (Klevay, 1975). This hypothesis stated that a "metabolic imbalance in regard to zinc and copper is a major factor in the etiology of coronary heart disease". This metabolic imbalance was noted to be
"either a relative or absolute deficiency of copper, characterized by a high ratio of zinc to copper (Klevay, 1975)".

With dietary copper deficiency, increases in plasma TC in rats have been observed (Allen et al., 1978). Increases in triglyceride (TG) concentrations, decreases in plasma HDL-C, and significant increases in LDL-C have also noted (Koo et al., 1981). Marginal copper deficiency produced abnormal and irregular features of arterial elastin in rats (Hunsaker et al., 1983).

It has been suggested that copper is a component of the metallo-enzyme system responsible for cholesterol and lipoprotein metabolism (Freiden, 1980). Inadequate copper nutriture therefore, may interfere with cholesterol and lipoprotein regulation which maintains or normalizes serum or plasma TC levels.

Zinc has been determined to interfere with copper absorption, thus, this mineral has been suggested to be associated with CVD risk (Klevay, 1975). Zinc has been found to compete with copper for binding to metallothionein, a transport protein (O'Dell, 1984). With increased levels of zinc, suppression of intestinal absorption of copper occurred along with increased copper biliary excretion (O'Dell, 1984). The ratio of zinc to copper may therefore be of potential significance in relation to cardiovascular health.

JUSTIFICATION

While research has advanced the information available regarding the cause(s), treatment(s), and prevention of CVD, it has been found that nutrition is of major importance. Nutrition, either alone or in combination with one or more risk factors, must continue therefore, to be considered in the study of the etiology of CVD.

Medical intervention and treatment is necessary, and may help to reduce incidence of CVD. However, prevention may be of equal, or greater importance. The extent to
which nutrition influences the development of CVD is also important in determining if, and how, diet modification can be implemented as a preventative measure.

Despite extensive research, the effects of zinc and copper on cholesterol and lipoprotein metabolism(s), have yet to be clearly defined. The relationship between dietary intake and CVD development found in various animal and epidemiological studies needs further investigation. This study will assist in determining the effects of dietary zinc and copper on cholesterol and lipoprotein levels in humans.

REVIEW OF LITERATURE

The role of epidemiology in the study of the prevalence of CVD began to emerge in the early part of the 20th century. Findings of subsequent and numerous investigations have helped to define the factors which appear to be associated with, or promote risk for CVD. These risk factors include elevated cholesterol levels (Gordon et al, 1977), altered lipoprotein levels (Castelli et al, 1986), cigarette smoking (Nesje et al, 1985), hypertension (Klevay, 1987), diabetes (Miller et al, 1977), obesity (Gordon et al, 1977), sedentary lifestyle (Allen and Klevay, 1978), and history of atherosclerotic disease (Castelli et al, 1986). However, hypercholesterolemia appears to be one the risk factors most strongly correlated to CVD.

Atherosclerosis has been determined to be the disorder which underlies most CVD's, including CHD and CVA (Castelli et al, 1986). From the accumulation of fatty deposits, primarily plaques of cholesterol and cholesteryl esters, the inner walls of the arteries have been observed to narrow. Smooth muscle and connective tissue have been found to surround the deposits, interfering with circulation efficiency. With a decrease in circulation, less blood is delivered to the tissues, which directly causes the heart, brain, or kidneys cells to cease normal functioning (Strong et al, 1973).
In 1949, the United States Public Health Service began a large-scale study, the Epidemiological Investigation of Cardiovascular Diseases, in Framingham, Massachusetts. More than 5000 healthy men and women, ages 30 to 62 years, were examined every year for evidence of CHD and other atherosclerotic conditions. Elevated levels of serum cholesterol were found consistently in association with increased CHD rates (Kannel et al, 1971). Kannel et al (1971), and Carlson and Bottiger (1972), found that the risk of developing CHD increased with increasing plasma cholesterol. Miller et al, (1975) also determined an association between elevated serum cholesterol and increased risk of CHD.

LIPOPROTEINS AND CHOLESTEROL:

While cholesterol has been determined to be the main component of atherosclerotic plaques, the actual source of the cholesterol has been determined to be highly important. Cholesterol has appeared in plasma or serum as a component of lipoproteins. Approximately 10-13% is found in very low density lipoprotein (VLDL), 70% in LDL, and 17-20% in HDL (Nesje et al, 1985). However, this distribution has varied individually. There is increasing evidence that CVD is more strongly related to distribution of serum TC among the different lipoprotein classes than to total serum cholesterol alone.

Studies have emphasized LDL-C and HDL-C in association with serum cholesterol levels and risk of atherosclerosis and CVD. Increased LDL-C appears to increase the risk of the development of atherosclerosis (Brown and Goldstein, 1976), while increased HDL-C has been found to inhibit the atherogenic process (Miller et al, 1977).

LOW DENSITY LIPOPROTEINS AND CHOLESTEROL METABOLISM:

The cholesterol of atherosclerotic plaques is derived from circulating LDL in the
bloodstream (Brown and Goldstein, 1976). The blood level of LDL has been determined to be affected by the number of specialized proteins, LDL receptors, that project from the surface of animal cells (Brown and Goldstein, 1976). The lipoproteins were bound by the receptors and taken into the cells, thus removing LDL from the bloodstream. The LDL was noted to be broken down, and cholesterol made available for cell metabolism and maintenance. Alterations in this receptor-mediated process affected LDL levels. With an increased LDL level, the risk of atherosclerosis is increased (Brown and Goldstein, 1976).

HIGH DENSITY LIPOPROTEINS AND CHOLESTEROL METABOLISM:

Cholesterol has been found to be synthesized in most tissues of the body, including arterial wall and atheromatous tissue. During cholesterol turnover, transportation from the tissues to the liver has been noted, where catabolism and excretion occurred (Miller et al, 1977). HDL facilitated the uptake of cholesterol from extra-hepatic tissues and transport to the liver (Miller et al, 1977), and were rapidly cleared through a high affinity, receptor-mediated process (Lei, 1983). Reduction of HDL concentration accelerated the development of atherosclerosis by impairing the clearance of cholesterol from arterial wall, and therefore, transport to the liver was inhibited (Miller et al, 1977).

High density lipoproteins consist of 2 subfractions: 1) HDL with apolipoprotein-E (apo-E), or HDL₁, which interacts with specific receptors of fibroblasts; 2) HDL with no apo-E, or HDL₂, which does not interact with receptors (Mahley, 1981). HDL₁ has been found to be formed from HDL₂ as the particles become enriched in cholesteryl esters and acquire apo-E either from de novo synthesis or VLDL (Mahley, 1978).

Cholesterol has been determined to exist within the body in esterified and unesterified forms, but only the latter can exchange readily between plasma lipoproteins and tissues
(Gordon et al, 1977). The activity of the plasma cholesterol esterifying enzyme, lecithin-cholesterol acyltransferase (LCAT), has been shown in vitro to promote the transfer of cholesterol from erythrocytes to plasma by maintaining a shift in the equilibrium between plasma and cell membrane unesterified cholesterol (Gjone et al, 1978). LCAT has been found to preferentially esterify HDL unesterified cholesterol, and apolipoprotein A of HDL, the principal LCAT activator (Glomet, 1979). Therefore, HDL unesterified cholesterol and the role of LCAT in esterifying this cholesterol, were found to be highly important in the efflux of cellular cholesterol and the transport of cholesterol to the liver (Glomet, 1979).

HDL-C has been observed to have an inverse relation to the risk of CHD. In 1968, the Framingham Study initiated an investigation of lipoproteins in relation to CHD. It was determined that there was an inverse correlation of HDL-C to incidence of CHD, and that of the lipoproteins and lipids measured, HDL-C had the largest impact on risk (Gordon et al, 1977). A more recent report of the Framingham Study, a 12 year update, also found that the proportion of TC carried by HDL-C was a consistent and important indicator of CHD risk (Castelli et al, 1986).

The Tromso Heart Study, a 2 year case control study of males, 20 to 49 years of age, found that coronary risk was inversely related to HDL-C (Miller et al, 1977). Subjects with CHD had decreased HDL-C levels. Berg et al (1976) and Hsia et al (1975) found that plasma from CHD patients had decreased concentrations of HDL-C. Hsia et al (1975) also determined that along with this decrease in HDL-C, there was less capacity in CHD patients to bind additional exogenous cholesterol than in healthy subjects.

Bondjers and Bjorkerud (1975) observed that in human arterial tissue, HDL had a significant effect on elimination of cholesterol from tissue sites. Bailey (1965) also observed that HDL was necessary for the facilitated transport of cholesterol from cells. Based on the observed roles of HDL and LDL in extra-hepatic cellular efflux and influx of
cholesterol, it would appear that the percentage of cholesterol transferred by these lipoproteins would also change the net accumulation of cholesterol by arterial tissue, and risk of atherosclerosis (Allen and Klevay, 1980). Therefore, determination of LDL-C and HDL-C levels in the bloodstream would be important in the assessment of CVD risk.

RELATIONSHIP BETWEEN DIETARY COPPER AND ZINC, AND LIPID METABOLISM:

Essential micronutrient metals have been determined to be linked with cholesterol and lipoprotein metabolism. Through a considerable number of studies, dietary copper and zinc have been found to be related to serum and/or plasma levels of TC and lipoproteins (Klevay, 1973; Klevay, 1975; Petering et al, 1977; Eisemann et al, 1979; Looney and Lei, 1978; Frimpong and Magee, 1987; Caster and Parthemas, 1976).

COPPER AND CHOLESTEROL:

Dietary copper deficiency in rats has produced plasma hypercholesterolemia (Klevay, 1973, 1974, 1975), and it has been hypothesized that an absolute or relative deficiency of copper is a factor in the etiology of CHD (Klevay, 1975). The influence of copper nutriture on plasma cholesterol concentrations, therefore, is assumed to be of particular significance since an elevation of plasma cholesterol has been determined to be one of the principal indicators of CHD risk (Allen and Klevay, 1978).

The role(s) of copper in cholesterol and lipoprotein metabolism have not been well understood. Through mostly animal studies, hypercholesterolemia has been hypothesized to be due to an increase in cholesterol synthesis, a decrease in degradation, or a decrease in bile acid excretion (Allen and Klevay, 1978), in the presence of dietary copper inadequacy. Also, various studies have been conducted to determine the mechanism(s) which caused the observed hypercholesterolemia in copper deficiency. Reductions in
LCAT activity (Harvey and Allen, 1981, Lau and Klevay, 1981), and lipoprotein lipase activity (Lau and Klevay, 1982), have been suggested to contribute to copper deficiency induced hypercholesterolemia.

Copper inadequacy has been hypothesized to play a role in the atherogenic process by interfering with the functioning of the lysyl oxidase enzyme which is responsible for the extra-cellular cross-linking of elastin (Hunsaker et al, 1983). Alterations in this cross-linking affected blood flow and clot formation, leading to increased incidence of CVD (Hunsaker et al, 1983).

Allen and Klevay (1978) investigated the influence of copper deficiency on the appearance of newly synthesized cholesterol in the plasma lipids of male weanling rats following $^3$H mevalonate injection. Mevalonate has been observed to be an obligatory metabolite in cholesterol synthesis (Allen and Klevay, 1978), and $^3$H incorporation into plasma lipids following injection was a measure of net cholesterol influx to, and efflux from, the plasma pool during a given interval. Changes in $^3$H incorporation in the plasma pool were found to be dependent upon cholesterol synthesis, clearance to the plasma, degradation to bile acids in the liver with subsequent biliary excretion, and uptake of cholesterol by extra-hepatic tissues. Rats were fed either a copper deficient diet of 0.57 ug copper/g diet or a control diet of 5.0 ug copper/g diet. After 181 days, mean plasma TC was significantly elevated by 130% in the copper deficient rats, 109.6 mg/dl compared to the control group, 48.9 mg/dl. The percent dose of $^3$H mevalonate incorporated into plasma lipids was elevated by 100%, cholesteryl esters were elevated by 80%, and free cholesterol was elevated by 162% in the copper deficient rats (Allen and Klevay, 1978). The increase in both plasma cholesterol and $^3$H incorporation into plasma lipids in response to copper deficiency suggested a role for dietary copper in regulating the clearance of newly synthesized cholesterol from the liver to the plasma pool.

Shao and Lei (1980) also examined the effects of copper deficiency on the
conversion of injected mevalonate into cholesterol in male weanling rats. Rats were fed either a diet deficient in copper, $2 \text{ ug/g}$ diet, or $18 \text{ ug copper/g}$ diet for 8 weeks. Free serum TC of the copper deficient rats was significantly elevated at all times after injection of $^{14}\text{C}$ mevalonate. Mean TC levels of the copper deficient group at 40, 80, and 120 minutes after injection were 12.3, 13.6, and 15.4 mg/dl respectively. Mean TC levels of the copper adequate group were 5.94, 10.97, and 9.3 mg/dl at 40, 80, and 120 minutes respectively. Mean serum ester cholesterol concentration also appeared to be elevated in copper deficiency, but the trend was not significant. Mean liver ester cholesterol concentration was significantly reduced in the rats fed the copper deficient diet, mean level of $0.09 \text{ mg cholesterol/g}$ compared to $0.15 \text{ mg cholesterol/g}$ for the adequate group. Liver ester cholesterol specific activity reached a peak at or before 40 minutes in rats fed the copper deficient diet. The specific activity of liver ester cholesterol from the rats fed the copper adequate diet increased between 40 and 120 minutes. Shao and Lei (1980) suggested that in the rats fed the copper deficient, the liver ester cholesterol, newly synthesized from $[^{14}\text{C}]$ mevalonate, was leaving the liver pool at an increased rate. The serum ester cholesterol specific activity was elevated in rats fed the copper deficient diet at all times, therefore, suggesting that the newly synthesized ester cholesterol was entering the serum pool at an increased rate or leaving the serum pool at a reduced rate, or both. Shao and Lei (1980) hypothesized that these findings may explain the hypercholesterolemia found in copper deficient rats.

Lin and Lei (1981) observed the influence of copper deficiency on the turnover and kinetics of serum TC metabolism in male weanling rats. For 16 weeks, rats were fed either a diet consisting of $<2 \text{ ug copper/g}$ diet or $8 \text{ ug copper/g}$ diet. Copper deficient and copper adequate rats were injected with $[4-^{14}\text{C}]$ cholesterol, and the serum cholesterol specific activity was determined by disappearance curves. Disappearance curves of the specific activity was subjected to a kinetic two-pool analysis. The amount of cholesterol in
pool A in copper deficient rats was larger than that of copper adequate rats. The half-life of the rapidly exchangeable pool A was increased in the presence of copper deficiency. A reduction in the rate of cholesterol degradation in pool A, or in the removal of cholesterol was indicated, which may have been responsible for the hypercholesterolemia found in the copper deficient rats (Lin and Lei, 1981).

Lei (1978) studied the effects of copper deficiency on in vivo catabolism and excretion of [26-14 C] cholesterol in male weanling rats. Rats were fed either a copper deficient diet of <2 ug copper/g diet, or a copper adequate diet of 18 ug copper/g diet for 8 weeks. A significant increase in mean serum TC and ester cholesterol was observed in the copper deficient rats, 112.4 mg/dl and 86.9 mg/dl respectively, when compared to serum TC and ester cholesterol of copper adequate rats, 83.2 mg/dl and 64.8 mg/dl, respectively. Liver free cholesterol was also found to be significantly reduced in copper deficiency, 1.55 mg/g liver compared to 1.80 mg/g liver for the copper adequate rats. Reduction of free cholesterol in the liver may have contributed to the increase in serum TC in the rats fed the copper deficient diets (Lei, 1978). Decreases of serum free, ester, and total cholesterol specific activities were determined. These reductions may have resulted from a small increase in the serum pool size, or a small increase in the turnover of these metabolites. Rates of oxidation and excretion of [26-14 C] cholesterol were not influenced by dietary copper. Therefore, Lei (1978) concluded that a shift of cholesterol from the liver to the serum pool appeared to be responsible for copper deficiency induced hypercholesterolemia, not reduced cholesterol degradation.

Cunnane et al (1986) have observed the effects of copper deficiency in male mice pups on liver cholesterol levels. Mice were fed either a copper deficient diet with 0 ug copper/ml water, or a copper supplemented diet of 2 ug copper/ml water for 4 weeks. Results indicated that the liver TC of copper deficient mice, 1.95 mg/g liver, was not significantly lower than the copper adequate mice, 1.87 mg/g, as found in a previous
study (Harvey and Allen, 1985). These researchers determined that unless dietary copper deficiency is severe and prolonged, the concentration of TC in mice liver does not change. Plasma or serum cholesterol levels were not studied.

Research with adult monkeys has shown that high levels of ascorbic acid have an adverse effect on copper metabolism (Milne et al, 1981). Since dietary copper deficiency has been implicated in producing hypercholesterolemia in rats (Klevay, 1973, 1975; Lei, 1978), Milne et al (1981) have studied consumption of 1 mg ascorbic acid/kg body wt/day or 25 mg ascorbic acid/kg body wt/day on serum TC in monkeys fed a depletion diet of 0.1 ug copper/ml water for 28 weeks, and then repleted with 0.5 ug copper/ml water. Serum TC significantly increased from week 1 to week 28, 81.6 mg/dl to 111.2 mg/dl, in rats that consumed 1 mg ascorbic acid/kg body wt/day. A significant increase in mean TC was also noted in rats that consumed 25 mg ascorbic acid/kg body wt/day with a level of 79 mg/dl noted at week 4 to 105.3 mg/dl at week 28. With 0.5 ug/ml copper added to the diets from weeks 28 to 32, serum TC decreased to 99.1 mg/dl in the group consuming the low ascorbic acid supplement, while in the high ascorbic acid supplement group, mean TC continued to increase to 113.4 mg/dl. The researchers concluded that copper metabolism was adversely affected by ascorbic acid and therefore, cholesterol metabolism was also impaired.

Klevay et al (1984) have studied the effects of copper deficiency on cholesterol metabolism in a young adult man. The dietary copper intake of adults has been recommended to be approximately 2-3 mg/day (Committee on Dietary Allowances, Food and Nutrition Board, 1989). However, an high intake of a component such as zinc which has been found to interfere with copper absorption or availability, would increase the requirement to over 2 mg/day. A depletion diet consisting of 0.83 mg/day of copper was consumed for 105 days, an amount which has been found to be similar to that in some contemporary diets (Klevay, 1975; Guthrie, 1973), and then supplemented with 4 mg
copper/day for 39 days. Plasma TC significantly increased during depletion, 202 to 234 mg/dl, and significantly decreased during repletion to 198 mg/dl.

COPPER AND LIPOPROTEINS:

In the rat, and other animals resistant to atherosclerosis, the majority of plasma cholesterol has been found to be carried in HDL's (Klevay, 1975). However, in man and animals susceptible to the atherogenic process, LDL's carry the major portion of plasma cholesterol (Brown and Goldstein, 1976). Allen and Klevay (1980), found that dietary copper deficiency in the rat produced a significant decrease in the percentage of plasma cholesterol transported by HDL, and a significant increase in LDL bound cholesterol. However, because plasma cholesterol was markedly elevated, the absolute amount of cholesterol carried by HDL was markedly increased by copper deficiency (Allen and Klevay, 1980).

HDL's and LDL's have been found to play roles in extra-hepatic cellular transport of cholesterol (Miller et al, 1975; Brown and Goldstein, 1976). The deposition of cholesterol in arterial tissue would then be affected by changes in the percentage of cholesterol carried by the lipoproteins. Therefore, CVD risk would not only be associated with total serum or plasma cholesterol, but would also be associated to the distribution of cholesterol among lipoproteins (Brown and Goldstein, 1976).

Gjone et al (1978) found that individuals with familial type II hyperlipoproteinemia had elevated plasma LDL's caused by a reduction in LDL degradation, rather than by an abnormality in LDL synthesis. These researchers made the observation that the hypercholesterolemia found in the presence of copper deficiency may be caused therefore, by this noted reduction in lipoprotein degradation, rather than an increase in cholesterol synthesis (Gjone et al, 1978).

Lei and Lin (1981), investigated the role of copper deficiency on the process of
cholesterol degradation, and examined the effects of copper deficiency on the half-life of HDL-C in male weanling rats. Rats, fed either a copper deficient diet, 1.6 ug copper/g diet, or a copper adequate diet, 8 ug copper/g diet for 16 weeks, were injected with a tracer dose of [4-14 C] cholesterol after 5 weeks of treatment. Disappearance curves of the specific activity of free cholesterol or TC associated with serum HDL were determined. Mean levels of free cholesterol and TC in serum HDL were also observed. The mean level of free cholesterol in HDL was found to be elevated in the copper deficient group, 13.93 mg/dl, compared to the copper adequate group, 10.71 mg/dl. Mean serum HDL-C was also elevated in the copper deficient group, 48.40 mg/dl, compared to the copper adequate group, 36.37 mg/dl. Increases in heart weight, and heart to body ratio were also found in the copper deficient group, an indication of copper deficiency as found previously (Hill, 1969). In the copper deficient group, the mean disappearance curve of the specific activity of TC in the HDL was significantly lower than that of the adequate group. Similar results were observed for the specific activity curve of free cholesterol in HDL. The half life of the free cholesterol and TC associated with HDL was also prolonged in rats fed the copper deficient diet. These results suggested that a slower turnover of the HDL-C may have resulted in the increase in HDL-C concentration.

Lei (1983) investigated the alterations in the lipid, lipoprotein, and apolipoprotein concentrations induced by copper deficiency in male weanling rats. Rats consumed either a copper adequate diet, 8 ug copper/g diet, or a copper deficient diet, 0.85 ug copper/g diet for 7 weeks. A significant increase in LDL-C and HDL-C was observed in copper deficient rats. In the copper deficient group, mean plasma LDL-C was 43.1 mg/dl, and HDL-C was 60.5 mg/dl. The copper adequate group had mean plasma LDL-C of 23.1 mg/dl, and HDL-C of 45.3 mg/dl. Apolipoprotein-E of HDL was found to be significantly elevated in copper deficiency by 43%, 17.4 mg/dl, compared to 12.2 mg/dl for the copper adequate rats. The increased concentration of apo-E in the plasma HDL
fraction observed in this study suggested that copper deficiency may have resulted in an increased concentration of HDL₁. Since plasma HDL's are cleared rapidly by the liver, the copper deficient animal may have responded to hypercholesterolemia by increasing the plasma level of the HDL with apo-E. Lei (1983) suggested that the hypercholesterolemia, and prolonged half-life of cholesterol as found in a previous study (Lei and Lin, 1981) found with copper deficiency, is more likely associated with an impairment in the cholesterol degradation process, possibly in the uptake of HDL by hepatic receptors.

Allen and Klevay (1980) observed the effects of copper deficiency on total plasma cholesterol and lipoproteins in male weanling rats. Rats were fed a copper deficient diet of 0.57 ug copper/g diet, or a copper supplemented diet of 5.0 ug copper/g diet, for 48 days. Copper deficient rats had a significantly higher mean plasma TC, 147.8 mg/dl, than the copper supplemented rats, 72.5 mg/dl. Mean plasma HDL-C content of the copper deficient group was significantly higher, 114.2 mg/dl, than the copper supplemented group, 61.7 mg/dl. Mean plasma LDL-C level was also higher in the copper deficient group, 19.4 mg/dl, compared to the supplemented rats, 6.8 mg/dl. The authors concluded that dietary copper deficiency in the rat produced a significant decrease in the percentage of plasma cholesterol transported by HDL and a significant increase in the LDL-C. Based on Brown and Goldstein's (1976) findings on the role of HDL and LDL bound cholesterol in extra hepatic efflux and influx of cholesterol, changes in the percentage of cholesterol transported by these lipoproteins would also change the net accumulation of cholesterol by arterial tissue.

Lefevre et al (1986) studied the effects of copper deficiency induced hypercholesterolemia on HDL subfractions and hepatic lipoprotein receptor activity in the adult male rat. Copper deficient rats consumed a diet with only a trace amount of copper, and copper adequate rats were fed 5 ug copper/g diet for 6 weeks. In the copper deficient group, mean plasma TC was 57.4 mg/dl and HDL-C was 44.0 mg/dl. These values were
significantly increased over mean plasma TC levels of 48.8 mg/dl, and HDL-C of 36.4 mg/dl for the copper adequate rats. HDL fractions were shown to be enriched with apo-E, as found in a previous study (Lei, 1983). Through kinetic analysis, hepatic membranes from the copper deficient rats bound significantly fewer lipoproteins than the copper adequate rats. A strong negative correlation was observed between hepatic lipoprotein receptor activity and HDL₁ levels. Marcel et al (1980) had noted in a previous study that HDL₁ was a poor substrate for LCAT, decreasing LCAT activity. The HDL₁ subclass has been found to bind to at least two distinct hepatic receptors, LDL or apo-B, E receptor, and chylomicron remnant or apo-E receptor (Gordon et al, 1983). With decreased HDL binding, there was a HDL cholesteryl ester accumulation, and subsequently, an increase in apo-E enriched HDL (Lefevre et al, 1986). Higher HDL in copper deficiency therefore appeared to be due to lower HDL binding by the liver (Lefevre et al, 1986). VLDL-C, LDL-C, nor protein concentrations were affected by copper levels, suggesting that HDL metabolism is preferentially affected by copper deficiency. In other studies (Allen and Klevay, 1980; Lei, 1983), copper deficient rats were found to have increased LDL-C as well as HDL-C. Lefevre et al (1986) noted that these studies were more severely copper deficient. It has therefore been suggested that high LDL-C may represent a later occurrence in the development of copper deficiency induced hypercholesterolemia (Lefevre et al, 1986).

LECTHIN CHOLESTEROL-ACYLTRANSFERASE REGULATION OF CHOLESTEROL AND LIPOPROTEINS:

Plasma LCAT has been found to catalyze the transfer of fatty acids from the C-2 position of lecithin to the hydroxyl group of unesterified cholesterol (Glomset, 1968). LCAT preferentially esterifies HDL bound free cholesterol (Gjone et al, 1978), and apo-A
of HDL has been determined to be the principal activator of the enzyme (Glomset, 1979). By the esterification of cholesterol, transfer of cholesteryl esters to LDL and VLDL occurred in association with apo-E (Harvey and Allen, 1981). Receptor-mediated uptake at the extra-hepatic cells then removed the cholesteryl esters. Hepatic mechanisms may also have facilitated removal (Goldstein and Brown, 1977).

The esterification of HDL free cholesterol by LCAT, as well as the role of HDL bound cholesterol, has been hypothesized to be important in the transport of cholesterol to the liver for catabolism (Insull, 1973). It has been determined that alterations in LCAT activity therefore, affected plasma cholesterol levels.

COPPER AND LCAT ACTIVITY:

Copper deficient animals have been found to be hypercholesterolic (Klevay, 1975), as well as hypocupremic (Klevay, 1980), have fibrotic hearts (Allen and Klevay, 1978), abnormal electrocardiograms (Klevay et al, 1981), and arteries with abnormal connective tissue (Klevay, 1980; O'Dell, 1961). Atherosclerotic patients have been observed to have similar manifestations, as well as low LCAT activity (Liu et al, 1979).

Lau and Klevay (1981) studied the relationship of copper nutriture and LCAT activity in male weanling rats. Rats were fed a diet containing either 2 ug copper/ml of water, or 0 ug copper/ml of water. Mean plasma TC concentrations at 26, 44, and 46 days were elevated in the copper deficient rats, 93, 121, and 153 mg/dl respectively, as compared to the copper supplemented rats, 77, 92, and 125 mg/dl, respectively. A significant reduction of 22 to 32% of enzyme activity was observed in the plasma of copper deficient rats, with mean values of 11, 11, and 10% labeled cholesterol esterified/100 ul plasma/hour, respectively. Mean values of LCAT activity for copper supplemented rats were 17, 16, and 14% labeled cholesterol esterified/100 ul plasma/hour, respectively. The authors concluded that copper may therefore be required for the
synthesis of LCAT or as a constituent of the enzyme (Lau and Klevay, 1981).

Harvey and Allen (1981) also found hypercholesterolemia in copper deficient weanling rats, along with a significant increase in plasma free cholesterol, and markedly decreased LCAT activity. Rats were fed a diet containing either 0.6 ug copper/g diet or 5.9 ug copper/g diet. Mean plasma TC was significantly elevated in the copper deficient rats, 110.8 mg/dl, while the copper supplemented rats were found to have a level of 70.5 mg/dl. Mean plasma free cholesterol was significantly elevated in the copper deficient rats, 22.2 mg/dl, as compared to 15.4 mg/dl for copper supplemented rats. Fractional LCAT activity for the copper deficient group was determined to be 0.8 % labeled cholesterol esterified/hour and significantly lower than the copper supplemented group, 3.1% labeled cholesterol esterified/hour. Due to the observed depressed LCAT activity and an increase in plasma free cholesterol with copper deficiency, Harvey and Allen (1981) hypothesized in vivo roles for copper in LCAT activity. Altered LCAT activity in copper deficiency may include a direct role for copper in the LCAT process, either as a possible co-factor, regulator, or in the association of LCAT and HDL (Harvey and Allen, 1981). LCAT was noted to be synthesized in the liver (Lau and Klevay, 1981), and a decrease in liver copper, evident of copper deficiency, has been found to impair adequate synthesis (Allen and Klevay, 1978). LCAT has been suggested to be a metallo-enzyme, requiring a metal for activity, or for further activation (Suzue et al, 1980). If copper is necessary for activation, deficiency of the metal will affect activity of the enzyme.

Decreased LCAT activity may be associated with reductions in specific apolipoprotein synthesis, in particular, apo-A and apo-E (Harvey and Allen, 1981). Apo-E allows for cholesteryl ester transport from HDL following LCAT esterification of free cholesterol, to other lipoproteins (Glomset, 1979). Apo-A has been hypothesized as being the principal activator of LCAT (Glomset, 1979). Allen and Klevay (1980) found that the percentage of HDL-C was reduced in copper deficient rats, while VLDL-C and LDL-C were
increased, and therefore suggested that alterations in lipoprotein metabolism may affect LCAT activity in the presence of copper deficiency.

COPPER AND LIPOPROTEIN LIPASE ACTIVITY:

Goldstein and Brown (1977) have hypothesized that the absorption and intracellular synthesis of cholesterol are controlled by the LDL receptor pathway. The LDL was noted to bind to the receptor on the extra-hepatic cellular surface and a series of steps was initiated, leading to the suppression of cholesterol synthesis. Lipoprotein lipase (LPL) was determined to act on the VLDL's to produce particles resembling LDL, and therefore, cholesterol regulation was affected (Cataprano et al, 1980).

Lipoprotein lipase was found in the vascular endothelial surface of adipose tissue, heart, skeletal muscle, lung, and mammary gland (Tan, 1978). The enzyme was determined to be tightly bound to the endothelial wall, and released by heparin which has a high affinity for the enzyme molecule (Chohan and Cryer, 1980). Heparin, normally present in the bloodstream of humans, along with apo-C-II and divalent cations form a complex which is necessary for activation of the enzyme (Strinivasan et al, 1975). Copper may be one of the metals required for enzyme structure and activation (Klevay, 1975).

Lau and Klevay (1982) have investigated the effects of copper deficiency on LPL activity in male weanling rats. Rats were fed diets containing either adequate copper, 2 ug/ml water, or deficient in copper, 0 ug/ml water. At 21, 26, and 39 days, mean plasma TC in the copper deficient groups was determined to be 111, 131, and 145 mg/dl, respectively, and significantly higher than the copper supplemented rats, 88, 101, and 105 mg/dl, respectively. LPL activity was significantly decreased by 40-47% in copper deficient rats. In copper deficient rats at 21, 26, and 39 days, LPL activity was 1200, 700, and 900 cpm 14C-oleic acid, respectively, versus values of 2400, 1000, and 1500 cpm 14C-oleic acid, respectively, for the copper supplemented groups. Lau and
Klevay (1982) have hypothesized that if the activation of LPL is dependent on copper, receptors with reduced copper content would have impaired function, and caused elevations in serum or plasma cholesterol.

COPPER AND AORTAL MORPHOLOGICAL CHANGES:

The effects of copper nutriture in adult male rats, dams, and male pups fed diets containing 2 ug copper/g diet and 10 ug copper/g diet were studied (Hunsaker et al, 1983). The copper requirement determined by the National Research Council subcommittee on Laboratory Animal Nutrition falls within the range of 5-9 ug/g diet (National Research Council, 1978). Therefore, the levels of copper given were considered marginal, not deficient. Upon analysis, mean serum TC differences failed to be statistically significant. Mean serum TC for the dams consuming 2 ug copper/g diet was 82.2 mg/dl versus 83.1 mg/dl for the dams consuming 10 ug copper/g diet. Serum copper was only slightly depressed in the lower copper diet, while liver copper was reduced to a significant degree. Dams on the copper deficient diet had a mean liver copper of 11.8 ug/g, while those on the higher copper diet had a level of 15 ug/g. This suggested an increase in turnover from the liver to the serum pool. Despite the lack of effect of low dietary copper on serum cholesterol, electron micrographs of the aorta in marginally deficient animals revealed morphologic abnormalities. Elastin was clumped, and of irregular size and shape. Collagen was found to be loosely arranged, with evident discontinuities. Narrower arteries were also observed (Hunsaker et al, 1983).

As previously noted, increasing age has been determined to be a risk factor for CVD (Allen and Klevay, 1978). As aging proceeds, the arterial wall has been noted to show disorder and degradation, including diminished cross-linking of elastin (Gerrity and Cliff, 1978). These morphological changes have been attributed to decreased activity and/or amount of the copper metallo-enzyme, lysyl oxidase (Kitano, 1980; Coulson and
Carnes, 1967). Lysyl oxidase mediates the extra-cellular cross-linking of 2 elastin molecules to form mature elastic laminae (Kitano, 1980). The activity of lysyl oxidase and the extent of elastin cross-linking have been found to be reduced in swine (Coulson and Carnes, 1967), and chicks (O'Dell, 1961) consuming diets deficient in copper. Diminished cross-linking of elastin has been positively associated with degree of atheroma (Gerrity, 1972). Therefore, insufficient dietary copper may promote or enhance the age and/or atherosclerosis related changes in arterial elastin.

ZINC AND LIPID METABOLISM:

The metabolism of cholesterol and lipoprotein has been found to be influenced by the nutritional status of zinc as evidenced by alterations in plasma and serum levels of cholesterol and lipoproteins during zinc depletion or repletion (Schneeman et al, 1986; Koo and Ramlet, 1983; Koo et al, 1986; Freeland-Graves et al, 1982; Hooper et al, 1980; Koo et al, 1981). However, the mechanisms by which zinc status affects these levels has yet to be determined.

The effect of zinc status on the distribution of serum cholesterol among the major serum lipoproteins was assessed in adult male rats fed a zinc deficient diet of 0.37 ug zinc/g diet as compared with those fed a control diet of 41 ug zinc/g diet (Koo et al, 1981). Within 4 weeks of dietary treatment, the group consuming the zinc deficient diet demonstrated a significant reduction from 78 mg/dl to 63.8 mg/dl in mean serum TC, mainly due to a decline in HDL-C from 53.0 mg/dl to 45 mg/dl. Mean plasma TG levels also significantly decreased from 85.5 mg/dl to 64 mg/dl. No significant alterations were noticed in VLDL-C or LDL-C, while a positive correlation between serum zinc and HDL-C were determined. These observations were found to be of significance in view of the epidemiological evidence for the inverse relationship between HDL-C and the incidence of CHD (Miller et al, 1977; Castelli et al, 1977; Gordon et al, 1977;
Miller et al, 1975). However, the biochemical explanation for the close association between serum zinc and HDL-C has not been defined, but possible hypotheses have been suggested (Freeland-Graves et al, 1982; Pories et al, 1974; Koo et al, 1986; Schneeman et al, 1986; Hooper et al, 1980; Koo et al, 1983).

The role of zinc in protein synthesis has suggested a mechanism by which zinc deficiency may produce alterations in the apoprotein of HDL (Pories et al, 1974). In zinc deficiency, there was a decreased incorporation of amino acids into proteins. The cause of the decreased protein synthesis was decreased synthesis of ribosomal RNA due to decreased activity of DNA dependent RNA polymerase, an enzyme which requires zinc. Zinc has also been found to be required for the activity of thymidine kinase which is essential for DNA synthesis and apoprotein synthesis (Pories et al, 1974).

An investigation of the relationship between serum levels of zinc and HDL-C was observed in adult male rats (Koo et al, 1983). One control diet containing no cholesterol and an experimental diet containing 1% cholesterol were formulated with an equal level of 30 ug zinc/g diet. Rats fed the 1% cholesterol diet had a significantly decreased mean serum HDL-C level, 70.7 mg/dl, as compared to the control group, 79.5 mg/dl. Mean serum zinc levels were also significantly different with values of 177 mg/dl for the cholesterol fed rats, and 197 mg/dl for the control rats. Mean serum TC levels were not significant. Mean serum zinc was found to be positively correlated with serum HDL-C (r=0.57, p<0.001). It was not determined however, whether the decrease in serum zinc by cholesterol intake was due to altered intestinal absorption processes or by changes in the tissue distribution, or by turnover and excretion of zinc. The positive correlation observed between serum zinc and HDL-C was found to exist under conditions where dietary cholesterol is the only variable with an adequate level of zinc. However, it was not suggested that the cholesterol-induced decline in HDL-C was secondary to the change in serum zinc or vice versa. The suggestion made was that there may be a specific metabolic
link between zinc and HDL-C (Koo et al, 1983).

Plasma clearance and hepatic uptake of chylomicron cholesterol were investigated in adult male rats fed 3 ug zinc/g diet as compared with those fed 30 ug zinc/g diet (Koo et al, 1986). Through intravenous injection, rats received lymph chylomicrons labeled in vivo with 14-C-cholesterol. The rats who consumed 3 ug zinc/g diet, were found to demonstrate a significant delay in plasma chylomicron cholesterol clearance. This delayed removal was suggested to be due to induced alterations in the morphological characteristics and apoprotein makeup of chylomicrons as found in another study (Henderson et al, 1984). Koo et al (1986) found that intestinal chylomicrons were abnormally large and exhibited a strong tendency to coalesce into even larger droplets. Lymph chylomicrons showed marked decreases in the relative contents of apo-C and apo-E which are known to be important in the metabolism of chylomicrons (Schaefer et al, 1978; Green et al, 1981). Apoprotein C has been determined to activate endothelial lipoprotein lipase, inhibit hepatic uptake, and facilitate the extra-hepatic lipolysis of chylomicrons (Schaefer et al, 1978). Apo-E was found to participate in the hepatic recognition and removal of the remnants formed subsequent to peripheral lipolysis (Schaefer et al, 1978). Therefore, the abnormally large sizes of chylomicrons, and the marked reduction in apo-C and apo-E induced by zinc deficiency may cause a delay in the extra-hepatic lipolysis and/or the subsequent receptor-mediated uptake of the remnants by the liver, leading to a slow removal of chylomicrons from the plasma.

Schneeman et al (1986) studied alterations in HDL composition due to zinc deficiency, or to reduction in food intake. Three groups of male adult rats were fed a zinc adequate diet of 100 ug zinc/g diet ad libitum, a zinc deficient diet of < 1 ug zinc/g diet ad libitum, or a zinc adequate diet of 100 ug zinc/g diet with restricted feeding. Mean TC levels were not significant. The mean plasma zinc level of the zinc deficient rats, 0.69 mg/dl, was found to be significantly lower than the restricted feeding and zinc adequate groups with
values of 1.61 mg/dl and 1.49 mg/dl, respectively. Mean plasma HDL-C was significantly lower in the restricted feeding and zinc deficient groups, 38.6 mg/dl and 35 mg/dl respectively, compared to the zinc adequate group, 47.7 mg/dl. Mean plasma TG's were significantly lower in the restricted feeding and zinc deficient groups with levels of 50.4 mg/dl and 58.7 mg/dl respectively, as compared to the zinc adequate group, 82.3 mg/dl. It was hypothesized that these alterations may be due to either the reduction in food associated with zinc deficiency (Thompson et al, 1979), or by the zinc deficiency induced decrease of chylomicron formation (Koo et al, 1986). In both circumstances, plasma TG's were found to be reduced by zinc deficiency, and were associated with a reduction in the plasma pool of apo-C's since these apoproteins are important in the metabolism and clearance of TG's. The apo-C's have been found to be exchanged between the HDL fraction and the VLDL and chylomicron fractions during alimentary hyperlipemia (Havel et al, 1973). Apo-C-II was determined to activate lipoprotein lipase, and its level in plasma was correlated with plasma TG. (Huff et al, 1981). Schneeman et al (1986), noted that lower ratio of C-II to C-III was observed and may be related to the reduction in plasma TG of the zinc deficient and restricted intake groups, concluding that the reduced food intake associated with zinc deficiency may be an important factor in lipoprotein alterations.

Hooper et al (1980) studied the effect of zinc supplementation on serum lipoprotein values in man. Twelve subjects ingested 160 mg of elemental zinc per day for 5 weeks. Plasma HDL-C levels significantly decreased 25%, from 40.5 mg/dl to 30.1 mg/dl, while plasma TC, TG, and LDL-C did not change significantly. Mean plasma zinc significantly increased with zinc administration from 94.8 ug/dl to 145.7 ug/dl. However, the increase in plasma zinc did not correlate with the decrease in HDL-C. The researchers suggested that zinc may be atherogenic in humans due to its association with
lowering HDL-C levels, not because of an elevated plasma TC level as had been found previously in rats (Klevay, 1973; Klevay, 1975).

Freeland-Graves et al (1982) studied the effects of elemental zinc supplementation at levels of 0, 15, 50, or 100 mg/day on mean HDL-C plasma concentrations in 32 young women for 8 weeks. No significant differences were seen in HDL-C over the 8 weeks except in the 100 mg zinc group at week 4, when a transient decrease from baseline to week 4, 57 to 48 mg/dl, was observed. A weak, but significant negative correlation was noted between levels of plasma HDL-C and plasma zinc \( r = -0.22, p < 0.005 \). The concentrations of plasma zinc peaked at the time plasma HDL-C levels significantly dropped, suggesting that a certain concentration of plasma zinc is necessary to induce a pharmacological response in HDL-C. A homeostatic regulation at the level of intestinal absorption or saturation of zinc-binding proteins in plasma may be possible (Freeland-Graves, 1980).

The effects of zinc supplements on serum lipids in young adult white males has recently been investigated (Black et al, 1988). Forty five subjects were given 50 mg zinc/day, 75 mg zinc/day, or a placebo tablet for 12 weeks. Mean serum TC, LDL-C, and TG levels were not affected by zinc supplements. However, serum HDL-C levels in subjects receiving 75 mg zinc/day were significantly lower at weeks 6 and 12, 53.36 and 53.75 mg/dl, respectively, than for the placebo group, 60.32 and 63.42 mg/dl, respectively. HDL-C at weeks 6, 8, and 12, 53.36, 52.59, and 53.75 mg/dl, respectively, were also significantly lower than at baseline, 61.87 mg/dl, for the group consuming 75 mg zinc/day. The group receiving 50 mg zinc/day had a lower mean serum HDL-C level at week 12, 53.36 mg/dl, than the placebo group, 63.42 mg/dl. The mean serum HDL-C level at week 12 of the 50 mg zinc/day group, 53.36 mg/dl, was also significantly lower than at baseline, 59.55 mg/dl. These findings were similar to
Freeland-Graves et al (1982), which showed a temporary, but significant decline in serum HDL-C in females consuming 100 mg zinc/day.

FACTORs AFFECTING ZINC AND COPPER METABOLISM:

Both zinc and copper have been reported to be involved in cholesterol and lipoprotein metabolism. However, the bioavailability of these minerals from the diet to participate in these functions, have been found to be affected by various factors.

COPPER ABSORPTION, DISTRIBUTION, AND BIOAVAILABILITY:

The primary sites of copper absorption in the human have been determined to be the stomach and the duodenum (Solomons, 1980). The energetics of copper absorption has been found to involve two mechanisms (Bremner, 1987). The first has been found to be energy dependent and involves the absorption of copper-amino acid complexes. The second has been determined to be similar to that for zinc absorption. Copper has been found to bind to a ligand (metallothionein) in the intestine, and then released to the epithelial cell (Solomons, 1981). Normal circulating plasma concentrations of copper are between 80 to 150 ug/dl, 94% of which is present in ceruloplasmin (Solomons, 1981). Dietary copper has been reported to be 25 to 75% absorbed (WHO, 1973). However, copper absorption has been found to be affected by interactions with other nutrients.

Ascorbic acid has been found to be antagonistic towards copper absorption. Finley and Cerkluwski (1983), reported that supplementation of the self-selected diets of young men with 1500 mg of ascorbic acid/day for 2 months resulted in a significant drop in serum ceruloplasmin activity. A similar, but nonsignificant downward trend in serum copper levels was also observed.

Apparent reductions were found in serum ceruloplasmin activity and serum copper levels in adult monkeys fed 25 mg ascorbic acid/kg body wt/day compared to controls fed
1 mg ascorbic acid/kg body wt/day for 32 weeks (Milne et al, 1981). Both diets contained 0.1 ug copper/ml water. Within 1-2 weeks, a significant 20% drop in both ceruloplasmin activity and serum copper was observed in the group consuming 25 mg ascorbic acid/kg body wt/day. Milne et al (1980) also observed decreased copper absorption during high ascorbic acid supplementation in a previous study.

Molybdenum decreased copper bioavailability in ruminants, probably by forming a complex with tetrathiomolybdate (Price and Chesters, 1985). In sheep, molybdenum significantly decreased copper bioavailability in grass diets from 43 to 75 % (Price and Chesters, 1985). The bioavailability of copper in the sheep duodenal digesta was 43% compared to 12% in the rumen, and 28% in the ileal digesta. Duodenal digesta of molybdenum-treated grass had a copper bioavailability 8 times less than untreated grass.

Sources of carbohydrate have affected copper bioavailability. Weanling rats were fed a low copper diet of 1 ug copper/g diet or a copper supplemented diet of 5 ug copper/ml drinking water, in combination with carbohydrate sources of sucrose, starch, or fructose for 7 weeks (Reiser et al, 1983). Regardless of the nature of the carbohydrate, the low copper diet significantly decreased serum ceruloplasmin activity. For the rats that consumed the low copper diet, mortality rates were found to be 10% for starch-fed rats, 30% for sucrose-fed rats, and 35% for fructose-fed rats. Hemoglobin and hematocrit values were significantly lower in rats fed fructose. Copper deficient rats have previously been shown to have a significantly greater hemoglobin breakdown, causing anemia (Paynter and Martin, 1980). Therefore, these results indicated that the fructose-containing diet markedly increased the severity of copper deficiency in rats (Reiser et al, 1983).

To observe the effects of fructose or starch on copper status in humans, a diet comparatively low in copper, 1.03 mg copper/day, containing either 20% fructose or starch, was consumed by male subjects for 8 weeks (Reiser et al, 1985). Fructose
ingestion had no effect on serum ceruloplasmin activity or serum copper concentration, but did significantly reduce cupro-zinc superoxide dismutase (SOD) activity of erythrocytes, 31.6 U $10^9$ erythrocytes, as compared to the starch diet, 38.6 U $10^9$ erythrocytes. Repletion of the subjects with 3 mg copper/day for 3 weeks significantly increased SOD levels in subjects fed fructose, 40.4 U $10^9$ erythrocytes, but not starch, 33.4 U $10^9$ erythrocytes. SOD has been found to be a copper metalloenzyme found in tissues such as liver and erythrocytes, and may be the most sensitive indicator of copper status (Winterbourn et al, 1975). Based on a study conducted by Klevay (1984), serum copper levels and ceruloplasmin activity were found not to be significantly affected by a short term diet less than 12 weeks.

ZINC ABSORPTION, DISTRIBUTION, AND BIOAVAILABILITY:

In the human, dietary zinc has been found to be primarily absorbed in the duodenum and jejunum, facilitated by an intraluminal ligand presumably of pancreatic origin (Evans et al, 1975). Zinc can be absorbed against a concentration gradient. The regulation of zinc absorption has been demonstrated to be largely affected by the induction of mucosal metallothionein (Cousins et al, 1979). Presumably, only part of the zinc taken up by the enterocyte is released into the portal bloodstream. Newly absorbed zinc has been found to be cleared from the portal blood with an efficiency of greater than 60% (Solomons, 1981). The liver has been found to process zinc for release into the peripheral circulation. Forty percent of circulating zinc is tightly bound to $\alpha_2$ globulins and is not exchangeable with the tissues; 60% of the circulating zinc is loosely bound to albumin and amino acids (Solomons, 1981). Normal zinc concentration in the plasma has been determined to be 70 - 125 mg/dl (Evans et al, 1975). A circadian pattern of zinc concentration has been observed, with a peak in the pre-breakfast hours of the morning (O'Dell, 1984).
In humans, Ritchey (1985) has summarized the bioavailability of zinc as the following:

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<th>Subjects</th>
<th>% Apparent Absorption</th>
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<tr>
<td>Preadolescent girls</td>
<td>7.1 - 62.1</td>
</tr>
<tr>
<td>Adolescent girls</td>
<td>7.8 - 11.3</td>
</tr>
<tr>
<td>Adult females</td>
<td>7.8 - 11.5</td>
</tr>
<tr>
<td>Adult males</td>
<td>9.8 - 14.4</td>
</tr>
<tr>
<td>Elderly adults</td>
<td>1.5 - 2.4</td>
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</tbody>
</table>

An important aspect of zinc absorption and bioavailability is the interaction with other dietary components. Fiber has been found to bind zinc and therefore contribute to zinc loss (Ismail-Beigi et al, 1977). By increasing the rate of passage of food along the gastrointestinal tract, fiber may further decrease zinc absorption. Reinhold et al (1986) observed that consumption of 10 grams of cellulose/day reduced zinc balance in humans.

Diets high in soybeans or unleavened whole wheat bread have been found to result in zinc deficiency due to phytate interference with absorption in rats (Davies and Nightingale, 1975). Zinc in seed protein was found to be less available than in animal protein, correlated with the phytate content of the vegetable products (O'Dell, 1984). Likuski and Forbes (1965), noted that calcium may compete with zinc-phytate compounds, decreasing zinc absorption further. However, studies with human subjects fed diets based on natural foods low in phytate have shown that by increasing calcium intake by a factor of 3 or 4 has no effect on zinc absorption (O'Dell et al, 1972).

A strong interaction has been found between zinc and copper (Van Campen and Scarfe, 1967; Storey and Greger, 1987). Although the nutritional effect of excess zinc on copper metabolism has been determined to be much stronger than vice versa (Taper et al, 1980; Klevay, 1973; Sandstead, 1978; Solomons, 1979) evidence has been found that excess copper decreases zinc absorption, possibly due to competition between copper and zinc for binding sites at the surface or within intestinal epithelium (Solomons, 1980).

Two ug copper/ml in drinking water consumed by weanling rats, resulted in a significantly lower mean serum zinc level, 84 ug/dl, when compared to rats who
consumed 1 ug copper/ml of water, 131 ug/dl, for 60 days (Murthy et al, 1974). Water content of zinc was 25 ug zinc/ml for both groups.

One uM of copper decreased 65-zinc absorption in the intestines of weanling rats that consumed supplements of 50 ug zinc/day, while no effect was observed in those not supplemented with zinc and consuming a basal diet of 0.6 ug zinc/g diet (Evans et al, 1974). Copper was also found to inhibit 65-zinc absorption from isolated rat intestinal segments (Van Campen, 1970).

In the human diet, the interference of copper on zinc absorption or utilization has been suggested to be highly unlikely (Greger et al, 1978; Pories et al, 1974). Holden et al (1979) found that based on 14 day intake records, 81% of participating subjects consumed less than 2/3's of the accepted adequate allowance of 2 mg of copper/day. Klevay et al (1979) determined the average daily copper intake of hospital and conventional United States diets to be 0.76 mg/day and 0.78 mg/day respectively, well below a level which would affect zinc absorption.

**EFFECTS OF DIETARY COPPER ON PLASMA OR SERUM COPPER:**

As with zinc assessment, the concentration of copper in plasma or serum has been noted to be the most widely used index of copper nutrure (Solomons, 1981). With copper nutrure estimates however, there are also factors which have been determined to affect circulating levels of copper. These include external contamination of samples; estrogens; corticosteroids; infections; stress; and diurnal variation (Solomons, 1981). Also, 94% of copper in circulation has been determined to be bound to ceruloplasmin, and through studies, most of the aforementioned factors have been found to influence ceruloplasmin production or release (WHO, 1973). Other biochemical and functional procedures for copper assessment are erythrocyte superoxide dismutase activity; serum amine oxidase activity; leukocyte cytochrome c oxidase activity; red cell copper
concentration; skin elastin morphology; tensile strength of skin; radioisotopic copper turnover and pool size; copper balance (Solomons, 1981).

Klevay et al (1984) studied the effects of copper depletion on a young adult male for 188 days. The control phase, during which a diet containing 0.8 mg copper was supplemented with 0.5 mg copper/day, lasted 43 days. The depletion phase lasted 105 days; no copper supplement was given. The repletion phase lasted 39 days; the diet was supplemented with 4 mg copper/day. Plasma copper and ceruloplasmin decreased with time during depletion, and most of the decrease in plasma copper occurred late in depletion. The lowest values for plasma copper and ceruloplasmin in the depletion period were 48 ug/dl and 16.4 mg/dl, respectively, which were significantly lower than baseline plasma copper, approximately 70 ug/dl, and baseline ceruloplasmin, 22 mg/dl (Values obtained from graph). Although not significant, both values increased during repletion which indicated a sensitivity to dietary copper. Superoxide dismutase activity was also noted to decline significantly during depletion and then increased during repletion.

Sloane et al (1985) studied a biracial sample of teenaged females for copper status. Twenty-four hour recall data was collected and plasma copper levels determined. Mean daily copper intakes were 0.92 mg for blacks and 0.83 mg for whites. Although mean plasma copper tended to be higher for blacks at 121 ug/dl, as compared with whites at 116 ug/dl, no significant correlation was found between plasma copper levels and dietary copper.

Allen and Klevay (1978) studied the effects of copper deficiency in male weanling rats. Control rats were given a diet which contained 5.0 ug copper/g diet, while the experimental diet contained 0.57 ug copper/g diet. After 181 days, a significant difference was observed when the mean plasma copper concentration of the copper deficient rats, 0.8 mg/dl, was reduced by 95% as compared to the controls, 1.58 mg/dl. This was an indication that plasma copper was a reliable index of copper status.
Lefevre et al (1986) studied the effects of copper deficiency in adult male rats for 6 weeks. The control group was fed a diet containing 5 ug copper/g diet, while the deficient diet contained only a trace amount of copper. Plasma and liver copper concentrations in the copper deficient group were significantly lower than the controls, which provided evidence of a dietary induced deficiency. Plasma and liver copper for the control group was 1.08 ug/ml and 5.30 ug/g respectively, and in the deficient group, 0.45 ug/ml and 2.71 ug/g respectively.

The effects of dietary copper on serum copper values in weanling rats were studied using water containing 0.25, 0.50, 1.0, or 2.0 ug copper/ml (Murthy et al, 1974). After 60 days, mean serum copper levels of 11.9, 12.7, 30.3, and 51.7 ug/dl were noted for diets containing 0.25, 0.50, 1.0, and 2.0 ug copper/ml water, respectively, and a significant variation in serum copper due to dietary copper was indicated.

For 42 days, weanling rats were fed diets containing less than 1 ug copper/g diet, or 5 ug copper/g diet to study the effects of copper deficiency (Johnson et al, 1987). Those rats which consumed the copper deficient diet of less than 1 ug copper/g diet had a mean serum copper value of 14.2 ug/dl, while the copper adequate rats which consumed the diet containing 5 ug copper/g diet had a significantly higher value of 149.2 ug/dl. Liver copper was determined to be 0.8 ug/g dry weight for the copper deficient rats, and significantly lower than the copper adequate group, 2.0 ug/g dry weight. These findings demonstrated that plasma and liver copper levels were directly effected by the dietary intake of copper.

Korte et al (1987) studied the effects of copper deficiency on several parameters in male mice pups, including ceruloplasmin and hematocrit. For 7 weeks, mice were fed either a basal copper deficient diet of 0.5 ug/g diet, or the basal diet supplemented with 20 ug copper/ml drinking water. At the end of the study, the activity of ceruloplasmin in the copper supplemented group was determined to be 39.3 units/l, significantly greater than the value of the copper deficient group, 2.30 units/l. The development of anemia in the
copper deficient group was evidenced by the large variance in hematocrit values. The copper supplemented group had a mean hematocrit value of 49.7% compared to the copper deficient group, 28.8%. These findings were similar to previous studies (Prohaska et al, 1983; Prohaska et al, 1984).

Stuart and Johnson (1986) fed varying levels of dietary copper to 4 groups of weanling rats for 60 days. Dietary treatments consisted of 2.5, 5, 10 or 20 ug copper/g diet. In the 4 groups, plasma levels were 103, 98, 98, and 104 ug/dl respectively, and no significant difference was noted. These results showed that plasma copper was not a sensitive index for copper status.

A study was conducted in which rats were fed either a diet containing 2 ug copper/g diet or 10 ug copper/g diet (Hunsaker et al, 1983). Serum copper levels of dams, male pups, and adult males were determined after 92 days. The dams on the 10 ug copper/g diet had a mean serum copper concentration of 120 ug/dl, not significantly different from the group which consumed 2 ug copper/g diet, 90 ug/dl. Male pups were also not significantly different with values of 64 ug/dl for the 10 ug copper/g diet group, and 60 ug/dl for the 2 ug copper/g diet group. No significance difference was found with the adult males who consumed 10 ug copper/g diet, 130 ug/dl, and those fed the 2 ug copper/g diet, 110 ug/dl.

Eisemann et al (1979) studied the effects of a 8 or 125 ug copper/g diet on plasma copper in 6-8 week old swine for 16 weeks. Plasma copper was not found to be significantly affected by dietary copper. Animals on both levels of copper had increasing levels of plasma copper over time, but results indicated no correlation with diet and plasma levels.

Rabbits were fed a diet containing either 2 or 6 ug copper/g diet for 12 weeks to observe the effects on plasma copper (Samman and Roberts, 1986). Although the mean
plasma copper level for the 6 mg copper/g diet, 67 ug/dl, tended to be higher than the 2 ug copper/g diet group, 48 ug/dl, no significant effect was noted.

EFFECTS OF DIETARY ZINC ON PLASMA OR SERUM ZINC:

The circulating concentration of zinc in serum or plasma is considered the most widely employed index of zinc nutrure (Solomons, 1981). However, several factors have artificially elevated or reduced apparent levels of circulating zinc. These include: blood sample contamination; hemolysis; veno-occlusion; prolonged fasting; albumin concentration; inflammatory processes or infections (Solomons, 1981). Other biochemical and functional procedures have been used to assess zinc nutrure. Due to zinc's role in many enzymatic functions, serum alkaline phosphatase activity, erythrocyte carbonic anhydrase activity, and serum ribonuclease activity are indices also used to assess zinc status (Solomons, 1981). Taste acuity, dark adaptation, sperm count, lymphoblast formation, fecal and urine content, and buccal mucosal morphology are other measures of assessment (Solomons, 1981). Hair, sweat, skin, and fingernail zinc content measures are considered less reliable (Solomons, 1981).

The effect of dietary zinc on plasma zinc has been studied in humans (Fischer et al., 1984). Twenty six adult males consumed either 50 mg of elemental zinc/day, or a placebo for 6 weeks. Plasma zinc was significantly elevated in the supplemented group by 2 weeks to a level of 112 ug/dl, as compared to the placebo group level of 90 ug/dl, and remained significant at the 4th and 6th week.

Hooper et al (1980) administered a daily zinc supplement containing 160 mg of elemental zinc to 8 adult men while 8 others received a placebo with meals for 5 weeks. Mean plasma zinc concentration rose significantly from a baseline value of 94.8 ug/dl, to 145.7 ug/dl after 5 weeks in the supplemented group, while no significant change was noted in the placebo group. The supplemented group level returned to a near base-line
value of 89.7 ug/dl 11 weeks after zinc administration had stopped, indicating a plasma sensitivity to dietary zinc.

Freeland-Graves et al (1982) studied the effects of either a placebo, 15, 50, or 100 mg zinc supplement per day on 32 young adult women over 8 weeks. Concentrations of plasma zinc increased significantly for each of the supplemented groups, with the highest values of approximately 111, 125, 145, and 157 ug/dl (values obtained from graph), respectively, obtained at week 4. Values then declined toward initial levels. Peak values of plasma zinc were found to be directly related to the level of the zinc dose since the highest value of 157 ug/dl occurred in the 100 mg supplemented group and the lowest value of 111 ug/dl was noted in the 0 mg group. However, mean initial values of plasma zinc in the four groups differed and when changes in plasma zinc were expressed as an increase from the initial value, the mean increases in plasma zinc levels in the 15, 50, and 100 mg groups were 41, 46, and 44 ug/dl respectfully. These results indicated all 3 supplemented groups had a similar magnitude of response regardless of dose level. It was also noted that the decline in plasma zinc over time regardless of a continuous zinc administration may reflect a homeostatic regulation, presumably at the level of intestinal absorption or saturation of zinc-binding proteins in the plasma (Freeland-Graves et al, 1982).

Hollingsworth et al (1987) studied the relationship between dietary zinc and serum zinc in the elderly, aged 66-87. Subjects were given either a daily total mineral supplement containing 15 mg zinc and 3 mg copper, or a zinc sulfate supplement of 100 mg elemental zinc/day. Administration of 100 mg zinc/day produced a significant increase in serum zinc over the 3 month period of supplementation, with a mean base-line serum zinc value of 93 ug/dl, and upon conclusion of the study, 131 ug/dl. The slight trend upward from the baseline serum zinc value of 87 ug/dl for the total mineral supplement group to the final mean value of 99 ug/dl was not significant.
Forty five adult young males consumed 50 mg zinc/day, 75 mg zinc/day, or a placebo for 12 weeks (Black et al, 1988). By week 2, the mean serum zinc level of the 50 mg zinc/day group increased significantly from baseline, 107 ug/dl to 133 ug/dl. The group consuming 75 mg zinc/day had a baseline level of 103 ug/dl, which was significantly lower than the level at week 2, 120 ug/dl. Subjects in the 50 mg zinc/day group had a peak serum zinc level at week 2, and the peak serum zinc level of the 75 mg zinc/day group was noted at week 8. Serum zinc concentrations were noted to decline slightly after reaching the peak level, but leveled off thereafter.

Wada et al (1985) studied the effects of diets containing 16.5 or 5.5 mg elemental zinc/day on serum zinc levels in 6 adult men. The 75 day study was divided into 3 metabolic periods. Subjects received 16.5 mg zinc/day during the first period, 5.5 mg zinc/day during the second period, and 16.5 mg zinc/day during the third period. Although there was a trend for lower mean serum zinc from 114 ug/dl in the first period to 108 ug/dl in the second period, the difference was not significant. After 7 weeks of the study and 24 days of consuming 5.5 mg zinc/day, the lowest individual serum zinc value was 100 ug/dl; the values for the 6 men ranged from 100-117 ug/dl. It appeared that an intake of 5.5 mg zinc/day was adequate for maintenance of normal circulating serum zinc levels. In period 3 when the subjects again received 16.5 mg zinc/day, no significant difference was noted in serum zinc levels. The researchers concluded that serum zinc levels were not distinguished by dietary zinc consistently (Wada et al, 1985). Results therefore question the validity of serum zinc as an indicator of zinc status, although it is a widely used assessment tool.

Medeiros et al (1983) compared the zinc intakes of males and females by 3 day diet records and serum zinc values. Mean dietary zinc intake was determined to be significantly different between the sexes. The mean serum value for males was found to be 13.5 mg zinc/day, and for females, 9.5 mg zinc/day. However, no significant
difference was found between the serum values of zinc of 101 ug/dl for males, and 96 ug/dl for females. The lack of correlation found between dietary zinc and serum levels of this mineral indicated that either the dietary recall information is an inaccurate reflection of intake or that serum levels do not accurately represent zinc status.

Eisemann et al (1979) studied the effect of dietary zinc on plasma zinc concentration in 6 to 8 week old swine. Diets contained zinc levels of 100 or 500 ug/g diet for 16 weeks. Blood samples obtained indicated an increase in plasma zinc during the first 4 weeks before hitting a plateau. Plasma zinc was significantly and consistently higher in animals supplemented with 500 ug zinc/g diet with a mean level of approximately 1.2 ug/ml during the 4 weeks, while the swine consuming the lower zinc diet 100 ug zinc/g diet, had a zinc level of approximately 0.95 ug/dl. The increase in plasma zinc was considered to be reflective of increased absorption of zinc.

Petering et al (1977) studied the effects of water supplemented with 2.5, 10 or 40 ug zinc/ml in weanling rats for 109 days. Increases in serum zinc were found to be correlated with zinc content. The treatment of 2.5 ug zinc/ml of water produced the lowest mean level of serum zinc of 65.8 ug/dl. The mean value of serum zinc for the 10 and 40 ug zinc/ml of water groups were 167.3 and 180 ug/dl, respectively. These results demonstrated that the dietary zinc content of the diet directly influenced serum zinc with a leveling off effect at the higher levels.

Zinc at levels of 2.5, 5, 10, 20, and 40 ug/ml of water were consumed by male weanling rats for 60 days (Murthy et al, 1974). A significant lower level of serum zinc due to dietary intake of 2.5 ug zinc/ml of water was noted, 119 ug/dl, when compared to the other diets, 148, 150, 139, and 135 ug/dl, respectively.

Koo et al (1986) fed 4 groups of weanling rats either a zinc deficient diet of 3 ug zinc/g diet or a control diet of 30 ug zinc/g diet to observe the effects on plasma zinc. The plasma levels of zinc were significantly decreased in the zinc deficient groups with means of 154
and 165 ug/dl as compared with levels for the control groups of 185 and 209 ug/dl. This study also demonstrated an effect of dietary zinc on plasma zinc concentration.

Male weanling rats were placed into 2 groups and fed a zinc adequate diet of 100 ug zinc/g diet or a zinc deficient diet of less than 1 ug zinc/g diet for 21 days (Schneeman et al, 1986). Mean plasma zinc for the zinc adequate rats was determined to be 149 ug/dl, and significantly higher than the zinc deficient rats with a mean plasma level of 69 ug/dl.

Koo and Williams (1981) studied the effects of dietary zinc on serum zinc levels in weanling male rats fed either a deficient diet of 0.37 ug zinc/g diet, or a control diet of 41 ug zinc/g diet. After 4 weeks, the control rats had a significantly higher mean serum zinc level of 136 ug/dl as compared to 86 ug/dl for those rats on the deficient diet.

Weanling rats were placed on diets containing the following levels of zinc: 7.5, 10, 30, 45, or 60 ug zinc/g diet (Fischer et al, 1980). At five weeks, mean serum zinc levels were significantly lower only in those fed the 7.5 ug zinc/g diet, approximately 90 ug/dl, when compared to the higher zinc diet, approximately 153, 130, and 160 ug/dl, respectively (values obtained from graph). By 10 weeks, the mean zinc level was still lower for the group consuming 7.5 ug zinc/g diet although the difference was no longer significant. By 15 weeks, no difference was noted among the groups.

EFFECTS OF ZINC:COPPER RATIO ON PLASMA OR SERUM ZINC AND COPPER:

As previously noted, the nutritional effect of excess zinc on copper metabolism has been determined to be greater than vice versa. Van Campen (1970) found that in vitro, zinc and copper are mutually antagonistic during absorption, and high levels of dietary zinc repress the levels of tissue and plasma copper in rats. Inhibition of copper absorption was observed when dietary zinc to copper ratios were from 500:1 to 1000:1 (Van Campen, 1970). While these ratios have been considered high, Osgro et al (1974),
determined that a ratio as low as 30:1 significantly decreased copper absorption in rats.

Storey and Greger (1987) studied zinc and copper interactions in weanling rats with 3 experimental diets of varying intakes of the two minerals. Separate control groups were assigned for comparison to each experimental diet. Diet 1 consisted of 2441 ug zinc/g diet and 6.4 ug copper/g diet, for a ratio of 381:1, while the diet of the control group was 14.6 ug zinc/g diet and 6.4 ug copper/g diet, for a ratio of 2:1. Diet 2 consisted of 2441 ug zinc/g diet and 6.7 ug copper/g diet, for a ratio of 364:1. The control diet contained 14.8 ug zinc/g diet and 6.7 ug copper/g diet, for a ratio of 2:1. Diet 3 consisted of 2470 ug zinc/g diet and 3.7 ug copper/g diet, for a ratio of 667:1. A control diet containing 14.6 ug zinc/g diet and 3.7 ug copper/g diet, for a ratio of 7:1, was fed to rats for comparison. After a 21 day feeding period, mean serum copper level of rats fed diet 1, 13 ug/ml, was significantly lower than the control group, 75 ug/ml. Serum copper level of rats fed diet 2, 28 ug/ml, was significantly lower than the controls, 93 ug/ml. Rats fed diet 3 had a serum copper level of 14 ug/dl, significantly lower than the controls, 82 ug/ml, respectively. Results indicated that ingestion of excess levels of zinc consistently and significantly depressed serum copper levels to less than 30% of the controls.

Murthy et al (1974) studied the metabolic interrelationships resulting from varying the dietary intake of copper and zinc in weanling rats. Significant variations in serum copper due to dietary intake of both minerals was noted. The higher zinc to copper ratios of 160:1 and 80:1 produced serum levels of copper of 6.0 and 8.0 ug/dl, respectively, significantly different from lower ratios of 40:1, 20:1, and 10:1 which produced serum copper levels of 11.3, 10.0, and 22.7 ug/dl, respectively. However, serum zinc was not significantly affected by varying levels of copper. Also noted were changes in the copper contents of liver and heart tissue in response to variations in dietary zinc and copper. Liver copper steadily decreased from 11.3 ug/g dry tissue with the lowest ratio of 10:1, to 6.7, 6.7, 6.0, and 5.7 ug copper/g dry tissue produced by the higher ratios of 20:1, 40:1, 80:1, and
160:1, respectively. This trend was also noted with heart copper levels. With increasing zinc to copper ratios, heart copper levels decreased from 14.0 ug/g dry tissue to 8.0, 7.0, 7.3, and 8.0 ug/g dry tissue, respectively. The observance that copper in these organs tended to be highest when zinc intake was lowest strongly suggested an interrelationship between the two minerals.

Seven experimental diets were fed to groups of male weanling rats for 15 weeks which contained varying amounts of copper and zinc (Fischer et al., 1980). These diets contained: 1) 30 ug zinc/g diet and 6 ug copper/g diet, for a ratio of 5:1; 2) 7.5 ug zinc/g diet and 1.5 ug copper/g diet, for a ratio of 5:1; 3) 30 ug zinc/g diet and 1.5 ug copper/g diet, for a ratio of 20:1; 4) 45 ug zinc/g diet and 1.5 ug copper/g diet, for a ratio of 30:1; 5) 60 ug zinc/g diet and 6.0 ug copper/g diet, for a ratio of 100:1; 6) 30 ug zinc/g diet and 3.0 ug copper/g diet, for a ratio of 10:1; 7) 10 ug zinc/g diet and 3.0 ug copper/g diet, for a ratio of 3:1. After 5 weeks, serum copper levels were 20 ug/dl or less, and significantly lower in animals fed diets 2, 3, and 4 which contained 1.5 mg copper/kg diet, when compared with those fed higher levels of copper, 3 or 6 ug copper/g diet, which had serum values of 50 ug/dl and greater. At 10 weeks, groups 2, 3, and 4 still had significantly lower levels of serum copper of 50 ug/dl or less, compared with 80 ug/dl or greater for the other groups. By 15 weeks, those animals fed diet 2 which contained low copper and low zinc, had an average serum copper level of 80 ug/dl which was no longer significantly different from the other groups. Animals fed diet 3 which contained the same amount of copper as diet 2, but with a greater amount of zinc, had a significantly lower serum copper concentration of 50 ug/dl. Rats consuming diet 4, which also had a low amount of copper but had a higher amount of zinc than diet 3, had an even lower serum copper level of 30 ug/dl. Since zinc has been found to compete with copper for binding to metallothionein for absorption, at higher dietary zinc levels, less copper was absorbed. Due to this competition for absorption between zinc and copper, it was hypothesized that
the larger amounts of dietary copper were sufficient to overcome the inhibitory effect of zinc on copper absorption. Therefore, dietary zinc had no effect on serum copper levels in those animals fed diets containing adequate copper levels of 3.0 and 6.0 ug/g diet.

Eisemann et al (1979) studied the effects of dietary zinc and copper on plasma and tissue concentrations in swine. Diets consisted of zinc levels at either 100 or 500 ug/g diet, and copper at 8 or 125 ug/g diet. These levels were then given in zinc to copper ratios of 12.5:1, 0.8:1, 62.5:1, or 4:1. Plasma zinc was consistently and significantly higher in animals supplemented with 500 ug zinc/g diet over the 16 weeks. No influence of dietary copper on plasma zinc was found even at a zinc to copper ratio of 0.8:1. This was similar to what had been observed in a previous study (Van Campen, 1969). Presumably, the increased plasma zinc was reflective of increased absorption due to an isolated intestinal zinc binding protein which earlier had been found to correlate with changes in serum zinc in the rat (Richards et al, 1976). Plasma copper was not significantly influenced by dietary copper levels although a consistent increase with time was observed for all diets. In the liver, zinc accumulation was responsive to diets containing high levels of zinc. Copper accumulation in the liver was influenced by dietary copper, dietary zinc and a zinc:copper interaction was observed. The researchers hypothesized that the increase in both zinc and copper content of the liver with increasing dietary levels of each mineral, reflected an increase in the protein bound mineral in the cytosol. In consideration of liver copper levels, the zinc:copper interaction probably represented displacement of copper by zinc from a cytosolic binding protein (Eisemann et al, 1979).

Petering et al (1977) studied the effect of dietary copper and zinc on serum copper and zinc, serum ceruloplasmin, and liver and kidney concentrations of these minerals in male weanling rats. Dietary copper ranged from 0, 0.25, 2.0, and 16.0 ug/ml of drinking water, and dietary zinc ranged from 2.5, 10.0, and 40.0 ug/ml of drinking water. Serum
copper significantly increased with an increase in dietary copper. However, no significant effect was noted by the varying levels of zinc. Serum zinc levels also increased significantly with increasing levels of dietary zinc, but was unaffected by dietary copper. Serum ceruloplasmin, liver copper, and kidney copper were found to be positively correlated with different levels dietary copper, with no apparent effect of dietary zinc.

Fischer et al (1984) studied the effect of zinc supplementation on the copper status of adult men by assessing the activities of copper metalloenzymes, plasma ferrooxidase (ceruloplasmin), and erythrocyte copper, zinc-superoxide dismutase. Copper, zinc-superoxide dismutase has been found to be dependent on copper, but not zinc status, and therefore is believed to be an accurate measure of copper status (Bettger et al, 1979). Subjects were given either 50 mg elemental zinc/day or placebo for 6 weeks while on their normal diets. No significant difference was noted in the ferrooxidase activity or plasma copper between the 2 groups. However, erythrocyte superoxide dismutase activity decreased after 4 weeks in the supplemented group to 280 units/ml packed cells, and was significantly lower than the placebo group with 340 units/ml packed cells by 6 weeks. These results indicated that the ingestion of moderately high amounts of zinc for a 6 week period by humans resulted in a decreased copper status, using the activity of erythrocyte copper, zinc-superoxide dismutase as an index of the metabolically available copper. Also observed was that the decrease in liver superoxidase dismutase activity was linear with respect to increasing amounts of dietary zinc. This suggested that copper status may be directly related to zinc intake.

The effect of feeding two different levels of zinc on nutrient utilization, including zinc and copper balance was observed in 11 adolescent females during a 30 day period (Greger et al, 1978). Diet 1 provided 11.32 or 11.64 mg zinc/day. Diet 2 provided 14.52 or 14.84 mg zinc/day. Average copper content for both diets was 1.25 mg/day. When subjects were fed the higher zinc diets, significantly more zinc was lost in the feces, 13.56
mg/day, as compared to subjects who consumed the lower zinc diet, 10.20 mg/day. Fecal copper excretion was also significantly higher in the group which consumed the higher zinc diet, 0.90 mg/day, as compared to subjects fed the lower zinc diet, 0.79 mg/day. The increased loss of copper due to a higher level of dietary zinc agreed with previous findings that zinc and copper are mutually antagonistic during the absorption process (Van Campen, 1970).

ZINC:COPPER RATIO AND LIPID PARAMETERS:

Based on animal studies, Klevay (1973) hypothesized that both zinc and copper are major factors in the etiology of CVD. Increases in plasma TC levels were found to be attributable to a high ratio of dietary zinc to copper in rats (Klevay, 1973). Over a testing period of 3 years, 3 studies were conducted in which intakes of zinc and copper were varied by altering the ratio of these elements in drinking water (Klevay, 1973). In the first study in 1970, 36 male weanling rats were fed either a diet containing 10 ug zinc/ml drinking water and 2 ug copper/ml drinking water, a ratio of 5:1, or 20 ug zinc/ml drinking water and 0.5 ug copper/ml drinking water, a ratio of 40:1. Mean plasma TC concentration of the rats consuming the zinc to copper ratio of 5:1, 168 mg/dl, was significantly lower than the rats consuming a ratio of 40:1, 223 mg/dl. In 1971, diets consisted of the same zinc:copper ratios of 5:1 or 40:1. Plasma TC levels were determined to be 94 mg/dl for the rats consuming the zinc:copper ratio of 5:1, and significantly lower than the 40:1 ratio, 111 mg/dl. In 1972, ratios remained as 5:1 or 40:1. Plasma TC levels were noted to be significantly different with 96 mg/dl for the rats consuming a zinc:copper ratio of 5:1, and 125 mg/dl for the group consuming a ratio of 40:1. Klevay (1973) concluded that the zinc to copper ratio of 40:1 had significantly and consistently produced higher concentrations of plasma TC than the lower ratio of 5:1. Based on the significant and reproducible findings, Klevay hypothesized that increases in plasma TC
were associated with an increased zinc:copper ratio, while a lower ratio was associated with reduced plasma TC (Klevay, 1975).

Petering et al (1977) investigated the effects of varying both dietary zinc and copper on lipid metabolic parameters in male weanling rats. For 119 days, groups of rats were fed diets consisting of 2.5, 10.0, or 40.0 ug zinc/ml of water and 0, 0.25, 2.0, or 16.0 ug copper/ml of water for a combination of 9 different ratios. A significant interaction was found between serum TC and the zinc to copper ratio. Significant decreases in serum TC occurred with decreasing zinc to copper ratios of 160:1, 20:1, and 2.5:1, with levels of 99.4, 58.9, and 46.7 mg/dl, respectively.

Eisemann et al (1979) studied the effects of dietary zinc and copper on cholesterol levels in swine for 16 weeks. Diets consisted of 1) 100 ug zinc/g diet and 8 ug copper/g diet, a ratio of 12.5:1; 2) 100 ug zinc/g diet and 125 ug copper g diet, a ratio of 0.8:1; 3) 500 ug zinc/g diet and 8 ug copper/g diet, a ratio of 62.5:1; 4) 500 ug zinc/g diet and 125 ug copper/g diet, a ratio of 4:1. Plasma TC was determined at 2, 4, 8, and 12 weeks of dietary treatment. At week 4 of feeding, the effect of the zinc to copper ratio was highly significant. Animals on the zinc: copper diets of 62.5:1 and 12.5:1 had higher plasma TC levels of 99.5 and 89.2 mg/dl, respectively, compared to the levels of 82 and 74.4 mg/dl, respectively, for the zinc:copper ratios of 4:1 and 0.8:1. However, significance was no longer apparent at weeks 8 and 12. Although not significant, the lowest zinc:copper ratio of 0.8:1 produced the lowest levels of plasma TC throughout the study with levels of 70.3, 74.4, 83.4, and 84.7 mg/dl, respectively, at weeks 2, 4, 8, and 12. These researchers concluded that it was possible that there was a changing relationship between dietary zinc and copper and plasma TC over time (Eisemann et al, 1979).

Looney and Lei (1978) observed the effects of marginal and abundant levels of zinc, 10 ug/g diet and 120 ug/g diet, respectively; and deficient and adequate levels of copper, 2 ug/g diet and 18 ug/g diet, respectively, on serum TC in male weanling rats. For 9
weeks, rats were fed the following diets: 1) 10 ug zinc/g diet and 2 ug copper/g diet, a ratio of 5:1; 2) 120 ug zinc/g diet and 2 ug copper/g diet, a ratio of 60:1; 3) 10 ug zinc/g diet and 18 ug copper/g diet, a ratio of 0.5:1; 4) 120 ug zinc/g diet and 18 ug copper/g diet, a ratio of 6:1. Significantly elevated serum TC levels were observed in the copper deficient rats (2 ug copper/g diet) as compared to the copper adequate rats (18 ug copper/g diet). Serum TC for the rats which consumed the diet deficient in copper and marginal in zinc (10 ug zinc/g diet), a ratio of 5:1, was noted to be 98.2 mg/dl. Rats fed the diet deficient in copper and abundant in zinc (120 ug zinc/g diet), a ratio of 60:1, had a serum TC level of 108.3 mg/dl. The group which consumed the diet adequate in copper and marginal in zinc, a ratio of 0.5:1, was noted to have a serum TC level of 85.3 mg/dl, while rats fed the diet adequate in copper and abundant in zinc, a ratio of 6:1, had serum TC of 78.9 mg/dl. These findings were in agreement with Klevay (1973).

Frimpong and Magee (1987) observed the effects of dietary copper and zinc on serum lipid parameters in young male rats for 6 weeks. In one experiment, copper supplements of 0.56, 1.68, and 5.04 ug/g diet, and zinc supplements of 5, 10, and 20 ug/g diet were added to a basal diet which contained 0.30 ug copper/g diet and 0.34 ug zinc/g diet. No significant effect was noted on serum TC or HDL-C at any of the dietary levels of zinc or copper. For a second experiment, levels of 5.6, 16.8, and 50.4 ug copper/g diet were used in combination with 50, 100, and 200 ug zinc/g diet. None of the levels of zinc supplementation had any significant effects on TC or HDL-C levels. Increases in dietary copper supplements however, were associated with highly significant declines in TC. The basal diet without copper supplementation, and zinc supplements of 50,100 and 200 ug/g diet produced TC levels of 81, 103, and 86 mg/dl, respectively. With 5.6 ug copper supplemented to the basal diet and diets containing 50, 100, and 200 ug zinc/g diet, TC levels decreased to 71, 68, and 81 mg/dl respectively. Additional increases in dietary
copper (up to 50.4 ug), however, did not result in any statistically significant changes in TC or HDL-C.

Caster and Parthenas (1976) observed a positive correlation between dietary zinc and copper, and plasma TC levels in male weanling rats fed 16 common breakfast cereals for 45 days. Dietary zinc:copper ratios ranged from 1.8 to 6.5. Ratios of 2.2 and 2.9 produced the lowest plasma TC concentrations of 32 mg/dl and 38 mg/dl, respectively. Higher ratios of 4.9 and 5.1 produced the highest plasma TC levels of 92 mg/dl and 95 mg/dl respectively. The correlation between the dietary zinc:copper ratio and the plasma TC level was r=0.58 for 11 of the cereals and r=0.10 for all 16 diets. Neither correlation was statistically significant, but the value for the 11 cereals was close enough (r=0.60 for p=0.05) for the authors to suggest that the zinc:copper ratio was an important factor in relation to plasma TC levels in this study.

Fischer et al (1980) observed the effect of dietary copper and zinc on cholesterol metabolism in male weanling rats for 5 weeks. Rats were fed one of the following diets: 1) 30 ug zinc/g diet and 6 ug copper/g diet, a ratio of 5:1; 2) 7.5 ug zinc/g diet and 1.5 ug copper/g diet, a ratio of 5:1; 3) 30 ug zinc/g diet and 1.5 ug copper/g diet, a ratio of 20:1; 4) 45 ug zinc/g diet and 1.5 ug copper/g diet, a ratio of 30:1; 5) 60 ug zinc/g diet and 6 ug copper/g diet, a ratio of 10:1; 6) 30 ug zinc/g diet and 3 ug copper/g diet, a ratio of 10:1; 7) 10 ug zinc/g diet and 3 ug copper/g diet, a ratio of 3.3:1. TC ranged from 100 to 130 mg/dl for all groups, with no significant change in serum TC levels throughout the study. The researchers concluded that an extreme copper deficiency as used by Klevay (1973), was necessary before an increase in serum TC occurred. Lei (1978) observed an elevation in serum TC in rats fed less than 2 mg copper/kg diet and 120 mg zinc/kg diet when compared to animals fed 18 mg copper/kg diet and 120 mg zinc/kg diet. The low copper level was similar in this particular study (Fischer et al, 1980) however, zinc levels were much lower. The higher amount of zinc could have decreased the absorption of
copper to the point that it had a hypercholesterolemic effect.

Woo et al (1981) conducted a study similar to Fischer et al (1980), but used a larger range of zinc, 10, 20, 100, and 500 ug/g diet, and a constant range of copper, 15 ug/g diet. Male weanling rats consumed zinc:copper ratios of 0.66:1, 1.3:1, 6.6:1, or 33:1 for 12 weeks. Serum TC levels of 43.6, 48.5, 50, and 46.7 mgidl, respectively, were not found to be significant. Also, HDL-C levels of 28.7, 30.3, 33.0, and 32.2 mgidl were not significant. These results supported Fischer et al (1980) and indicated that even very high zinc:copper ratios, up to 33:1, did not affect lipid metabolism when copper levels were adequate.

For 27 days, Caster et al (1979) fed male weanling rats diets in which the zinc content was 11, 29, or 100 ug/g diet, and copper content was 0.5, 1.3, and 5.1 ug/g diet. These intakes provided zinc:copper ratios of 2:1, 6:1, 7:1, 20:1, 21:1, 22:1, 62:1, 78:1, and 220:1. Over the entire range of zinc:copper ratios, there were no significant correlations between zinc and copper, the ratio in the diet, or plasma TC levels. The slope of the least squares straight line through these data, was if anything, negative, and provided no support for the suggestion that an increase in the zinc:copper ratio in the diet was responsible for an increase in plasma cholesterol.

Effects of dietary zinc and copper on TC and HDL-C in adult male rats were studied for 6 weeks (Lefevre et al, 1985). Diets consisted of 0 or 5 ug copper/g diet combined with 1, 10, 100, or 1000 ug zinc/g diet. Plasma TC was found to be negatively correlated with plasma copper levels (r=-0.60; p<0.01). Plasma TC was not significantly effected by neither dietary copper or zinc, nor the copper times zinc interaction term. At ratios of 1:0, 10:0, 100:0, and 1000:0, plasma TC levels were noted to be 58, 65, 65, and 68 mgidl, respectively. At zinc:copper ratios of 1:5, 10:5, 100:5, and 1000:5, plasma TC levels were found to be 55, 60, 50, and 55 mgidl, respectively. Although a trend was noted for the group fed the 0 ug copper/g diet to have higher HDL-C levels than the group
consuming the 5 ug copper/g diet, no significance was observed by the dietary copper level or the copper times zinc interaction term. At zinc:copper ratios of 1:0, 10:0, 100:0, and 1000:0, plasma HDL-C levels were 50, 35, 45, and 55 mg/dl, respectively. Ratios of 1:5, 10:5, 100:5, and 1000:5 produced HDL-C levels of 45, 30, 35, and 40 mg/dl, respectively.

The association of copper and zinc intake with serum TC and HDL-C was studied in 59 young adults (Medeiros et al, 1983). Three day diet records and fasting blood samples were collected and analyzed. Mean zinc:copper ratios for males was determined to be 6:1, and for females, 5.5:1. No correlation was found between the zinc:copper ratio and mean TC levels of 177.8 mg/dl for 27 males, and 173 mg/dl for 32 females, or between the mean HDL-C levels of 50.9 mg/dl for males and 49.1 mg/dl for females.
EFFECT OF DIETARY ZINC AND COPPER ON
PLASMA ZINC, COPPER, TOTAL CHOLESTEROL, AND
HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN YOUNG
ADULT MALES

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George E. Bunce, Mary Ann Novascone

Forrest W. Thye
Human Nutrition and Foods
VPI & S1
Blacksburg, Virginia 24061
An eight week controlled study was conducted to determine the effects of dietary zinc and copper on plasma zinc, copper, total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) levels in young adult males receiving two levels of zinc. Source of calcium was also varied, however, possible interactions or effects of calcium were not determined in this study. Subjects consumed zinc supplements of 10 mg/day, in combination with 1 of 3 diets, resulting in zinc:copper ratios of 30:1 (Dairy Calcium, or DC group, 20:1 (Control or CO group), and 20:1 (Calcium Carbonate or CC group). Copper content of all diets was approximately 1 mg/day. Plasma levels of zinc, copper, TC, and HDL-C did not differ significantly among the groups (p<.05). However, plasma levels of copper, zinc, and HDL-C were found to be significantly affected by the week of controlled feeding across all 3 groups (p<.05). Plasma copper at baseline was significantly lower than at weeks 2, 4, 6, 8, and post treatment. Plasma zinc at baseline, and weeks 2 and 4, was found to be significantly lower than at weeks 6, 8, and post treatment. At week 6, plasma HDL-C was noted to be significantly higher than at baseline, weeks 2, 4, 8, and post treatment. Spearman correlation coefficients determined negative correlations between plasma copper and TC (r=-0.39, p<.04), and plasma copper and zinc (r=-0.43, p<.02) in the DC group. A positive correlation was also noted between plasma zinc and TC (r=0.32, p<.10) in the DC group. Plasma copper and HDL-C were determined to be negatively correlated in the CO group (r=-0.48, p<.005). Plasma zinc and HDL-C were found to be negatively correlated in the CC group (r=-0.58, p<.001).

(Key Words: zinc, copper, total cholesterol, high-density lipoproteins)
INTRODUCTION

Cardiovascular disease (CVD) has been determined to still be the leading cause of death in the United States, as well as in other industrialized nations (1). This major cause of mortality causes 54% of all deaths, twice as many as are caused by cancer (2).

Various factors have been found to be associated with increased incidence of CVD, and include: diabetes (3); abnormal electrocardiograms (4); smoking (5); male gender (6); physical inactivity (3); obesity (6); hypertension (7); increasing age (4). However, increased serum or plasma low density lipoprotein cholesterol (LDL-C)(60-70% of total cholesterol (TC)), and decreased high density lipoprotein cholesterol (HDL-C) have been determined to be the most strongly correlated with coronary heart disease (CHD)(6) (8) (9).

Alterations in lipoprotein and cholesterol concentrations have been linked to certain dietary minerals. Sodium, magnesium, sulfur, calcium, and manganese have been observed to be associated with cholesterol and lipoprotein metabolism (10). In 1975, Klevay developed the zinc:copper hypothesis in relation to CHD based on 3 years of study with rats consuming varying dietary zinc:copper ratios (11). Klevay suggested that "a metabolic imbalance in regard to zinc and copper is a major factor in the etiology of coronary heart disease (11)". This metabolic imbalance is "either a relative or absolute deficiency of copper, characterized by an increase ratio of zinc to copper (11). Several researchers have supported Klevay's hypothesis (11) (12) (13) (14) (15) (16) (17).

This study was designed to observe the effects of two dietary zinc:copper ratios on plasma zinc, copper, TC and HDL-C in young adult men receiving different sources of calcium with all other nutrients remaining constant under controlled feeding conditions.
METHODS

SUBJECTS:

A metabolic feeding study was conducted on the Virginia Polytechnic Institute and State University campus in Blacksburg, Virginia. Participants included 23 male subjects, 20 to 37 years of age (mean age of 26), who responded to recruitment ads and posters. Volunteers were given a written and oral explanation of the study, and meetings were held to allow for questions and answers. Upon written consent, a fasting blood sample was collected from each prospective subject, and plasma TC was determined. Individuals with values of 190 mg/dl or higher were notified of qualification for the study. In addition to the minimum plasma TC level necessary for qualification, participants were also required to be non-smokers, of normal weight, and in good physical condition as determined by a personal medical history questionnaire and a physician's written approval. Guidelines outlined by the Institutional Review Board for Research Involving Human Subjects at Virginia Polytechnic Institute and State University were followed throughout the study period.

EXPERIMENTAL DESIGN:

The total time of the study was 90 days, consisting of a 20 day pretreatment or baseline period, 56 days of controlled feeding, and a 14 day post-treatment or follow-up period. No dietary limitations were in effect during the baseline and follow-up periods. A total of 16 blood samples were drawn per subject at 8 different times for 2 consecutive days. During the pretreatment baseline period, 3 blood samples (2 consecutive days each time) were collected per subject at 10 day intervals, 4 samples every 2 weeks during the controlled feeding period, and 1 sample 14 days after treatment ended. A randomized block design ranking plasma TC levels was used for subject assignment to one of three
dietary treatment groups. Some subject food preference and tolerance were also taken into consideration for assignment.

During the controlled feeding period of the study, the following treatment groups were observed (Table 1): Dairy Calcium diet (DC) (calcium source primarily from dairy products), consisting of 1.02 mg copper/day, 30.34 mg zinc/day, and zinc:copper ratio of 30:1; Control diet (CO) (calcium source from dairy products and calcium carbonate), consisting of 0.93 mg copper/day, 19.76 mg zinc/day, and zinc:copper ratio of 20:1; Calcium Carbonate diet (CC) (calcium source primarily from calcium carbonate), consisting of 0.96 mg copper/day, 19.63 mg zinc/day, and zinc:copper ratio of 20:1. Zinc supplements of 10 mg/day and magnesium supplements of 200 mg/day were given to the 3 groups. All 3 diets provided approximately 2805 kcal, and fat, carbohydrate, and protein provided 40, 49, 11% of kcals, respectively (Table 2). Each diet contained approximately 500 mg of cholesterol per day, with a P/S ratio of 0.45 as determined by food composition tables (18). The 3 dietary treatments either met or exceeded the RDA for all nutrients, with the exception of copper. The Food and Nutrition Board has established a provisional recommendation range for the dietary intake of copper, but no RDA has been determined. This provisional range is 2-3 mg/day for adults (19).

Preparation and consumption of all meals was supervised by trained individuals at the Department of Human Nutrition and Food metabolic unit. All food and drink for each individual for each meal were weighed to the nearest 0.1 g to achieve a high standard of accuracy. Duplicate meals for all 3 diets were prepared and composited for analysis. Supervisors checked each meal tray before and after consumption to insure each was correct, and then entirely eaten.

Subjects were weighed each morning to note any weight fluctuations. If extra kcals were necessary due to weight loss, cookie supplements were given to maintain beginning weights. Cookie supplements provided 250 kcal each, and contained the same % kcal of
Table 1. Average daily intake of copper and zinc, and zinc:copper ratios.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DC</th>
<th>CO</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (mg)</td>
<td>1.02</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>30.34</td>
<td>19.76</td>
<td>19.63</td>
</tr>
<tr>
<td>Zinc:Copper ratio</td>
<td>30:1</td>
<td>20:1</td>
<td>20:1</td>
</tr>
</tbody>
</table>
Table 2. Average daily nutrient content of dietary treatments.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DC</th>
<th>CO</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcal</td>
<td>2809</td>
<td>2805</td>
<td>2805</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>126</td>
<td>124</td>
<td>125</td>
</tr>
<tr>
<td>%Kcal</td>
<td>40.3</td>
<td>39.9</td>
<td>40.0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>345</td>
<td>345</td>
<td>343</td>
</tr>
<tr>
<td>%Kcal</td>
<td>49.1</td>
<td>49.2</td>
<td>48.9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>%Kcal</td>
<td>11.4</td>
<td>11.4</td>
<td>11.4</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>488</td>
<td>511</td>
<td>493</td>
</tr>
<tr>
<td>P:S</td>
<td>0.445</td>
<td>0.446</td>
<td>0.448</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1839</td>
<td>737</td>
<td>1723</td>
</tr>
</tbody>
</table>
protein, fat, and carbohydrate and P/S ratio as the dietary treatments. They were also prepared at the metabolic unit, and were determined to contain only trace amounts of zinc and copper.

BLOOD COLLECTION AND ANALYSIS:

Subjects fasted for 12-14 hours before blood specimens were collected. Blood was collected into Na$_2$EDTA-containing vacutainers for 2 consecutive days for each period and then averaged. Until centrifugation at 3000 rpm at 4 degrees C for 30 minutes for plasma and red blood cell determination, specimens were held on ice. All determinations were done in duplicate. Addition of a heparin-manganese chloride solution caused precipitation of the plasma very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) (20). Plasma high-density lipoproteins (HDL) were suspended in the supernatant. Using the LRC ultracentrifuge method, the plasma VLDL fraction was separated (21). Plasma samples were spun at 40,000 rpm at 4 degrees C for 18 hours in a Beckman Preparative Ultra Centrifuge (Beckman model L2-65). Plasma LDL and HDL fractions remained upon removal of the VLDL layer. Plasma total cholesterol (TC), HDL-cholesterol (HDL-C) and the combined LDL-cholesterol (LDL-C) and HDL-C samples were analyzed using the Lieberman-Burchard colorimetric reaction (22). Sigma Diagnostics cholesterol standards with a Serachem Clinical Chemistry Control (Fisher Scientific) were included in each run. Plasma LDL-C and VLDL-cholesterol (VLDL-C) concentrations were determined indirectly by the following equations:

$$LDL-C = (LDL-C + HDL-C) - HDL-C$$

$$VLDL-C = TC - (LDL-C + HDL-C)$$

For plasma copper levels, samples were diluted at a ratio of 1 ml plasma to 1 ml
distilled water. For plasma zinc concentration, samples were diluted at a ratio of 1.4 ml plasma to 0.6 ml distilled water. Concentrations of copper and zinc were then determined using atomic absorption spectroscopy methods (Perkin - Elmer Model 503, Perkin Elmer Co., Mountain View, Ca.). All samples were diluted and read in duplicate.

STATISTICAL ANALYSIS:

Differences between mean plasma values of copper, zinc, TC, and HDL-C were assessed using analysis of variance (ANOVA), within and among the three treatment groups. A Duncan post-hoc test was performed if F-ratios were significant. Statistical significance was determined at p<0.05. Due to unequal group size, Spearman correlation coefficients were calculated to observe relationships between pairs of variables. Statistics were analyzed using the SAS computer program.

RESULTS

Mean plasma levels of copper, zinc, HDL-C, and TC are shown in Table 3 according to treatment groups and weeks of controlled feedings. An assessment of the dependence of the plasma levels of copper, zinc, HDL-C, and TC on group, subject within a group, week, and group within a week was conducted by using an ANOVA. Based on this analysis, groups were not different (p<0.05) apparently due to large variations within each group. No trends were observed within treatment groups for TC and HDL-C levels. However, mean plasma copper was noted to increase from baseline through 4 weeks of controlled feeding, and then stabilize at 6 and 8 weeks across all treatment groups. Mean plasma zinc was observed to increase from baseline through 6 weeks of controlled feeding, and then stabilize at 8 weeks across all treatment groups. The largest increase from baseline in plasma zinc was observed in the DC treatment group (zinc:copper ratio of 30:1), while plasma copper was noted to increase the least in the same group (Table 3). Trends for plasma copper levels were similar between the CO and CC groups from
baseline, with plasma zinc levels not as consistent. In addition, plasma levels of copper, zinc, and HDL-C were found to be affected by the week of controlled feeding for all groups.

Duncan's multiple range test was used to determine if there were significant differences in the mean plasma levels between weeks of controlled feeding across all treatments for all variables (Table 4). Mean baseline and post treatment values were included in the analysis to observe changes caused by controlled feeding during weeks 2, 4, 6, and 8. Mean plasma copper at baseline was found to be significantly lower than at weeks 2, 4, 6, 8, and post treatment. Mean plasma zinc at baseline, and weeks 2 and 4, were found to be significantly lower than at weeks 6, 8, and post treatment. Mean plasma HDL-C at week 6 was found to be significantly higher than at baseline, weeks 2, 4, 8, and post treatment. No significant differences were found between mean total plasma cholesterol levels from baseline through post treatment.

Spearman correlation coefficients were calculated to observe the relationship between pairs of variables for each group at weeks 2 through 8. For subjects on the DC diet, a significant negative correlation was found between plasma copper and plasma zinc levels (r=-0.43, p<0.02) (Figure 1), and plasma copper and plasma TC (r=-0.39, p<0.04) (Figure 2). In addition, a significant positive correlation was observed between plasma zinc and plasma TC for the DC group (r=0.32, p<0.10) (Figure 3). Plasma copper and plasma HDL-C were determined to be negatively correlated in the CO group (r=-0.48, p<0.005) (Figure 4), while plasma zinc and plasma HDL-C were found to be negatively correlated in the CC group (r=-0.58, p<0.001) (Figure 5).
Table 3. Mean plasma levels of copper, zinc, total cholesterol, HDL-cholesterol for each treatment at BL, weeks 2, 4, 6 and 8 of controlled feeding, and post treatment.

<table>
<thead>
<tr>
<th></th>
<th>DC (n=7)</th>
<th>GROUP CO (n=8)</th>
<th>CC (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cu (ug/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL(^1)</td>
<td>103 ± 9</td>
<td>117 ± 19</td>
<td>115 ± 28</td>
</tr>
<tr>
<td>2</td>
<td>105 ± 11</td>
<td>120 ± 17</td>
<td>119 ± 23</td>
</tr>
<tr>
<td>4</td>
<td>106 ± 9</td>
<td>121 ± 18</td>
<td>120 ± 23</td>
</tr>
<tr>
<td>6</td>
<td>106 ± 9</td>
<td>121 ± 16</td>
<td>120 ± 22</td>
</tr>
<tr>
<td>8</td>
<td>106 ± 9</td>
<td>122 ± 17</td>
<td>120 ± 22</td>
</tr>
<tr>
<td>POST</td>
<td>106 ± 7</td>
<td>118 ± 7</td>
<td>122 ± 7</td>
</tr>
<tr>
<td><strong>Zn (ug/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>90 ± 13</td>
<td>89 ± 9</td>
<td>95 ± 9</td>
</tr>
<tr>
<td>2</td>
<td>97 ± 9</td>
<td>92 ± 5</td>
<td>96 ± 9</td>
</tr>
<tr>
<td>4</td>
<td>102 ± 5</td>
<td>95 ± 5</td>
<td>100 ± 6</td>
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<tr>
<td>6</td>
<td>104 ± 5</td>
<td>99 ± 6</td>
<td>103 ± 5</td>
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<tr>
<td>8</td>
<td>105 ± 6</td>
<td>100 ± 5</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>POST</td>
<td>105 ± 3</td>
<td>103 ± 3</td>
<td>100 ± 3</td>
</tr>
<tr>
<td><strong>Total Cholesterol (mg/dl)(^3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>185 ± 37</td>
<td>207 ± 26</td>
<td>210 ± 23</td>
</tr>
<tr>
<td>2</td>
<td>192 ± 7</td>
<td>198 ± 26</td>
<td>213 ± 17</td>
</tr>
<tr>
<td>4</td>
<td>188 ± 18</td>
<td>203 ± 21</td>
<td>208 ± 16</td>
</tr>
<tr>
<td>6</td>
<td>201 ± 14</td>
<td>201 ± 25</td>
<td>215 ± 20</td>
</tr>
<tr>
<td>8</td>
<td>192 ± 16</td>
<td>194 ± 24</td>
<td>203 ± 18</td>
</tr>
<tr>
<td>POST</td>
<td>194 ± 3</td>
<td>201 ± 3</td>
<td>201 ± 3</td>
</tr>
<tr>
<td><strong>HDL-Cholesterol (mg/dl)(^3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>51 ± 8</td>
<td>47 ± 11</td>
<td>51 ± 10</td>
</tr>
<tr>
<td>2</td>
<td>47 ± 9</td>
<td>50 ± 12</td>
<td>51 ± 12</td>
</tr>
<tr>
<td>4</td>
<td>50 ± 8</td>
<td>48 ± 11</td>
<td>53 ± 12</td>
</tr>
<tr>
<td>6</td>
<td>52 ± 6</td>
<td>56 ± 10</td>
<td>56 ± 12</td>
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<tr>
<td>8</td>
<td>51 ± 7</td>
<td>51 ± 14</td>
<td>53 ± 9</td>
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<tr>
<td>POST</td>
<td>51 ± 1</td>
<td>54 ± 1</td>
<td>52 ± 1</td>
</tr>
</tbody>
</table>

\(^1\)Baseline average of 3 pretreatment values. \(^2\)Standard error of the mean. \(^3\)From Koenig, V. (23)
Table 4. Mean plasma levels of copper, zinc, total cholesterol, and HDL-cholesterol for all treatment groups at BL, week of controlled feeding, and post treatment.

<table>
<thead>
<tr>
<th>Week</th>
<th>Cu (ug/dl)</th>
<th>Zn (ug/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL-Cholesterol (mg/dl)</th>
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</table>

\(^{1}\) Baseline average of 3 pretreatment values. Values in same column not sharing a common superscript are significantly different (p<0.05).
Figure 1. Plasma zinc versus plasma copper for DC diet (Zn:Cu = 30:1) (weeks 2-8).

Figure 2. Plasma TC versus plasma copper for DC diet (Zn:Cu = 30:1) (weeks 2-8).
Figure 3. Plasma zinc versus plasma TC for DC diet (Zn:Cu = 30:1) (weeks 2-8).
Figure 4. Plasma HDL-C versus plasma copper for CO diet (Zn:Cu = 20:1) (weeks 2-8).

$r=-0.48$

$p<0.005$

$N=32$
Figure 5. Plasma HDL-C versus plasma zinc for CC diet (Zn:Cu = 20:1) (weeks 2-8).
DISCUSSION

A consistent increase in plasma copper was noted for all 3 treatment groups from baseline through 4 weeks of controlled feeding (Table 3). Mean plasma copper values of the Dairy Calcium (DC) treatment group (zinc:copper ratio of 30:1) were noted to be lower than the Control (CO) and Calcium Carbonate (CC) treatment groups (zinc:copper ratio of 20:1) throughout the study period. However, based on the results of an ANOVA, no significance was determined among treatment groups (p<.05). This observation may have been due to variations in copper levels within each group. A significant increase in plasma copper was noted from baseline to week 2 for all treatments, with a leveling off effect in the following weeks of the study and post treatment (Table 4). Since copper intakes were consistent throughout the study period, a homeostatic regulation may have stabilized plasma copper levels.

Klevay et al. (24) demonstrated plasma sensitivity to dietary copper when an intake of 4 mg copper/day for 39 days increased plasma copper in a young adult man after a depletion period of 105 days receiving 0.83 mg copper/day. Copper levels significantly increased from approximately 48 ug/dl during depletion, to 80 ug/dl after repletion (values obtained from graph), with levels then returning to near baseline, approximately 75 ug/dl. Various animal studies have also indicated a significant relationship between dietary copper and plasma or serum copper levels (15) (16) (25) (26) (27) (28) (29).

Plasma copper values obtained were within the normal circulating concentration range of 80-150 ug/dl as noted by Solomons (30). Dietary copper content of the DC, CO, and CC diets were 1.02, 0.93, and 0.96 mg/day, respectively (Table 1), and were noted to be lower than the provisional recommendation range of 2-3 mg/day for adults (19). However, Klevay et al. (31) found that the average daily copper intake of hospital and conventional diets were 0.76 and 0.78 mg/day respectively. Mean copper intakes of a biracial group of teenaged females were observed to be 0.92 mg/day for blacks, and 0.83 mg/day for whites (32). Therefore, the copper intake of the subjects involved in this study was not
considered below normal when compared to other populations. A consistent but not significant rise in plasma copper occurred for all 3 treatment groups from baseline through the weeks of controlled feeding (Table 3). This observance may have been affected by a slightly greater than average intake of copper than the subjects usually consumed.

The increase in plasma copper for the DC group was approximately 3 mg from baseline to week 8 of controlled feeding, while increases in plasma copper for the CO and CC groups were approximately 5 mg over the same period. While no significant difference was observed among groups, it is noteworthy that the DC group with the higher zinc:copper ratio of 30:1, produced the least increase in plasma copper levels. This may have resulted from the competition of zinc with copper for absorption.

Although plasma or serum copper has been the most widely used index of copper status (30), recent studies have determined that ceruloplasmin levels may be a more accurate indicator of copper nutriture (28) (33) (34) (35). Since 94% of copper in circulation is bound to ceruloplasmin (36), the measurement of ceruloplasmin may have produced more consistent results, and provided greater understanding of the zinc:copper relationship in this study.

Increases in plasma zinc levels from baseline were noted across all 3 treatment groups, however, no significant difference was observed between the groups (Table 3). For all groups, mean plasma zinc at baseline, and at weeks 2 and 4, were significantly lower than at weeks 6 and 8, and post treatment (Table 4). The stabilization of plasma zinc regardless of consistent supplementation may have reflected a homeostatic regulation as noted in previous studies (37) (38). Baseline plasma zinc group means of young adult women consuming a placebo, 15, 50, or 100 mg zinc/day, significantly increased from approximately 98, 100, 110, and 117 ug/dl (values obtained from graph), respectively, to approximately 111, 125, 145, and 157 ug/dl (values obtained from graph), respectively, after 4 weeks (37). After week 4, levels declined toward initial values. Peak values of plasma zinc were found to be directly related to the level of zinc dose since the highest
value of approximately 157 ug/ml occurred in the 100 mg supplemented group, and the lowest value of approximately 111 ug/dl was noted in the placebo. All 3 supplemented groups had a similar magnitude of response regardless of dose level. Black et al (38) observed a significant increase in plasma zinc from baseline levels in young adult males who consumed 50 or 75 mg zinc supplements/day. Mean serum zinc increased from baseline, 107 ug/dl, to 133 ug/dl at week 2, in subjects consuming 50 mg zinc/day. Mean serum zinc increased from 103 ug/dl at baseline, to 120 ug/dl at week 2, in subjects consuming 75 mg zinc/day. Levels for both groups then reached plateaus, possibly due to homeostatic control as noted by the researchers (38). Young adult men who consumed 160 mg zinc supplements/day for 5 weeks demonstrated an increase in mean plasma zinc levels from 94.8 ug/dl at baseline, to 145.7 ug/dl at week 5 (39). Zinc concentrations then returned to near-baseline levels after zinc administration had stopped. In the elderly, a zinc supplement of 100 mg/day produced a significant increase in serum zinc from 93 ug/dl at baseline, to 131 ug/dl after a 3 month period (40). Animal studies have also demonstrated a significant increase in plasma zinc with zinc supplementation (12) (15) (26) (41) (42) (43). Based on these previous studies, perhaps higher zinc supplementation or a longer study period may have produced more significant results regarding the relationship between dietary zinc and plasma zinc in this present study.

Plasma zinc levels were within the normal range of 70-125 ug/dl (44) (Table 3). Dietary zinc content, which included a 10 mg supplement/day, of the DC, CO, and CC groups were 30.34, 19.76, and 19.63 mg/day respectively (Table 1), and higher than the RDA of 15 mg/day for adult males (12 mg/day for females) (19). The mean dietary zinc intake of young adult males has been previously noted as 16.1 mg/day (45). Therefore, the increase in plasma zinc levels from baseline observed in all 3 groups (Table 4), may have been affected by a greater than average intake of zinc than that which subjects usually consumed.

The increase in plasma zinc for the DC treatment group was approximately 15 mg from baseline to week 8 of controlled feeding, while plasma zinc levels of the CO and CC
groups increased approximately 11 and 7 mg, respectively, over the same period. While no significant difference was found between groups, it is noteworthy that the DC group with the higher zinc: copper ratio of 30:1 produced the greatest increase in zinc levels during the study. This apparent increase may have been due to greater availability of zinc for absorption.

Due to varying calcium content of the treatment groups (Table 1), it is necessary to discuss zinc-calcium interactions. Dietary calcium levels have been associated with zinc bioavailability, but the detrimental effect of a high calcium intake has been determined to be dependent upon the presence of phytate in the diet (46). Forbes et al (47) found that by increasing calcium content of a diet consumed by rats from 0.4 to 1.2%, and with zinc intake constant at 9 mg/kg, zinc bioavailability was decreased by 50% in the presence of soy protein. However, when all the protein was supplied by egg whites, little or no effect was noted by increasing the percentage of calcium in the diet. The researchers contributed this effect to the presence of phytate in soy protein, and the ability of calcium to complex with phytate and decrease zinc availability. Morris and Ellis (48) found that high dietary calcium significantly reduced zinc bioavailability in rats after a 4 week period. In rats with phytate/zinc molar ratios of 20:1 and 10:1, and 0.75% dietary calcium, femur zinc levels were 98 and 208 ug/g, respectively. With phytate/zinc ratios of 20:1 and 10:1, and 1.75% dietary calcium, femur zinc levels were reduced to 56 and 42 ug/g, respectively, indicating a significant effect by calcium content. However, Sandstrom et al (49) found no significant negative effect on zinc absorption due to increased calcium in 3 soy containing diets of human men and women when phytate levels were fairly constant for all treatments. Snedeker et al. (50) also found no negative effect on zinc absorption by an increased level of calcium intake in young adult males for 39 days. Subjects fed 780 mg Ca/day had a mean plasma zinc level of 85 ug/dl, while those who consumed 2382 mg Ca/day were found to have a mean plasma zinc level of 88 ug/dl.
In this present study, the DC treatment group consumed the highest levels of calcium and zinc (Table 1). The greatest increase in plasma zinc from baseline through week 8 of controlled feeding (Table 3) was also observed in the DC group. Since plasma zinc levels were used in this study as measurements of zinc absorption, based on the results obtained, calcium did not affect zinc bioavailability. Effects of phytate cannot be ruled out, however, phytate content was not determined in this study.

As noted with copper, plasma or serum zinc levels have been found to be the most widely used indicator of zinc status (30). However, validity and accuracy have been questioned (30) (51). Solomons (30) noted that circulating zinc concentrations may be affected by many factors which may artificially elevate or reduce actual levels. These factors include external contamination of blood samples, hemolysis, veno-occlusion, inflammation or infection, albumin concentration, and prolonged fasting. Wada et al. (51) found that serum zinc levels were not significantly different for young adult men consuming either 16.5 or 5.5 mg zinc supplements/day. However, based on apparent absorption data using $^{70}\text{ZnCl}_2$ or $^{67}\text{ZnCl}_2$, the amount of zinc absorbed significantly increased for the subjects consuming the higher zinc supplement in the same study.

Based on ANOVA ($p<.05$), the zinc x copper interaction term was not significant, indicating zinc:copper ratios of 30:1 or 20:1 did not affect plasma zinc or copper levels in this study. Similar results were noted in animal studies (12) (15). In a previous study (52), both copper, zinc superoxide dismutase levels and plasma copper and zinc levels were obtained from young adult males who consumed either a placebo or a supplement of 50 mg zinc/day for 6 weeks. While no significant difference was noted for plasma copper levels, the mean copper, zinc superoxide dismutase level of the 50 mg zinc group at week 6, approximately 280 units/ml packed cells, was observed to be significantly decreased from baseline, approximately 340 units/ml packed cells (values obtained from graphs). Since the activity of copper, zinc superoxide dismutase is dependent on copper but not zinc status, it is considered a viable index of copper status (53). L'Abbe and Fischer (54) have
demonstrated that reductions in the copper metalloenzyme activities of rats fed either a copper deficient or high zinc diet were greater and preceded changes in plasma and tissue levels of copper. Greger et al. (55) noted a significantly increased level of fecal copper excretion in adolescent females who consumed 15 mg zinc/day, 0.90 mg/day, while those who consumed 11.5 mg zinc/day had a mean fecal copper excretion of 0.79 mg/day. These results indicated an antagonism between copper and zinc during the absorption process. Based on these previous studies, perhaps assessment of copper, zinc superoxide dismutase levels, or mineral balance may have produced more significant results regarding the relationship of dietary copper and zinc on absorption or bioavailability of these minerals.

No significant difference between plasma HDL-C levels was found among groups (Table 3), however, an effect was noted across the combined 3 treatments by week of controlled feeding (Table 4). Plasma HDL-C levels of all 3 groups peaked at week 6, and were significantly higher than at baseline, weeks 2, 4, and 8, and post treatment. It is not clear why HDL-C levels were increased at week 6 and then declined at week 8 despite controlled feeding conditions and stable mineral consumption. Individual variations, exercise patterns (56), or laboratory error may have affected results.

The lack of effect by dietary zinc, copper, or the zinc:copper ratio on TC levels within or between groups (Tables 3 and 4) was consistent with previous animal (57) (58) (59) (60), and human studies (61).

A negative correlation was found between plasma copper and zinc in the DC group (r=-0.43, p<.02) (Figure 1), which consumed the higher zinc:copper ratio of 30:1. Due to competition between zinc and copper for absorption, higher levels of zinc have been found to repress plasma copper in rats (26) (62) (63). Therefore, the negative correlation between plasma copper and zinc in the DC group may have been due to a suppression of copper by zinc during the absorption process at the surface or within the intestinal epithelium.
Plasma copper was negatively correlated with TC in the DC treatment group ($r=-0.39$, $p<0.04$) (Figure 2). Similar findings were found in rats (58). The activity of the plasma cholesterol esterifying enzyme, LCAT, has been suggested to promote transfer of cholesterol from erythrocytes to plasma in vitro (64). Researchers have observed that copper deficiency was correlated to decreases in LCAT activity in rats (65) (66), suggesting that copper may be required for the synthesis or activation of the enzyme. Lipoprotein lipase has also been found to be a necessary enzyme for the absorption and synthesis of cholesterol (67), possibly requiring copper for structure and activation (11) (65). Therefore, if enzyme systems were impaired due to inadequate copper, cholesterol metabolism may have been altered, resulting in higher cholesterol levels.

Also in the DC group, a positive correlation approached significance between plasma zinc and TC ($r=0.32$, $p<0.10$) (Figure 3). As previously noted in this study, plasma zinc was negatively correlated with plasma copper in the DC group. Due to the competition of zinc with copper for binding to metallothionein, less copper may have been available, decreasing plasma copper levels, while increasing plasma zinc levels. With less copper available for possible use as a component of the enzymes necessary for cholesterol metabolism, TC levels may have been affected.

A fairly high negative correlation ($r=-0.48$) that was highly significant ($p<.005$) was found between plasma copper and HDL-C in the CO treatment group (Figure 4). Because copper has been determined to be a necessary component of enzymes responsible for cholesterol metabolism (11) (65) (66) (67), it is unclear why HDL-C levels would be higher when plasma copper was lower, and vice versa, particularly at the more favorable zinc: copper ratio of 20:1. This observation may have been due to individual variations or laboratory inaccuracy.

A negative correlation between plasma zinc and HDL-C was observed in the CC treatment group, with a zinc: copper ratio of 20:1 ($r=-0.58$, $p<0.001$) (Figure 5). A similar negative correlation was noted by Freeland-Graves et al. (37). Hooper et al. (39) has
suggested that zinc may be atherogenic in humans due to an association with lowering plasma HDL-C levels, possibly by interfering with copper availability for cholesterol and lipoprotein enzyme systems.

While results of this study were inconsistent to support the zinc:copper ratio hypothesis (11), the trends and correlations observed are noteworthy. The largest increase in plasma zinc from baseline through weeks of controlled feeding, and the lowest increase in plasma copper were both observed in the DC group, which consumed the higher zinc:copper ratio of 30:1. These results, in combination with the negative correlation between plasma copper and plasma zinc in the same treatment group, strongly indicated a suppression of copper by zinc due to zinc-copper antagonism during absorption. Also, the negative correlation between TC and plasma copper, and the positive correlation between plasma zinc and TC in the DC group, demonstrated that the competition for absorption between zinc and copper affected TC levels.

Increases in plasma copper from baseline through weeks of controlled feeding, were similar for the CO and CC groups, zinc:copper ratios of 20:1. However, despite stable mineral consumption, the increase in plasma zinc from baseline through weeks of controlled feeding was slightly greater for the CO group than the CC group. Correlations for the CO and CC groups were also inconsistent. A negative correlation was observed between plasma copper and HDL-C in the CO treatment. This result was not expected based on the suggested role of copper in cholesterol and/or lipoprotein metabolism. However, the negative correlation found between plasma zinc and HDL-C in the CC group supported previous studies (37) (39) that higher levels of zinc lowered HDL-C. Since intakes of all nutrients were identical with the exception of calcium for the CO and CC treatment groups, further research with the relationship between calcium and zinc and/or copper may have allowed for a better understanding of the results in this study.
REFERENCES


SUMMARY AND CONCLUSIONS

Through various animal and human studies, dietary copper and zinc have been found to affect serum and/or plasma levels of TC and HDL-C (Klevay, 1973; Klevay, 1975; Caster and Parthemas, 1976; Petering et al, 1977; Eisemann et al, 1979; Looney and Lei, 1978; Frimpong and Magee, 1987). Results of these studies have supported the zinc:copper hypothesis which states, "a metabolic imbalance in regard to zinc and copper is a major factor in the etiology of coronary heart disease" (Klevay, 1975). This metabolic imbalance has been noted to be "either a relative or absolute deficiency of copper characterized by a high ratio of zinc to copper" (Klevay, 1975). While no significant differences were found between treatment groups' levels of plasma TC and HDL-C due to dietary effects to support the zinc:copper hypothesis, distinctive trends and correlations were noted in the study groups. Results of plasma zinc and copper levels were also found to be noteworthy.

The negative correlation between plasma copper and zinc in the DC group supported the antagonism theory that high levels of zinc suppress copper availability (Van Campen, 1970). Also in the DC group, the negative correlation between plasma copper and TC may have been affected by an inadequate amount of available copper for normal cholesterol metabolism. Furthermore, the positive correlation between plasma zinc and TC in the DC group supported the previous two correlations, due to a possible antagonism between zinc and copper. The negative correlation between plasma zinc and HDL-C in the CC group also supported the zinc:copper antagonism theory. If less copper were available, enzyme systems which regulate lipoprotein metabolism may have been impaired, affecting concentrations of both plasma copper and HDL-C.

Plasma copper and zinc levels were similar for all groups regardless of zinc:copper ratio. Mean plasma copper peaked in the 4th week of controlled feeding, while mean plasma zinc peaked in the 6th week. Both mineral levels then stabilized in the subsequent
weeks. This observation strongly supports regulation by a homeostatic mechanism for both zinc and copper.

An effect of dietary zinc on plasma levels of zinc was apparent in the DC group, although not significant. The largest increase in plasma zinc from baseline occurred in this group which consumed the higher amount of zinc, indicating a sensitivity to dietary intake. This higher zinc intake also appeared to repress copper levels, with a smaller increase in plasma copper from baseline than the groups with the lower zinc intake.

Although this study did not demonstrate strong conclusions regarding the effects of dietary zinc and copper on plasma levels of zinc, copper, TC, and HDL-C, the findings noted would benefit future research. Recommendations include:

1) Increase the length of the study period to include a controlled feeding period of 12 weeks. Previous human studies have shown more consistent results with longer time spans (Klevay et al., 1984; Hollingsworth et al., 1987; Black et al., 1988).

2) Incorporate zinc supplements of 10, 25, or 50 mg/day into the dietary intakes of the study groups, with a constant copper intake at 2 mg/day to meet the established provisional range (Committee on Dietary Allowances, 1989). Wider ranges of zinc supplementation have been shown to effect plasma levels of copper, zinc, TC, and HDL-C (Hooper et al., 1980; Freeland-Graves et al., 1982; Fisher et al., 1984; Black et al., 1988).

3) Analyze LCAT or LPL activity in addition to plasma copper and zinc levels to observe possible correlations. Previous studies have suggested a role for copper in these enzyme systems (Lau and Klevay, 1981; Harvey and Allen, 1981).
REFERENCES


APPENDIX A: METHODOLOGY

I. SUBJECT QUALIFICATION:

Study participants responded to recruitment ads and posters placed on Virginia Polytechnic and State University's campus. Subject qualifications included:

1. male
2. 20-35 years old
3. in good health
4. non-smoker
5. normotensive
6. sedentary to moderate exercise pattern
7. no personal history of heart disease
8. taking no high blood pressure medication
9. taking no blood lipid medication
10. blood cholesterol level greater than 200 mg/dl (determined at study screening)

II. STUDY FORMAT: (3 periods)

A. Preliminary period (20 days)
   • subjects eat usual diets
   • subjects keep 5-day dietary records
   • subjects have blood drawn, and blood pressure and body weights measured bi-weekly

B. Controlled feeding period (56 days)
   • subjects randomly assigned to 1 of 3 diet groups
   • subjects eat only food and beverages supplied by the study
• subjects eat meals at metabolic unit
• subjects have blood drawn and blood pressures measured bi-weekly
• subjects have body weights measured daily
• subjects provide total fecal and urine collections for three 7 to 9 day periods

C. Follow-up period (14 days)
• subjects eat usual diets
• subjects keep 5-day dietary records
• subjects have blood drawn, and blood pressures and body weights measured bi-weekly

III. SUBJECT CONSTRAINTS:
• must be in town every day during controlled feeding period
• must eat all meals (3 per day) at metabolic unit during feeding period
• must consume all food and beverages provided by study during controlled feeding period
• must consume no food or beverage other than those provided by study during controlled feeding period (distilled water was provided ad lib)
• must not change exercise habits during entire study period
• must not lose or gain weight during entire study period
• must collect all urine and feces during the 3 total collection periods during controlled feeding period
• must be present at all appointments to have blood drawn and blood pressures and body weights measured during entire study period
• must complete two 5-day dietary records
IV. DIRECT DETERMINATION OF TOTAL CHOLESTEROL (TC) IN PLASMA:

1. 0.100 ml of distilled water (blank), 0.100 ml of plasma, and 0.100 ml of direct
cholesterol working standards were transferred into appropriately labeled test tubes.
Three working standards of 100, 200, and 300 mg/dl were prepared from Sigma test
set standards and used for the TC standard curve.

2. 6.0 ml of cholesterol reagent* was added to each test tube and mixed well.

3. Test tubes were placed in 37 degree C water bath for 20 minutes.

4. All test tubes were removed from the water bath and mixed thoroughly. Contents of
each tube were transferred to cuvettes. Specimens and standards were read against the
reagent blank at 625 nm within 30 minutes.

5. Optical density of the three working standards were plotted on graph paper and the
values of controls and unknowns were read directly from the standard curve.

* Cholesterol reagent - 41.5% acetic anhydride, 13.6% sulfuric acid, 3.4% phosphoric
acid.
V. ISOLATION AND DETERMINATION OF HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C) IN PLASMA:

1. Plasma samples were removed from refrigerator and allowed to warm to room temperature.

2. Plasma samples were mixed well. Using a calibrated volumetric pipet, 1 ml of plasma was transferred into 5 ml glass conical centrifuge tubes in duplicate.

3. The HDL-C reagent** was mixed well. A calibrated micropipet was used to transfer 0.1 ml reagent to each centrifuge tube. Each tube was vortexed and covered with Parafilm, and allowed to stand at room temperature for 10 minutes.

4. Centrifuge tubes were placed in the refrigerated centrifuge at 4 degrees C. Samples were spun for 30 minutes at 3000 rpm. Supernatant was removed with a Pasteur pipet.

6. Standard solutions of 20, 50, and 100 mg/dl were prepared with appropriate dilutions of the test set standards for making the standard curve. Analysis was completed with the spectrophotometer as described in TC determination.

** HDL-C reagent - 0.6 ml 40,000 units heparin/ml, 10 ml 1.06 M MnCl₂·4H₂O
APPENDIX B: INFORMED CONSENT

SPRING 1986 FEEDING STUDY CONSENT OF PARTICIPATION

I have received an explanation of the Department of Human Nutrition and Foods human feeding study and understand the following:

As a subject, I will participate in the study from March 11 through June 10, 1986. I will be on a controlled diet during the period from April 1 through May 26. I understand that I am to consume only food and beverages provided by the study during the controlled feeding period. Diets will provide nutrients at or above levels recommended by the National Research Council, Food and Nutrition Board (1980). The diets will contain approximately 500 mg of cholesterol per day.

A physical examination or medical record check must be conducted on each subject prior to the study by university health services or a personal physician. If at any time, the study investigators, a physician, or the subject himself believes that his health may be impaired by remaining in the study, the subject may drop from the study.

No compensation or medical treatment, other than normally available through student health services and emergency service by the rescue squad, is available if injury is suffered as a result of the research.

Venous samples of blood, approximately 60 ml, will be drawn a total of 16 times throughout the study. Eight 2-consecutive day samples, separated by 7 or more days, will be the blood sampling schedule. Blood samples will be taken by qualified medical personnel. Individual serum cholesterol data will be available to each subject.

Mineral balance will be determined for three 9-day periods during the controlled feeding period. The data will be based on dietary intake and total urine and fecal output.

Subjects will be paid $400.00 for successful completion of the entire study. A subject may drop from the study at any time for health or personal reasons.

All subjects are invited to ask any questions about procedures at any time.

I understand the above and agree to participate in the Department of Human Nutrition and Foods human feeding and metabolic experiment from March 11 through June 10, 1986.

__________________________
Signature of Subject

F.W. Thye, Principal Investigator
231-6620

__________________________
Date

C.D. Waring, Chairman IRB
231-5284
APPENDIX C: STUDENT CLINICAL RECORD EVALUATION

I, ___________________________ authorize C.W. Schiffert, M.D., Director of Virginia Tech Student Health Services, to release requested information about my health to Forrest W. Thye, Ph.D.

Signed________________________

Social Security #________________

I have reviewed the Virginia Tech clinical record of

______________________________

and _______ Do not find a medical or physical condition

_______ Do find a medical or physical condition

that precludes his participation in the Department of Human Nutrition and Foods human feeding and metabolic study, Spring 1986.

______________________________

Director of Student Health Services
APPENDIX D: STUDY TIMETABLE - Spring 1986 HNF Feeding Study

Saturday, March 1
Subject Orientation

Tuesday, March 11
Beginning of Preliminary Period, Lab Day

Wednesday, March 12
Lab Day

Sunday, March 16 thru Thursday, March 20
Dietary Records

Thursday, March 20
Lab Day

Friday, March 21
Lab Day

Monday, March 31
End of Preliminary Period, Lab Day

Tuesday, April 1
Beginning of Controlled Feeding Period, Lab Day

Monday, April 14
Lab Day

Tuesday, April 15
Lab Day

Saturday, April 19 thru Tuesday, April 29
Total Urine and Fecal Collection

Monday, April 28
Lab Day

Tuesday, April 29
Lab Day

Tuesday, May 6 thru Tuesday, May 13
Total Urine and Fecal Collection

Monday, May 12
Lab Day

Tuesday, May 13
Lab Day

Tuesday, May 20 thru Tuesday, May 27
Total Urine and Fecal Collection

Monday, May 26
End of Controlled Feeding Period, Lab Day

Tuesday, May 27
Beginning of Follow-Up Period, Lab Day

Tuesday, June 3 thru Saturday, June 7
Dietary Records

Monday, June 9
Lab Day

Tuesday, June 10
End of Follow-up Period, Lab Day
APPENDIX E: RAW DATA

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VITA

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