EFFECTS OF COPPER SUPPLEMENTATION ON SERUM LIPIDS, SERUM GLUCOSE AND SERUM INSULIN IN POSTMENOPAUSAL WOMEN

By

JEANETTE MARIE DANIEL KORTE KNIESS

Bachelor of Science

Southeast Missouri State University

Cape Girardeau, Missouri

1997

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1999

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SERUM LIPIDS, SERUM GLUCOSE

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Thesis Approved:

Janice R Hermann

Thesis Advisor

Qiu Qates

Lee L Ebro

Wayne B. Powell

Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express sincere appreciation and gratitude to my advisor, Dr. Janice Hermann for her patience, guidance, and encouragement throughout the study and writing process. I am grateful for the valuable time she volunteered and her willingness to assist in every step of completing this degree. Her enthusiasm and commitment to education is inspiring. I also extend appreciation to Dr. Lea Ebro and Dr. Gail Gates for their professional advice and suggestions while serving on my thesis committee.

I must thank the Lord above for his presence in my life. I lean on His love, survive by His grace, and am grateful for the blessings He has given to me. Many thanks to my entire family, especially my parents for their endless love and support. To my brother, Chris, for setting a standard of excellence and providing words of encouragement and inspiration. To my sister, Kathy, for her unwavering belief in my ability and inspiring me to always seek more from life. To my best friend, Daniel, who has seen me through the best and the worst of times. I am grateful for his unyielding support, humor, and friendship.

Most importantly, my deepest appreciation and gratitude is extended to my husband Jason. I dedicate this thesis to him for encouraging me to follow my dreams, making numerous sacrifices along the journey, providing endless support and encouragement, and for continually lighting a candle in my darkness of doubt. He is my greatest inspiration and has been instrumental in helping me attain my goals. I am thankful for the blessing he has been in my life.

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CHAPTER I

RESEARCH PROBLEM

Introduction

Coronary heart disease (CHD) claims approximately 500,000 lives each year, nearly half of which are women (American Heart Association, 1998). In addition, CHD ranks as the leading cause of death in postmenopausal women in the United States and most industrialized countries. With the increase in the elderly population, CHD will become epidemic unless preventative measures are taken. (Wenger, 1997). Elevated plasma cholesterol concentration is strongly associated with the narrowing of coronary arteries by atherosclorosis, which is a primary cause of heart disease (Thomas & Braus, 1998; American Heart Association, 1998).

Studies have shown that women over the age of 55 have a greater risk for CHD due to elevated trigylcerides and low high-density lipoproteins (HDL) concentrations (Wenger, 1997; Thomas & Braus, 1998). Part of the reason for women's increased risk of CHD is the loss of estrogen after menopause, which has a protective effect during childbearing years. As women approach menopause, risk of heart disease begins to rise and continues to rise with age (American Heart Association, 1998). For reasons unknown, estrogen helps protect premenopausal women against heart disease. When menopause occurs, women's blood lipid concentrations also change. Low density lipoprotein cholesterol (LDL) and total cholesterol concentrations increase and HDL concentrations decrease as a direct result of estrogen deficiency (American Heart Association, 1998; Thomas & Braus, 1998; Vander Schouw et al., 1996).

Copper (Cu) is an essential trace element that is needed for cholesterol metabolism, myocardial contractility, and glucose metabolism (Nath, 1997; Olivaries & Uauy, 1996). Numerous studies have shown a relationship between cardiovascular mortality and copper deficiency in both rats and humans (Hoogeveaen et al., 1995; Jalili et al., 1996: Milne & Nielson, 1996; Olivaries & Uauy, 1996). These studies have shown that diets low in copper result in increased total cholesterol and LDL cholesterol and decreased HDL cholesterol concentrations.

According to the Food and Nutrition Board of the National Academy of Sciences, the estimated safe and adequate daily dietary intake (ESADDI) of copper is 1.5-3.0 mg (Food & Nutrition Board, 1989; Milne, 1994). According to the Food and Drug Administration's Total Diet Study, Americans' dietary copper intake is less than 80% of the suggested daily intake (Pennington & Young, 1991). Reiser et al. reported that the median copper intake of 22 men and women was 0.78 mg, well below the estimated safe and adequate range (Reiser et al., 1985). The third National Heath and Nutrition Examination Survey (NHANES III) reported that the mean copper intake of women age 50 to 59 was 1.11 mg/d, which is slightly below the estimated safe and adequate intake range of 1.5–3.0 mg/d (Alaimo et al., 1994). With the increased risk of CHD after menopause and the risk associated with a diet low in copper, an analysis of the effect of a diet adequate in copper on postmenopausal women could provide valuable information on protection against heart disease.

Objectives

The objectives of this study were to investigate the effects of twelve week copper supplementation on serum lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides), glucose, insulin, estradiol, apolipoprotein A-1 and apolipoprotein B concentrations in postmenopausal women with elevated cholesterol who were not using estrogen replacement therapy.

Null Hypotheses

The following null hypotheses were developed for this study:

1. There will be no statistically significant effect due to twelve weeks copper supplementation on serum total, LDL-cholesterol, HDL-cholesterol, or triglyceride concentrations in postmenopausal women.
2. There will be no statistically significant effect due to twelve weeks copper supplementation on serum glucose and serum insulin concentrations in postmenopausal women.
3. There will be no statistically significant effect due to twelve weeks copper supplementation on serum apolipoprotein A-1 and apolipoprotein B concentrations in postmenopausal women.

Assumptions

The following assumptions were made for this research:

1. It was assumed that the subjects took one supplement capsule two times each day for twelve weeks.

2. It was assumed that the subjects fasted for 12 hours before each blood collection.
3. It was assumed that the subjects did not alter their regular exercise or eating patterns during the course of the study.
4. It was assumed that the subjects did not take any lipid altering medication or estrogen replacement therapy.
5. It was assumed that the subjects were honest and accurate in reporting their food intake using the food frequency form.

Limitations

1. The food frequency forms were limited by the subjects' knowledge and understanding of food composition and portion sizes.
2. The results of the study are relevant to this sample group limiting extrapolation of results to the general population.
3. Dietary intake of other nutrients or trace minerals, such as zinc, might affect copper absorption or metabolism.
4. Dietary copper intake may have influenced absorption or metabolism of copper from the supplement.

Thesis Format

The tables and bibliographic citations in this thesis are written in manuscript form using the guide for authors for the Journal of Nutrition for the Elderly.

Chapter II

REVIEW OF LITERATURE

Women and Coronary Heart Disease

CHD is the single leading cause of death in America (American Heart Association, 1998). According to the American Heart Association, nearly 500,000 Americans died of CHD in 1995. In addition, close to 14 million Americans have a history of heart attack, angina pectoris or both, and 96.8 million American adults have blood cholesterol concentrations of 200 mg/dl or higher (American Heart Association, 1998). Plasma lipid concentrations are used as predictors of CHD risk, and elevated plasma total cholesterol, LDL-cholesterol and triglycerides are strongly associated with the development of CHD (American Heart Association, 1998; Wenger 1998).

While CHD is ranked as the leading cause of death in America, it is also the largest killer of American women (American Heart Association, 1998; Newman & Sullivan, 1996; Rose, 1998; Thomas & Braus, 1998; Villablanca, 1996; Wenger, 1997; Wenger, 1996). As women approach menopause, the risk of heart disease begins to rise and continues to rise with age. Data from the Third National Health and Nutrition Examination Survey (NHANES III) indicate that among people over age 65, women have higher blood cholesterol concentrations than men (American Heart Association, 1998). Wenger reported that two thirds of American women have at least one major risk factor for CHD, and the number of CHD risk factors increase with age (Wenger, 1997). More women now die from all types of cardiovascular diseases and heart attacks than men (American Heart Association, 1998; Rose, 1998; Wenger, 1998). With the continued aging of America and women's increased risk for heart disease with age, CHD will

probably become epidemic among women unless preventive measures are taken (Wenger, 1997; Wenger, 1998).

Estrogen and Coronary Heart Disease

The increased risk of CHD in postmenopausal women may be due to estrogen loss. Estrogen has been identified as a beneficial factor in the prevention of cardiovascular disease. This protective effect has been implicated through epidemiological studies that show an increased risk of CHD in postmenopausal and ovariectomized women. With menopause, and the loss of estrogen, changes occur in women's lipid profiles as total cholesterol and LDL-cholesterol concentrations increase and HDL-cholesterol concentration decrease (Liu & Bachmann, 1998; Newman & Sullivan, 1996; Thomas & Braus, 1998; Villablanca, 1996; American Heart Association, 1998). Women also experience an increase in triglyceride concentration, which when coupled with other lipid changes with menopause creates a greater risk for CHD (Thomas & Braus, 1998; Villablanca, 1996).

Estrogen administered to both pre-and post-menopausal women has been observed to significantly increase plasma HDL cholesterol, which is known to be antiatherogenic (Fahraeus & Wallentin, 1983; Prema et al., 1980). Previous studies involving oral administration of estradiol to premenopausal women have also reported an increase in HDL cholesterol, which was attributed to an enhanced synthesis of apolipoprotein A-1 (Schafer et al., 1983; Hazzard et al., 1984).

Brenner & Koo examined the effect of estradiol on serum HDL cholesterol in ovariectomized rats. They observed a significant increase in serum total and HDL

cholesterol in female rats injected daily with 7 μg estradiol benzoate in 0.1 ml cottonseed oil/100 g body weight for 5 weeks. The authors hypothesized that the observed increase in plasma HDL cholesterol was due to a decrease in HDL catabolism. This hypothesis was based on the delayed clearance of radiolabeled HDL. The authors also observed a significant decrease in hepatic lipase activity, which is involved in HDL clearance (Brenner & Koo, 1988).

Estrogen has also been observed to alter copper status in both humans and animals. Chilvers et al. studied the effect of estrogen on copper status in postmenopausal women. They observed an increase in plasma total copper concentration in postmenopausal women who consumed 15 μg of ethinyl estradiol daily for 3 months. The increase in plasma copper concentration was mainly due to an increase in ceruloplasmin synthesis, the main copper-binding protein (Chilvers et al., 1985).

Brenner & Koo also observed an increase in serum and hepatic copper concentrations in rats receiving 7 μg estradiol for five weeks (Brenner & Koo, 1988). In addition, Yang and Koo observed an increase in both serum HDL-cholesterol concentration and copper status of rats implanted with a 1.5 mg pellet of 17B-estradiol (Yang & Koo, 1998).

Thus, estrogen may play a protective role against CHD in women through an increase in HDL-cholesterol concentration and an improvement in copper status. However, with menopause, women lose the protective effects of estrogen. Hormone replacement therapy is often recommended to reduce CHD risk, however for some women it has been observed to increase cancer risk (Barrett-Conner et al., 1998). Thus,

adequate dietary copper intake may provide postmenopausal women with an alternative type of protection against CHD.

Copper and Coronary Heart Disease

Diets low in saturated fat and cholesterol as well as increased exercise are beneficial in lowering plasma lipids (Rose, 1998; Wenger, 1996; American Heart Association, 1998; Thomas & Braus, 1998). However, other dietary factors including trace minerals may affect lipid profiles. Numerous studies have established that poor copper status in both animals and humans increases the risk of CHD (Reiser et al., 1985; Klevay et al., 1984; Hermann et al., 1993; Medeiros et al., 1993). This is of particular interest because copper intakes are low in typical American diets. Reiser et al. reported a six day average copper intake among 22 men and women of 1.0 mg copper/day (Reiser et al., 1985). Klevay reported an average daily copper intake of 0.78 mg/d among American adults (Klevay, 1986). Furthermore, NHANES III reported a mean daily copper intake among women age 50-59 of 1.1 mg (Alaimo et al., 1994). Older adults are at particular risk for low mineral intakes (Tripp, 1997). Copper has an estimated safe and adequate daily intake (ESADDI) of 1.5 to 3.0 mg/day (Food & Nutrition Board, 1989). Thurman & Mooradian cite copper as one of the most frequent dietary deficiencies in the elderly population (Thurman & Morradian, 1997).

Copper deficiency has been implicated as a contributor to the development of CHD as it has been shown to produce hypercholesterolemia and alter lipoprotein profiles in copper deficient humans (Klevay et al., 1984; Reiser et. al, 1987; Hermann et al., 1993). Klevay et al., studied a young male during induced copper depletion in which the

subject consumed a diet without copper for 188 days. The authors observed an increase in total cholesterol and LDL-cholesterol concentrations during copper depletion. Total and LDL-cholesterol concentrations subsequently decreased during the copper repletion phase of the study where the subject received 3 mg of copper twice a day for 39 days (Klevay et al., 1984). Reiser et al., also investigated the effects of copper depletion on lipids in 24 males who consumed a copper deficient diet of 0.36 mg/1000 kcal for 11 weeks. In addition to an increase in LDL-cholesterol concentration, Reiser et al. observed a decrease in HDL cholesterol concentration with copper depletion (Reiser et al., 1987). Hermann et al. investigated the relationship between plasma lipids and mineral intake in 43 adults over fifty years of age. The authors observed elevated triglyceride concentrations in those adults with diets that contained less than 2/3 of the ESADDI for copper (Hermann et al., 1993).

In a human copper supplementation study, Hermann et al. observed a significant decrease in plasma total cholesterol, LDL cholesterol, and triglyceride concentrations in adults over fifty years of age who received a supplement of 3.0 mg copper/d for twelve weeks (Hermann et al., 1998). Milne & Neilson did not observe any changes in serum lipids in a study of 12 postmenopausal women who consumed a copper depletion diet containing 0.57 mg copper/day for 105 days followed by copper repletion with 2.0 mg copper/day for 35 days (Milne & Neilson, 1996).

Several studies investigating copper deficiency in animals have also reported alterations in lipid profiles. Allen & Klevay observed an increase in both HDL and LDL cholesterol concentrations in rats consuming a copper deficient diet of 0.57 μg/cu/g for 48 days. Although HDL-cholesterol increased 85% in copper deficient rats when the

HDL bound cholesterol was expressed as a percent of the total plasma cholesterol concentration, there was a 9.6% decrease in the percent of cholesterol associated with HDL. The bulk of the hypercholesterolemia observed in this study came from a 187% increase in plasma LDL-cholesterol (Allen & Klevay, 1980).

Harvey & Allen also observed a 91% increase in total cholesterol concentration and a 13% decrease in HDL-cholesterol concentration in rats consuming a copper deficient diet for 66 days. They observed a reverse of the normal 2:1 ratio of HDL: (VLDL + LDL) in the copper deficient rats. An increase in plasma triglyceride concentration in the copper deficient rats was also observed (Harvey & Allen, 1985).

Crosswel & Lei also investigated the effects of copper deficiency in rats for seven weeks. They observed an increase in all lipoprotein concentrations, with the largest increase occurring in VLDL and LDL concentrations. They also observed a significantly higher protein and cholesterol concentration in HDL (Crosswel & Lei, 1985).

Lefeve et al. noted a significant increase in total cholesterol and HDL cholesterol concentrations in rats consuming a copper deficient diet for six weeks. They also observed that copper deficient rats had significantly higher Apolipoprotein A-I (Apo A-1) concentrations in the HDL fraction than the control animals (Lefeve et al., 1986). Lee & Koo also observed an increase in total cholesterol with a marked increase in HDL in rats consuming a copper deficient diet for twelve weeks. They concluded that the hypercholesterolemia they observed was primarily due to an increase in cholesterol in the HDL subfraction that contained a large amount of Apo A-I but did not contain Apo E (Lee & Koo, 1988).

Al-Othman et al. also reported copper deficiency induced hypercholesterolemia, characterized by an increase in plasma HDL in rats fed a low copper diet for seven weeks. They reported an increase in HDL and LDL protein and cholesterol concentrations as well as an increase in triglyceride concentration in copper deficient rats. The plasma pool size of protein, triglycerides, phospholipids, LDL-cholesterol, and HDL-cholesterol significantly increased with copper deficiency. A uniform increase in HDL protein and lipid components indicated that there was an increase in the number of HDL particles but not an increase in the HDL particle size (Al-Othman et al., 1992).

Lei suggested that the hypercholesterolemia observed in copper deficient animals may be due to a shift in cholesterol from the liver pool to the plasma pool (Lei, 1978). Allen & Klevay also suggested that the hypercholesterolemia in copper deficient rats was due to an increased rate of cholesterol clearance from the liver pool to the plasma pool (Allen & Klevay, 1978). Shao & Lei demonstrated that cholesterol esters, newly synthesized from (2-14 C) mevalonate, cleared the liver faster in copper deficient rats compared to controls (Shao & Lei, 1980).

The role of regulation enzymes in cholesterol metabolism including hepatic 3-hydroxyl-3-methylglutaryl coenzyme A (HMGCoA) reductase and lipoprotein lipase (LPL) activity in copper deficient animals have been examined. HMGCoA reductase is the rate-limiting enzyme in cholesterol synthesis (Rodwell et al., 1976). LPL is responsible for the uptake of cholesterol and triglycerides from lipoproteins into tissues (Lau & Klevay, 1982). An increase in HMGCoA reductase activity was observed in copper deficient animals thus enhancing hepatic cholesterol synthesis (Yount et al., 1990; Nath, 1997; Sangbae et al., 1992). A decrease in LPL activity has been observed with

copper deficiency, which may result in decreased clearance and thus elevated plasma triglycerides and total cholesterol concentrations (Koo et al., 1988; Lau & Klevay, 1982). In a human copper deficiency study, Klevay et al. observed a decreased lipoprotein lipase activity. This observation agrees with decreased LPL activity in copper-deficient rats observed in a previous study (Klevay et al., 1984).

In addition to the effect on plasma lipid concentrations, copper may have a role in CHD through its effect on oxidation. It is well accepted that elevated LDL-cholesterol increases the risk for CHD and oxidation of LDL-cholesterol further increases that risk. Copper is a cofactor for a number of important metalloenzymes including Cu/Zn superoxide dismutase (SOD) (Nath, 1997). Cu/Zn SOD is a sensitive indicator of copper status. Changes occur in SOD concentration before changes occur in other indicators associated with severe copper deficiency such as plasma copper concentration (Milne & Nielsen, 1996; Reiser et al., 1985).

SOD along with glutathione peroxidase constitute the primary catalytic defense against metabolically generated free radicals (Nath, 1997). SOD is found in tissues such as the liver and erythrocytes with normal ranges for free living subjects of 500-700 μg/g hemoglobin or 30–80 U/10^9 erythrocytes. SOD functions to scavenge the intermediates of oxygen reduction such as the superoxide radical. In this capacity, SOD is involved in the prevention of tissue peroxidation, and decreased SOD activity is associated with enhanced cell damage (Reiser et al., 1985).

Dietary copper deficiency leads to decreased SOD activity in the liver, aorta, lung, and erythrocytes, as well as to increased cell membrane lipid peroxidation. In addition, increased peroxidation is correlated with low SOD and glutathione peroxidase

activities (O'Dell, 1990; Nath, 1997). Studies have shown that copper deficient tissues have decreased SOD activity. Jalili et al. observed a decrease in SOD activity in copper deficient rats fed less than 1.0 mg Cu/kg for 40 days (Jalili et al., 1996). Prohaska also observed decreased SOD activity in rats fed a low copper (0.6 ppm) diet for two weeks (Prohaska, 1983). Milne & Nielson studied rats consuming 0.57 mg Cu/d for 105 days followed by repletion with 2.0 mg Cu/d for 35 days. They reported SOD activity decreased significantly during low copper intake, but did not increase during repletion (Milne & Neilson, 1996).

Copper is also a component of ceruloplasmin. Ceruloplasmin is a plasma glycoprotein containing 6 atoms of copper that has ferro-oxidase activity. Most copper is converted to ceruloplasmin and released into the blood where it constitutes 90% of the plasma copper pool (O'Dell, 1990; Milne, 1994; Nath, 1997, Ehrenwald et al., 1994, Fox et al., 1995). Ceruloplasmin donates copper to nonhepatic tissues for synthesis of cuproenzmes such as cyctochrome c oxidase, superoxide dismutase and lysyl oxidase (O'Dell, 1990, Harris, 1987). It also has a role in copper transport to the heart muscle and aortic endothelium. In addition, copper from ceruloplasmin is believed to regulate aortic Cu/Zn SOD (Nath, 1997).

There are conflicting reports on the effect of copper intake on ceruloplasmin activity. Several studies have reported that ceruloplasmin activity is depressed with copper deficiency, but returns to normal when copper intake is adequate. Prohaska observed a decreased ceruloplasmin activity in rats fed a low copper diet (0.6 ppm) for two weeks (Prohaska, 1983). Klevay et al. studied a young male during induced copper depletion in which the subject consumed a diet without copper for 188 days. They

observed a decrease in ceruloplasmin activity during copper depletion and a return to normal during the copper repletion phase where the subject received 3 mg copper twice a day for 39 days (Klevay et al., 1984). Milne et al. also observed decreased ceruloplasmin activity in men consuming a low copper diet (0.89 mg/day) for 120 days with a return to normal with adequate copper intake (Milne et al., 1990). However, in a study of rats consuming 0.57 mg copper for 105 day followed by consumption of 2.0 mg copper for 35 days, no significant change in ceruloplasmin activity was observed during copper depletion, but rather ceruloplasmin activity decreased with copper repletion (Milne & Neilson, 1996).

Diabetes

An estimated 15.7 million people in the U.S. have diabetes, which is ranked as the seventh leading cause of death in the US. Type 2 diabetes, a metabolic disorder resulting from the body's inability to make enough or properly use insulin, is the most common form of diabetes as it accounts for 90 to 95% of all cases (American Diabetes Association, 1998). Type 2 diabetes is considered a strong risk factor for the development of CHD. Adults with diabetes are 2-4 times more likely to have heart diseases, and the prevalence of CHD with diabetes increases with age. Evidence of coronary heart disease is present in 75% of diabetes related deaths and in 7.5 to 20 % of people with diabetes over age 45 (American Diabetes Association, 1998). Elevated blood glucose concentrations much lower than those necessary to diagnose diabetes are also associated with an increased risk of CHD (Gerstein & Yusuf, 1996). Furthermore, individuals with type 2 diabetes generally have higher triglycerides and lower HDL-

cholesterol concentrations than individuals without diabetes. Insulin resistance associated with type 2 diabetes is believed to be a contributing factor to the development of hypertriglyceridemia (Syranne & Taskinen, 1997).

The prevalence of diabetes as well as the risk for type 2 diabetes increases with age. There are 6.3 million people age 65 and older that have diabetes and approximately half of all cases occur in people over the age of 55 (American Diabetes Association, 1998). People over the age of 45, those who are overweight, and people with low HDL-cholesterol or high triglycerides are at highest risk for type 2 diabetes (American Diabetes Association. 1998). Unfortunately, diabetes is reaching epidemic proportions due to the increasing number of older Americans and a greater prevalence of obesity and sedentary lifestyles. Reducing lifestyle risk factors through weight loss, improved nutrition and increased physical activity may delay or even prevent the onset of type 2 diabetes (American Diabetes Association, 1998). Trace minerals, in particular copper, may also have a role in diabetes risk.

Copper and Diabetes

Alterations in copper status have been observed in people with diabetes. Awadallah et al. reported serum copper concentrations in children with diabetes were slightly decreased compared to non-diabetic children. They also observed a decrease in ceruloplasmin concentration in children with diabetes compared to non-diabetic children (Awadallah et al., 1978). Car et al. observed lower serum copper concentrations in both non-insulin dependent and insulin dependent diabetics when compared to non-diabetics. They observed that changes in copper concentrations were not affected by a change in

glucose concentration in either type of diabetes, which suggests that the type of diabetes was not directly responsible for changes in serum copper concentration. They also observed increased urinary copper excretion, increased copper absorption, and decreased CuZn SOD activity in diabetic subjects (Car et al., 1992). In contrast, Rohn et al. did not observe any differences in serum copper concentrations between 45 diabetic children and 12 non-diabetic children (Rohn et al., 1993).

Copper deficiency has been shown to play a role in the regulation of glucose metabolism as it is associated with impaired glucose tolerance and characterized by increased blood glucose and glucose intolerance (Mertz, 1994). Bhathena et al. conducted a study with 11 healthy males and 9 carbohydrate sensitive males who followed a diet containing 1.03 mg copper/d for 11 weeks, followed by a diet of 3 mg copper/d for 3 weeks. They observed a delay in insulin secretion and an increase in plasma glucagon concentration with the consumption of a marginally deficient copper diet (Bhathena et al., 1988).

Klevay et al. studied two healthy men who were consuming diets providing 0.7-0.8 mg/d of copper. The subjects were initially supplemented with 0.5 mg copper for 30 days. After which, subjects consumed a copper deficient diet providing 0 mg copper for 120 days, followed by a copper repletion diet providing 3.5 mg copper for 30 days. Glucose tolerance tests at 20 to 60 minutes post glucose infusion were conducted after 30 days (initial supplementation), after 120 days (depletion) and after 30 days (repletion). The average insulin concentration at both 20 and 60 minutes post glucose infusion declined by 10 μU/ml during the copper depletion phase and further declined during the repletion phase. Average serum glucose concentration, however, increased during the

depletion phase and decreased during the repletion phase. Thus it was observed that after the copper repletion phase, glucose clearance was more rapid, even though insulin concentration was lower (Klevay et al., 1983).

Similar findings have been observed in several animal studies. Fields et al. studied rats fed 1.2 ug copper or 5 ug copper per mg body weight for 9 weeks. They observed that copper deficiency impaired glucose tolerance in rats fed both glucose and fructose. An increase in glycosylated hemoglobin, triglycerides and cholesterol was also observed in the copper deficient fructose fed rats. In addition copper deficiency decreased insulin binding, lipogenesis, and glucose oxidation (Fields et al., 1984).

Cohen et al. observed a delayed plasma insulin response to an oral glucose load in rats fed a copper deficient diet when compared to rats fed a copper-supplemented diet for four months. They also observed a significant increase in plasma glucose concentrations at 60 and 120 minutes post glucose load in the copper deficient rats compared to the copper-supplemented rats (Cohen et al., 1982).

Hassel et al. investigated the effects of rats fed 0.85 mg copper/d or 8.0 mg copper/d for five weeks. They observed that although there was no difference in the fasting glucose levels, at 30 minutes after glucose injection the copper deficient rats had higher plasma glucose concentrations compared to the copper supplemented. After 2 hours there were no significant differences between the two groups. They also investigated the effects of copper supplementation on plasma insulin concentrations. They did not observe a significant difference in mean fasting insulin levels between groups. Plasma insulin concentration peaked at 30 minutes for copper supplemented rats, but was delayed to 60 minutes for the copper deficient rats. The authors speculated that

the observed delay in insulin response was responsible for the glucose intolerance observed in copper-deficient rats (Hassel et al., 1983). In contrast, Fields et al. studied marginal copper deficient rats consuming 0.6 ug Cu/g for 2 weeks. They reported that marginal copper deficiency had no effect on either plasma insulin or plasma glucose concentrations (Fields et al., 1996).

Abdul-Ghani et al. fed rats $Cu(OAc)^2$ in doses varying from 10 to 100 mg/kg body mass. They observed copper repletion decreased serum glucose and decreased insulin concentrations. They reported that copper complexes were more active than inorganic copper. These complexes facilitate absorption, distribution and utilization of copper, which is essential for activation of copper-dependent enzymes required to maintain normal physiological processes (Abdul-Ghani et al., 1996).

Cohen et al. also observed impaired glucose tolerance and delayed plasma insulin response to oral glucose load in rats fed a copper poor diet containing 6.7 ppm copper. They observed that the effect of copper on glucose incorporation differed from that of insulin. However, they observed a synergistic effect as copper enhanced the effect of insulin, and insulin also enhanced the effect of copper. When copper was added to tissues incubated first by insulin, there was greater glucose incorporation suggesting that preconditioning cells with insulin was needed for maximal effect of copper on glucose incorporation. They also observed increased insulin binding in the presence of copper due to an augmented insulin receptor capacity and affinity (Cohen et al., 1986).

Researchers have also investigated the effect of copper on lipogenesis. Following insulin binding, glucose is transported across the cell membrane where post receptor processes such as lipogenesis occur. Copper stimulates lipogenesis and glucose oxidase,

however, copper combined with insulin has been observed to have a greater effect on glucose incorporation into glycogen and into lipids than copper or insulin alone. Fields et al. reported that insulin was required to stimulate copper in order to effect lipogenesis. They studied copper deficient rats for 7 weeks and found that copper and insulin combined significantly decreased peak blood glucose at 30 minutes and increased incorporation of glucose to lipids due to formation of a more stable complex which increased insulin binding and or decreased its degradation. This study revealed that insulin and copper combined significantly decreased blood glucose in vivo and significantly increased lipogenesis when compared with either copper or insulin alone. Copper increased insulin binding and stimulated deoxyglucose transport by isolated adipocytes suggesting that copper may interact with insulin to form a complex which has greater affinity for a receptor site than insulin alone and or increased the stability of insulin (Fields et al., 1983).

In another study, Fields et al. observed that copper stimulated glucose transport and increased specific insulin binding in rats. They hypothesized that the increased insulin binding is due to an increased number of receptor sites (Fields et al., 1984). Bhathena et al. studied nine carbohydrate sensitive and eleven normal subjects during marginal copper depletion and adequate copper intake for fourteen weeks. They reported increased insulin binding, an increased fasting plasma insulin concentration, and a higher insulin response to oral glucose in the carbohydrate sensitive subjects regardless of copper status. They hypothesize that the increased insulin binding was due to an increase in the number of insulin receptors without a change in their affinity. This suggests that there was an altered response of the insulin receptor, namely the failure of plasma insulin

to regulate the number of receptors. They did not observe any effect of copper deficiency on the number or affinity of insulin receptors even though previous reports have indicated that copper deficient rats had fewer insulin receptors (Bhatena et al., 1988).

The selective impairment of glucose induced stimulation of insulin in animals fed a copper poor diet may involve impairment in glucose metabolism in the beta cells. Another probable mechanism of the effect of copper on the permeability of the cell membrane and coppers oxidative effect is the reduced activity of the oxidative amine oxidases. Adults with type 2 diabetes appear to have a selective inability to secrete insulin following glucose stimulation. It is unclear whether this effect is genetically inherited or acquired. In rats fed a copper poor diet (1.2 ppm) it has been shown that the defect is not genetically inherited, but may be acquired as a result of changes in environmental conditions (Cohen & Miller, 1986).

It has been suggested that oxidation of the pancreatic beta cells contributes to the development of diabetes. Cu/Zn SOD may also play a role in the etiology of type 2 diabetes as it provides the body with defense from oxygen radicals and plays a role in oxidative metabolism. Car et al. observed a lower Cu/Zn SOD concentration in diabetic subjects compared to healthy subjects (Car et al., 1992). A decrease in Cu/Zn SOD activity has also been reported in both animals and humans with inadequate copper intake. Furthermore, an enhanced tolerance of pancreatic beta cells to oxidative activity has been observed in those with copper adequate diets (Kubisch et al., 1994).

Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders such as diabetes. It is known to accelerate aging, cardiovascular and microvascular lesions in diabetic patients (American Diabetes Association, 1998; Faure

et al., 1993). As previously cited, copper through Cu/Zn SOD has an antioxidant effect and thus copper status may be an important factor in the development of CHD and the complications of diabetes.

CHAPTER III

MATERIALS AND METHODS

Subjects

This study was approved by the Oklahoma State University Institutional Review Board for human subject research (Appendix A). Women past menopause with elevated total cholesterol, who were not taking medication to lower blood cholesterol or estrogen replacement therapy, were solicited for this study. Subjects were solicited by announcements through local physicians, college and university mailings, and personal contacts with churches, senior citizen organizations, and local stores as well as through flyers distributed throughout the community (Appendix B).

Nineteen subjects volunteered for this study; however, five subjects were not included in the data analysis. One subject was on estrogen replacement therapy, two subjects were diabetic, and two subjects were non-compliant taking the supplement. As a result, data from the remaining fourteen subjects were used in the data analysis. There were seven subjects in each treatment group (placebo and copper supplemented).

Subjects who volunteered to participate signed an approved informed consent (Appendix C). They were interviewed individually prior to the onset of the study in order to complete a health questionnaire (Appendix D) and were trained individually by a registered/licensed dietitian on how to complete a food frequency questionnaire (Appendix E).

Preparation of Supplements

Supplements for this study were prepared in the Department of Nutritional Sciences laboratory at Oklahoma State University. Number two gelatin capsules

(Apothcary Product, Inc., Burnsville, MN) were filled using a gelatin capsule filler machine (Quanterron, Inc., Burnsville, MN).

Placebo capsules contained approximately 0.1979 g U.S.P. grade lactose (Spectrum Quality Product, Inc., Gardene CA). The copper supplementation consisted of 29.47 g U.S.P. grade $CuSO_4 \bullet 5H_20$ (Professional Compounding Centers of American. Inc., Houston, TX) in 940.53 g U.S.P. grade lactose, so that 0.194 g of copper supplement mixture was calculated to contain 1.5 mg copper. The lactose and $CuSO_4 \bullet 5H_2O$ were mixed together for 8 hours in a ball mixture to obtain a homogeneous distribution of the copper in the lactose. The copper and placebo supplements were analyzed for copper content using atomic absorption spectrophotometer, Model 5100 PC (Perkin-Elmer Copr., Norwalk, CT). Ten samples of the copper and placebo mixtures, weighing 0.1 g, were wet and dry ashed using a modified wet and dry ash procedure (Hill et al., 1985). The average analyzed copper content of the placebo was negative 42 μg/g supplement mix. The average analyzed copper content of the copper supplement was 7004 μg/g supplement mixture. Since the analyzed copper concentration was lower than calculated values, the filled weight of the capsules was adjusted to 0.214 g per capsule in order to provide 1.5 mg of copper per capsule.

Experimental Design

This study followed a pre-test, experimental treatment, post-test design. Volunteers were screened for serum total cholesterol concentration three weeks prior to starting the study to ensure subjects had total cholesterol concentrations greater than 200 mg/dl. Subjects were randomly assigned to treatment groups. Pre and post-data

collections were performed at the beginning and the end of the 12-week supplementation period.

Data Collection

Screening

Subjects signed an informed consent at the beginning of the screening, after it was verbally explained to them by the principal investigator. Subjects also completed a Health Questionnaire to assess their health status, medication use, and exercise regimens (Appendix D). Subject's height was measured while standing erect against the wall with barefoot heels together. A scaled rule attached to the wall was read to the nearest centimeter. Weight was measured without shoes using a calibrated beam scale (Continental Health-O-Meter, Chicago, IL). Weight was read to the nearest pound.

Subjects' were trained by a registered/licensed dietitian on how to complete a modified version of the Willetts food frequency questionnaire (Eck et al., 1991; Appendix E). Subjects were provided with non-biasing bean bag models to illustrate one cup, ½ cup, and ¼ cup portion sizes. Wooden blocks were provided to illustrate 3-oz meat portions.

Subjects received a baseline supplement, which was the lactose placebo, to take for three weeks between the screen and the pre-data collection in order to allow the subjects to adjust to taking the study supplements twice a day. Subjects were instructed to take two supplements each day, one with a morning meal and one with an evening meal.

Pre-data Collection

At the pre-data collection, subjects brought in a complete one-week food frequency questionnaire, which reflected their dietary intake for the week before the pre-data collection. The subjects were then weighed to the nearest pound. Subjects came to the pre-data collection following a 12 hour fast, but were instructed to consume water. Due to variations in plasma mineral concentrations with the time of the day, all blood collections were performed between 7:30 and 9:30 a.m. A licensed phlebotomist drew a fasting blood sample in two 7.5 ml serum tubes (Monovette, Sarstedt, Newton, NC) using butterfly needles and trace mineral free syringes. After the blood collection, subjects were provided with nutritional support in the form of orange juice, muffins, and coffee and were asked to sit for 20-30 minutes.

The subjects were randomly assigned to treatment groups and provided with a one month supply of the assigned supplement. Subjects were instructed to take two capsules each day, one in the morning and one in the evening with meals. The second and third month's supply of supplements were delivered individually. The subjects received $ 50.00 for their participation in the study.

Post-data Collection

At the end of 12 weeks supplementation, subjects returned for the post-collection following a 12 hour fast. Subjects brought in a completed food frequency questionnaire, which reflected their food intake for the week prior to the post-collection. A Follow-up Health Questionnaire was completed to determine if there were any changes in their health status or medication usage (Appendix F). Subjects' weight was measured to the

nearest pound and a licensed phlebotomist drew a fasting blood sample in two 7.5 serum tubes. Following blood collection, subjects were asked to sit for 20 – 30 minutes where they were provided nutritional support in the form of orange juice, muffins, and coffee.

Blood Handling and Storage

The same blood handling techniques were followed at both the pre- and post-data collections. After drawing the blood, the serum tubes were allowed to sit in an ice bath for 30 minutes. The tubes were centrifuged using a TJ-6R Tabletop centrifuge (Beckman Instruments, Inc., Palo, CA) for 20 minutes at 1520 g at 4° C to separate the serum. The serum was carefully removed with a transfer pipette and transferred into individual plastic storage containers, covered with parafilm and stored for future analysis as follows:

1. 0.5 ml transferred into a 0.5 ml FARA tube for LDL cholesterol analysis
2. 0.5 ml transferred into a 0.5 ml FARA tube for total cholesterol, triglyceride, apolipoprotein A-1, apolipoprotein B, and glucose analysis
3. 250 ul transferred into a 0.5 ml FARA tube for HDL cholesterol analysis
4. 250 ul transferred into a 0.5 ml tube for insulin analysis
5. 500 ul transferred into a 0.5 ml siliconized tube for estradiol analysis

LDL-cholesterol tubes were stored at 4°C and kept for a maximum of ten days before analysis. Total cholesterol, HDL-cholesterol, apolipoprotein A-1, apolipoprotein B, triglyceride, glucose and estradiol tubes were stored at –20°C. Insulin tubes were frozen at –70°C.

Biochemical Analysis

Total cholesterol

Serum total cholesterol concentration was measured using a quantitative, photometric determination method using the Roche kit number 44307 (Roche Diagnostic System, Inc., Montclair, NJ). Total cholesterol concentration was determined by measuring absorbance at 500 nm using the FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ).

HDL-cholesterol

Serum HDL-cholesterol was obtained by a separating reagent for HDL using Roche kit number 44136 (Roche Diagnostic System, Inc., Montclair, NJ). The addition of physphotungstic acid and magnesium ions to the serum allows chylomicrons, VLDL, and LDL to precipitate. After certrifugation, only the HDL remains in the supernatant. HDL-cholesterol concentration was measured using the Roche kit number 44307 (Roche Diagnostic System, Inc., Montclair, NJ) cholesterol reagent and measured using the FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ).

LDL-cholesterol

Serum LDL-cholesterol was obtained by a direct, quantitative determination of LDL cholesterol in the serum using Roche kit number 47416 (Roche Diagnostic System, Inc., Montclair, NJ). The LDL cholesterol concentration was measured by performing an enzymatic cholesterol assay on the filtrate solution and was measured by the FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ).

Triglycerides

Serum triglyceride concentration was measured using a quantitative, spectrophotometric determination method using the Roche kit number 44119 (Roche Diagnostic System, Inc., Montclair, NJ). Triglyceride concentration was determined by measuring absorbance at 490-550 nm using the FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ).

Apolipoprotein A-1

Serum Apo A-1 concentration was measured using a quantitative, immunoturbidimetrical determination using the Roche kit number 44162 (Roche Diagnostic System, Inc., Montclair, NJ). The degree of turbidity was measured at 340 nm using the FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ).

Apolipoprotein B

Serum Apo B concentration was measured using a quantitative, immunoturbidimetrical determination using the Roche kit number 44161 (Roche Diagnostic System, Inc., Montclair, NJ). The degree of turbidity was measured at 340 nm using the FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ).

Glucose

Serum glucose was measured using a quantitative spectrophometric determination method using the Roche kit number 47383 (Roche Diagnostic System, Inc., Montclair, NJ). The glucose concentration was determined by measuring absorbance at 340 nm using the FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ).

Radioimmunoassay

Insulin

Insulin was measured using a double antibody radioimmunoassay (RIA). Insulin determination is based on the ability of an antibody to bind a fixed amount radioactive isotope. Frozen serum samples were thawed and inverted prior to analysis. All tests were run in duplicate except non-specific binding tubes (NSB), which were run in quadruplicate. For each sample, duplicate values were averaged. If duplicate values were not within 5%, samples were reanalyzed. The Equate (Equate RIA, South Portland, ME) option 1 procedure was followed. Insulin tracer and antiserum were added to all tubes using an Eppendorf repeating pipette. All tubes were vortexed, covered with parafilm, and incubated at room temperature for ten minutes. The supernatant of each tube was decanted using a blotting technique, so that only the pellet remained. The tubes were counted for one minute using a gamma counter (Packard Coba II, Auto Gamma, Packard Instrument Co., Meriden, CT).

Estradiol

Estradiol was measured using a double antibody radioimmunoassay (RIA). Insulin determination is based on the ability of an antibody to bind a fixed amount radioactive isotope. Frozen serum samples were thawed and inverted prior to analysis. All tests were run in duplicate except non-specific binding tubes (NSB), which were run in quadruplicate. For each sample, duplicate values were averaged. If duplicate values were not within 5%, samples were reanalyzed. The Equate (Equate RIA, South Portland, ME) option 1 procedure was followed. Insulin tracer and antiserum were added to all tubes using an Eppendorf repeating pipette. All tubes were vortexed, covered with parafilm, and incubated at room temperature for ten minutes. The supernatant of each tube was decanted using a blotting technique, so that only the pellet remained. The tubes were counted for one minute using a gamma counter (Packard Coba II, Auto Gamma, Packard Instrument Co., Meriden, CT).

Food Frequency Questionnaire Analysis

The one week food frequency questionnaires were analyzed and averaged using the Food Processor Plus Program (version 7.11, ESHA Research, Salem, OR). The same food codes were used for all subjects. The average dietary data included kilocalories, protein, carbohydrate, fiber, total fat, saturated fat, copper, and percent of calories from carbohydrates, protein, fat, and saturated fat.

Data Analysis

Data were analyzed using the Statistical Analysis System (SAS Inst. Inc., Cary, NC, 1987) version 6.06, available at Oklahoma State University. Subjects' age, weight, BMI, average dietary data, and serum measures were compared at baseline using the SAS general linear model (GLM) procedure to see if there were any differences between groups at baseline. Changes in serum lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, Apo A-1, Apo-B, and triglyceride), glucose, insulin and estradiol concentrations after 12 weeks of supplementation of copper were compared to changes in the placebo group using the SAS GLM procedure. Changes in average dietary data were analyzed across time within groups using the SAS repeated measures procedure to determine if there was any change in average dietary intakes across the supplementation period. Significance level was set at $P \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Description of the Subjects

Sample Size

The subjects in this study were free-living volunteers from the community of Stillwater, Oklahoma and the surrounding area. Nineteen subjects volunteered for this study; however, five subjects were not included in the data analysis. One subject was on estrogen replacement therapy, two subjects were diabetic, and two subjects were non-compliant taking the supplement. As a result, data from the remaining fourteen subjects were used in the data analysis. There were seven subjects in each treatment group (placebo and copper supplemented).

Age and Anthroprometric Measurements

Subjects' mean age, height, weight, and body mass index (BMI) are presented in Table I. The mean age of the placebo group was 58 years, while the mean age of the copper supplemented group was 59 years. There was no significant difference in age between the two groups.

Mean body weight for the placebo group was 79.0 kg and 84.0 kg for the copper supplemented group at baseline. The mean total body weights for both groups was greater than the reported average total body weight for elderly adults of 68.2 kg (Deurenberg et al., 1990). Mean body mass index for the placebo group was 28.6 kg/m^2 and 32.8 kg/m^2 for the copper supplemented group at baseline. The mean body mass index for the placebo group was similar to those reported for older women of 26 kg/m^2

TABLE I

Age and Anthropometric Measurements[1]

	Placebo	Copper
Age (years)		
0 Weeks (Baseline)	58 ± 4	59 ± 2
Height (cm)		
0 Weeks (Baseline)	166 ± 1	161 ± 2
Body Weight (kg)		
0 Weeks (Baseline)	79.0 ± 3.9	84.0 ± 5.3
After 12 Weeks Supplementation	80.8 ± 3.5	84.7 ± 5.2
BMI (kg/m^2)		
0 Weeks (Baseline)	28.6 ± 1.6	32.8 ± 2.7
After 12 Weeks Supplementation	29.3 ± 1.5	33.0 ± 2.6

[1]Means ± standard error.

[a]Values with different superscript letters in a row are significantly different at baseline, $p \leq 0.05$.

*Change in concentration after 12 weeks in copper supplemented group significantly different compared to change in placebo after 12 weeks, $p \leq 0.05$.

(Deurenberg et al., 1990). No significant differences were observed between the groups at baseline for total body weight or body mass index. Furthermore, no significant changes in total body weight or body mass index were observed during the twelve weeks of supplementation.

Dietary Intake

The average seven day dietary food frequency intakes at baseline and after twelve weeks supplementation are presented in Table II. There were no significant differences observed in the dietary intakes between the groups at baseline. In addition, there was no change in dietary intakes within supplement groups over time. There was however, a close to significant increase in percent of kilocalories from total fat ($p=0.06$) for the copper supplement group. Therefore, we presume that the significant changes observed in this study after twelve weeks supplementation were not due to change in dietary intake during the study.

The average seven day dietary intake consumed by the subjects in this study reflects the typical American diet. The placebo group consumed an average copper intake of 1.0 mg/day and the copper supplemented group consumed an average intake of 1.0 mg copper/day. However, the copper intake for subjects in both groups may be slightly low due to incomplete data on the copper content of foods in the Food Processor Database. Out of an average of 43 foods per person, an average of six foods per person were missing copper values. Using the U.S. Department of Agriculture (USDA) Nutrient Database for Standard Reference, Release 12, it was determined that on average those foods could have increased the reported average copper intake by 0.3 mg/d (U.S.

TABLE II

Average Seven Day Dietary Intake[1]

	Placebo	Copper
Kilocalories		
0 Weeks (Baseline)	1835 ± 257	1634 ± 195
After 12 Weeks Supplementation	1632 ± 269	1640 ± 72
Carbohydrates (g)		
0 Weeks (Baseline)	213 ± 30	197 ± 25
After 12 Weeks Supplementation	201 ± 29	186 ± 12
Protein (g)		
0 Weeks (Baseline)	79 ± 11	67 ± 10
After 12 Weeks Supplementation	71 ± 12	64 ± 6
Total Fat (g)		
0 Weeks (Baseline)	67 ± 15	53 ± 11
After 12 Weeks Supplementation	61 ± 14	72 ± 6
Saturated Fat (g)		
0 Weeks (Baseline)	24 ± 3	21 ± 3
After 12 Weeks Supplementation	20 ± 4	23 ± 3
Cholesterol (mg)		
0 Weeks (Baseline)	214 ± 39	220 ± 44
After 12 Weeks Supplementation	196 ± 31	215 ± 19

[1]Means ± standard error.

[a]Values with different superscript letters in a row are significantly different at baseline, $p \leq 0.05$.

*Concentration after 12 weeks supplementation significantly different compared to baseline concentration within supplement group, $p \leq 0.05$.

TABLE II

Average Seven Day Dietary Intake[1] (Continued)

	Placebo	Copper
Fiber (g)		
0 Weeks (Baseline)	17 ± 3	15 ± 2
After 12 Weeks Supplementation	14 ± 3	15 ± 1
Copper (mg)		
0 Weeks (Baseline)	1.0 ± 0.1	1.0 ± 0.1
After 12 Weeks Supplementation	1.0 ± 0.1	1.0 ± 0.1
% Calories from Carbohydrate		
0 Weeks (Baseline)	48 ± 2	50 ± 3
After 12 Weeks Supplementation	51 ± 2	45 ± 3
% Calories from Protein		
0 Weeks (Baseline)	19 ± 1	17 ± 1
After 12 Weeks Supplementation	17 ± 1	16 ± 1
% Calories from Total Fat		
0 Weeks (Baseline)	33 ± 5	33 ± 5
After 12 Weeks Supplementation	32 ± 3	39 ± 3
% Calories from Saturated Fat		
0 Weeks (Baseline)	12 ± 1	12 ± 1
After 12 Weeks Supplementation	11 ± 1	12 ± 1

[1]Means ± standard error.

[a]Values with different superscript letters in a row are significantly different at baseline, $p \leq 0.05$.

*Concentration after 12 weeks supplementation significantly different compared to baseline concentration within supplement group, $p \leq 0.05$.

Department of Agriculture, 1996). The dietary copper intake of the subjects was similar to the reported average intake of men and women of 1.0 mg copper/day (Reiser et al., 1985). Both groups consumed slightly more than the average daily intake of 0.78 mg copper/day by American adults reported by Klevay (Klevay, 1986). Both groups consumed slightly less than the average daily intake of 1.1 mg copper/day reported in NHANES III for women age 50-59 (Alaimo et al., 1994). While the average dietary copper intake of both groups is typical of the American diet, both groups consumed less than the ESADDI range of 1.5 to 3.0 mg copper/day.

Both groups consumed diets that contained less than 300 mg of cholesterol, which is typical for elderly women. The average dietary cholesterol intake of the placebo group was 214 mg while the intake of the copper supplemented group was 220 mg. The American Heart Association reports the average dietary cholesterol intake among elderly women ranges between 220 – 260 mg/day (The Nutrition Committee, 1988).

The placebo group consumed 48% of calories from carbohydrates while the copper supplemented group consumed 50% of calories from carbohydrates. Both groups consumed diets higher in percent of calories from carbohydrates than the 45.6% intake reported for women age 50-59 in the 1987-88 Nationwide Food Consumption Survey (NFCS) (FASEB, 1995). In addition, the subjects consumed diets with percent of carbohydrates similar to the 49.0% reported by the Continuing Survey of Food Intakes by Individuals (CSFII) in 1989-91 for women age 50-59 (FASEB, 1995). Both groups consumed diets in line with recommendations made by the American Heart Association Nutrition Committee for 45% of calories from carbohydrate sources.

Mean percent daily calories from protein consumed by both groups were similar to the 18.2% and 17.7% reported for women age 50-59 by NFCS 1987-88 and CSFII 1989-91 respectively (FASEB, 1995). The placebo group consumed 19% of calories from protein. The copper supplemented group consumed 17% of calories from protein. In addition, the protein consumption of both groups were similar to the American Heart Association recommendation of 16% of calories from protein.

Both groups consumed diets lower in fat than the 36.6% fat intake reported for women age 50-59 by NFCS 1987-88 (FASEB, 1995). The placebo group consumed 33% of calories from fat, which is slightly above the recommended fat intake of 30% or less of total caloric intake. The copper supplemented group also consumed 33% of calories from fat. Both groups consumed diets with percent of calories from fat similar to the 33.9% of calories from fat reported for women age 50-59 by CSFII 1989-1991 (FASEB, 1995).

Both groups consumed diets similar to the 11.8% of calories from saturated fat reported for women age 20-59 reported by NHANES III (FASEB, 1995). Yet, both groups had slightly higher saturated fat intake than recommended. The percent of calories from saturated fat was 12% in both the placebo and copper supplemented groups. The recommended percent calories from saturated fat is less than 10% (American Heart Association, 1998).

Finally, the fiber intake of both groups was lower than the 20-30 g recommended for adults (American Heart Association, 1998). However, the average intake of both groups was greater than the 13.71 g/d reported for women age 50-59 by NHANES III (FASEB, 1995). The placebo group had an average fiber intake of 17 g/d and the copper supplemented group had an average fiber intake of 15 g/d.

Thus, the diets of both groups reflect the typical American diet. Since there were no significant changes within the two groups over time, we presume that the significant changes observed in this study after twelve weeks supplementation were not due to their dietary intake, but rather due to the copper supplementation.

Serum Lipid and Apolipoprotein Concentrations

The effect of copper supplementation on serum lipids and apolipoproteins are presented in Table III. At baseline there were no significant differences observed in serum LDL-cholesterol, HDL-cholesterol, apolipoprotein A-1, or apolipoprotein B concentrations between the copper supplemented group and placebo group. However, the copper supplemented group had a significantly higher serum total cholesterol and triglyceride concentrations than the placebo group at baseline (Table III).

The initial total cholesterol concentrations for the placebo group was 239 mg/dl and 292 mg/dl for the copper supplemented group. Both groups had initial total cholesterol concentration higher than the average total cholesterol concentration of 217-235 mg/dl for women age 45-64 (American Heart Association, 1998).

Both groups' initial LDL-cholesterol concentrations were higher than the recommended 156 mg/dl for adults age 60-64 (National Cholesterol Education Program, 1993). The initial LDL-cholesterol concentrations were 180 mg/dl and 205 mg/dl for the placebo and copper supplemented groups respectively. The initial HDL-cholesterol concentration for both the placebo group and the copper supplemented group was 29 mg/dl. Both groups were below the recommend 35 mg/dl.

TABLE III

Effect of Copper Supplementation on Serum Lipids and Apolipoproteins[1]

	Placebo	Copper
Total Cholesterol (mg/dl)		
0 Weeks (Baseline)	239 ± 11[a]	292 ± 18[b]
After 12 Weeks Supplementation	230 ± 11	283 ± 19
LDL-cholesterol (mg/dl)		
0 Weeks (Baseline)	180 ± 11	205 ± 16
After 12 Weeks Supplementation	170 ± 12	201 ± 19
HDL-cholesterol (mg/dl)		
0 Weeks (Baseline)	29 ± 2	29 ± 5
After 12 Weeks Supplementation	28 ± 12	8 ± 5
Triglycerides (mg/dl)		
0 Weeks (Baseline)	148 ± 17[a]	289 ± 73[b]
After 12 Weeks Supplementation	161 ± 19	274 ± 73
Apolipoprotein A-1 (mg/dl)		
0 Weeks (Baseline)	143 ± 13	162 ± 12
After 12 Weeks Supplementation	154 ± 17	180 ± 16
Apolipoprotein B (mg/dl)		
0 Weeks (Baseline)	133 ± 23	158 ± 10
After 12 Weeks Supplementation	119 ± 23	171 ± 24

[1]Means ± standard error.

[a]Values with different superscript letters in a row are significantly different at baseline, $p \leq 0.05$.

*Change in concentration after 12 weeks in copper supplemented group significantly different compared to change in placebo after 12 weeks, $p \leq 0.05$.

The initial triglyceride concentration of the placebo group was 148 mg/dl and 289 mg/dl for the copper supplemented group. According to the Second Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, the recommended triglyceride concentration for adults is less than 200 mg/dl (National Cholesterol Education Program, 1993). The placebo group had an initial triglyceride concentration below the recommended concentration. The initial triglyceride concentration of the copper supplemented group was greater than the recommended concentration.

Both groups' initial apolipoprotein A-1 concentrations were within normal parameters, with an initial concentration of 143 mg/dl and 162 mg/dl in the placebo and copper supplemented groups respectively. The apolipoprotein B concentration in the placebo group was 133 mg/dl and within normal parameters. However, the copper supplemented group had an initial apolipoprotein B concentration of 158 mg/d, which is slightly above the normal range of 60-150 mg/dl (Brewer et al., 1988).

No significant differences were observed over time between groups in total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride apolipoprotein A-1, or apolipoprotein B concentrations. Previous human studies of copper deficiency have reported alterations of total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations. Klevay et al. reported an increase in total cholesterol and LDL-cholesterol with copper depletion (Klevay et al., 1984). Reiser et al. also reported an increase in LDL-cholesterol concentration with copper deficiency as well as a decrease in HDL-cholesterol concentration (Reiser et al., 1987). In addition, Hermann et al. reported a significant decrease in total cholesterol and LDL-cholesterol concentrations in

elderly adults supplemented with 3.0 mg copper/day (Hermann et al., 1998). However, our findings agree with Milne & Neilson who did not observe changes in serum lipid concentrations of postmenopausal women with supplementation of 2.0 mg copper/day (Milne & Neilson, 1996).

A slight decrease in mean serum triglyceride concentration was observed after twelve weeks copper supplementation; however, the decrease was not significant. Hermann et al. observed an elevated triglyceride concentration in adults with diets that contained less than 2/3 of the ESADDI for copper (Hermann et al., 1993). In a copper supplementation study, Hermann et al. reported a significant decrease in triglyceride concentrations of elderly adults receiving 3.0 mg copper/day (Hermann et al., 1998).

Although not significant, after twelve weeks copper supplementation, mean apolipoprotein A-1 concentration in the copper supplemented group increased compared to the placebo. The increase in apolipoprotein A-1 agrees with the observed increase in HDL-cholesterol concentration.

Serum Glucose, Insulin and Estradiol Concentrations

Subjects' mean serum glucose, insulin, and estradiol concentrations at baseline and after twelve weeks copper supplementation are presented in Table IV. Initial mean glucose concentration in the placebo group was 101 mg/dl and is within the normal parameter. The copper supplemented group had an initial mean glucose concentration of 112 mg/dl, which is slightly higher than normal parameter of 70 – 110 mg/dl (Grant & Dehoog, 1991). There were no significant differences observed between the groups at baseline for serum glucose concentrations.

TABLE IV

Effect of Copper Supplementation on Serum Glucose, Insulin and Estradiol[1]

	Placebo	Copper
Glucose (mg/dl)		
0 Weeks (Baseline)	101 ± 1	112 ± 8
After 12 Weeks Supplementation	99 ± 3	101 ± 7
Insulin (μIU/ml)		
0 Weeks (Baseline)	13 ± 1[a]	22 ± 4[b]
After 12 Weeks Supplementation	12 ± 2	11 ± 1*
Estradiol (pg/ml)		
0 Weeks (Baseline)	70 ± 19	85 ± 44
After 12 Weeks Supplementation	60 ± 9	87 ± 48

[1]Means ± standard error.

[a]Values with different superscript letters in a row are significantly different at baseline, $p \leq 0.05$.

*Change in concentration after 12 weeks in copper supplemented group

The recommended fasting serum insulin concentration is 10-12 μIU/ml (Glascow, 1990). The placebo group had an initial insulin concentration of 13 μIU/ml, which was slightly above the recommended concentration. The initial insulin concentration of the copper supplemented group was higher than recommended. They had an initial insulin concentration of 22 μIU/ml. There was a significant difference observed between the placebo and copper supplemented groups at baseline for serum insulin concentration with the copper group having a significantly higher insulin concentration at baseline.

The normal estradiol concentration in postmenopausal women is less than 30 pg/ml (Wallach, 1996). Both groups had initial estradiol concentrations above the normal estradiol concentration. The placebo group had a mean initial estradiol concentration of 70 pg/ml. The initial mean estradiol concentration of the copper supplemented group was 85 pg/ml. In both groups six out of seven subjects had initial estradiol concentrations greater than 30 pg/ml. It appears that the subjects were in the early stages of menopause. There was no significant difference between the placebo and copper supplemented groups at baseline for serum estradiol concentrations.

In this study, twelve weeks of copper supplementation resulted in a significant decrease in serum insulin concentrations compared to the placebo group (Table IV). There was no significant change in estradiol concentrations after twelve weeks of copper supplementation compared to the placebo group.

Although not significant, the tendency towards a decrease in serum glucose in the copper supplement group observed in this study was similar to the findings of other studies. Bhathena et al. reported an increase in the plasma glucose concentrations in adults consuming a marginally copper deficient diet (1.03 mg/day) (Bhathena et al.,

1988). Hassel et al. reported an increase in plasma glucose concentrations at 30 minutes post glucose injection in copper deficient rats (Hassel et al., 1993). Fields et al. however, did not observe an effect on glucose concentrations in rats consuming a marginal copper deficient diet for two weeks (Fields et al., 1996).

A significant decrease was observed in serum insulin concentrations in this study. Klevay et al. also observed a decrease in the average insulin concentrations of adults during the copper repletion phase of their study (Klevay, 1986). This observation is similar to reports from animals studies that investigated the effect of copper on insulin concentration. Abdul-Ghani et al. conducted a copper supplementation study with rats and observed a decrease in serum insulin concentration during copper repletion (Abdul-Ghani et al., 1992). Cohen et al. observed delayed plasma insulin responses to oral glucose loads in copper deficient rats (Cohen et al., 1982). Hassel et al. also observed a delay in the peak insulin concentration to 60 minutes in copper deficient animals (Hassel et al., 1983). However, Fields et al. did not observe an effect on plasma insulin concentrations in rats consuming a marginal copper deficient diet for two weeks (Fields et al., 1996).

CHAPTER V

SUMMARY AND CONCLUSIONS

This study investigated the effects of twelve weeks copper supplementation on serum lipids, apolipoproteins, glucose, insulin and estradiol concentrations in hypercholesterolemic postmenopausal women not using estrogen replacement therapy. The subjects in this study were free-living volunteers from the community of Stillwater, Oklahoma. Subjects were randomly assigned to either the copper supplement group or the placebo group. The placebo group (n=7) consumed lactose placebo for twelve weeks. The copper group (n=7) consumed a total of 3.0 mg copper daily as copper carbonate for twelve weeks. Subjects consumed independently chosen diets throughout the study.

Subjects participated in three data collections: screening, baseline, and after twelve weeks of copper supplementation. Serum lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides), apolipoproteins (apolipoprotein A-1 and apolipoprotein B), glucose, insulin and estradiol were measured at baseline and after twelve weeks of supplementation. Changes in serum concentrations after twelve weeks of supplementation were compared using the SAS GLM procedure. Significance level was set at $P \leq 0.05$.

Dietary intakes from average seven day food frequencies were obtained at baseline and after twelve weeks supplementation. Seven day food frequencies were analyzed and averaged using Food Processor. The average dietary data included kilocalories, protein, carbohydrate, fiber, total fat, saturated fat, copper intake and the percent daily calories from carbohydrates, protein, fat, and saturated fat. The average dietary intakes at baseline were compared to the average dietary intakes after twelve

weeks of supplementation within groups to determine if any changes in dietary intake occurred during the supplementation period.

In this study a slight, but not significant decrease in serum triglyceride concentration; a slight, but not significant increase in apolipoprotien A-1 concentration; and a slight, but not significant decrease in glucose concentration were observed after twelve weeks of copper supplementation compared to the placebo group. A significant decrease in serum insulin concentration was observed in the copper supplemented group after twelve weeks of copper supplementation when compared to the placebo group. There were no significant changes observed in dietary intake.

Test of Null Hypothesis

The null hypotheses were tested based on the results from this study.

Ho 1: There will be no statistically significant effects due to twelve weeks of copper supplementation on serum total cholesterol, LDL-cholesterol, HDL-cholesterol, or triglyceride concentrations in postmenopausal women.

There were no significant effects on serum total cholesterol, LDL-cholesterol, HDL-cholesterol, or triglyceride concentrations in postmenopausal women due to twelve weeks of copper supplementation. Therefore, the researchers failed to reject null hypothesis one.

Ho 2: There will be no statistically significant effects due to twelve weeks of copper supplementation on serum glucose or insulin concentrations in postmenopausal women.

A slight but not significant decrease in serum glucose concentration was observed after twelve weeks of copper supplementation. In addition, twelve weeks of

copper supplementation significantly decreased serum insulin concentrations in postmenopausal women. Therefore, null hypothesis two is rejected.

Ho 3: There will be no statistically significant effects due to twelve weeks of copper supplementation on serum apolipoprotein A-1 or apolipoprotein B concentrations in postmenopausal women.

There were no significant effects on serum apolipoprotein A-1 or apolipoprotein B concentrations in postmenopausal women due to twelve weeks of copper supplementation. Therefore, the researchers failed to reject null hypothesis three.

Limitations

1. The food frequency forms were limited by the subjects' knowledge and understanding of food composition and portion sizes.
2. The results of the study are relevant to this small sample group, one cannot extrapolate the results of this study to the population as a whole.
3. Dietary intake of other nutrients or trace minerals, such as zinc, might affect copper absorption or metabolism.
4. Dietary copper intake may have influenced absorption or metabolism of copper from the supplement.
5. The Food Processor Database did not contain complete data on the copper content of foods.
6. Subjects self reported their menopausal status. Estradiol concentrations were higher than normal estradiol concentration for postmenopausal

women. It is assumed most women were in the early stages of menopause.

Implications

In this study, after twelve weeks of copper supplementation a decrease, although not significant in serum glucose was observed and a significant decrease in serum insulin concentration was observed. Thus, adequate copper intake may be particularly important for individuals with abnormal glucose tolerance, or those at risk for type 2 diabetes.

Recommendations

This study focused on the effect of copper supplementation in postmenopausal women with elevated total cholesterol, who were not taking medication to lower blood cholesterol or estrogen replacement therapy. It was however, difficult to obtain an adequate sample from this population. Estrogen replacement therapy is a common among postmenopausal women. Regardless of the number of factors indicating the need for research on this population group, future research on the male population is recommended.

The greatest response to copper supplementation was observed in serum insulin concentrations. In addition, although not significant, a decrease in serum glucose concentrations was observed after twelve weeks of copper supplementation. Future research should focus on the effect of copper supplementation on serum insulin and glucose in a larger sample. An investigation on the effect of copper supplementation on individuals with type 2 diabetes and those in a pre-diabetic state would be beneficial.

Furthermore, differences in the response of copper supplementation within those groups should be explored.

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APPENDIXES

APPENDIX A

APPROVAL FORM FOR INSTITUTIONAL REVIEW BOARD FOR HUMAN SUBJECTS RESEARCH

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: February 24, 1998 **IRB #: HE-98-057**

Proposal Title: EFFECTS AND INTERACTIONS OF CHROMIUM AND COPPER ON LIPID METABOLISM IN HYPERCHOLESTEROLEMIC POST MENOPAUSAL WOMEN

Principal Investigator(s): Janice R. Hermann, Andrea B. Arquitt, Barbara J. Stoecker

Reviewed and Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING, AS WELL AS ARE SUBJECT TO MONITORING AT ANY TIME DURING THE APPROVAL PERIOD.
APPROVAL STATUS PERIOD VALID FOR DATA COLLECTION FOR A ONE CALENDAR YEAR PERIOD AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Disapproval are as follows:

Signature: _____ Date: March 4, 1998

Chair of Institutional Review Board

APPENDIX B

RECRUITMENT ANNOUNCEMENT

HIGH BLOOD CHOLESTEROL???

Female Participants Wanted

Did you know that the risk of heart disease increases for women after menopause?

Would you like to know if adequate chromium and copper lower blood cholesterol and heart disease risk among women after menopause?

Volunteers will receive $50 for participating in this study.

We have an opportunity for women if you are:

- Past menopause
- Have a blood cholesterol level over 240 mg/dl
- Not using estrogen replacement therapy
- Not using medications to lower blood cholesterol

This study will investigate the effects and interactions of chromium and copper supplementation on the risk of cardiovascular disease in post menopausal women. The aims of this study are to determine if twelve weeks supplementation with chromium, copper or a combination of chromium and copper lowers blood cholesterol and other indicators of heart disease risk in post-menopausal women.

Volunteers will be given either a placebo, chromium, copper, or a combination of chromium and copper. Volunteers will be asked to take one supplement each morning and one each evening with meals for twelve weeks. The total daily intake is 3 mg copper and 200 ug/day chromium, which are the upper level of the safe and adequate intake range for each nutrient, as set by the National Research.

Volunteers will participate in fasting blood and early morning urine collections. A light breakfast will be served after the fasting blood collection. Volunteers will also participate in routine measurements of wrist, waist and hip circumferences, skin fold, height and weight. Volunteers will be asked to keep diet records before blood collections.

This study has been approved by the Institutional Review Board For Protection Of Human Subjects at Oklahoma State University and is funded by the Oklahoma center For the Advancement of Science and Technology.

Sound like fun??? If you are interested or for further information please contact:

Janice R Hermann, PhD, RD/LD
Andrea B Arquitt, PhD, RD/LD
Department of Nutritional Sciences
425 Human Environmental Sciences
Oklahoma State University
Stillwater, Oklahoma 74078
(405) 744-6824

APPENDIX C

INDIVIDUAL'S CONSENT TO PARTICIPATE IN RESEARCH

Individual's Consent to Participate in Research

Women's Cholesterol Study: Effects and interactions of chromium and copper on lipid metabolism in hypercholesterolemic post menopausal women

I,_____, voluntarily agree to participate in the above titled research which is sponsored by Agriculture Experiment Station, College of Human Environmental Sciences at Oklahoma State University.

I understand that:

(1) the purpose of the study is the measure the effects of mineral supplementation on plasma lipids and trace mineral status in post menopausal women;

(2) I will receive supplement containing ONE of the following:

(a) 0.25 mg lactose as a placebo
(b) 1.5 mg copper
(c) 100 ug chromium
(d) 1.5 mg copper plus 100 ug chromium

(3) I will take one supplement with each morning and evening meal for 12 weeks;

(4) I will not take any new vitamin or mineral supplement other than those that are part of this study;

(5) a phlebotomist will draw fasting blood samples of 30 ml (about 6 teaspoons) by venipuncture at the beginning and end of the study, and that slight bruising or discomfort may result from the venipuncture;

(6) my blood will only be used for the study protocal, and any remaining blood tissue will be discarded and no further tests will be run.

(7) a urine sample will be collected at the beginning and end of the study;

(8) routine data will be collected or measured for age, height, body weight, wrist, waist and hip circumferences at the beginning and end of the study;

(9) I will complete a Health Questionnaire concerning health conditions, medication use, vitamin and mineral supplement use and exercise practices at the beginning of the study; and a follow-up health and exercise questionnaire after each month of supplementation;

(10) I will complete a 7 day food frequency questionnaire at the beginning and end of supplementation;

(11) as a reward for participation and as an incentive to complete the study, I will receive $25 at both the beginning and end blood collections.

(12) all records are confidential and that my name will not be associated with any reports or data records at the end of the study;

(13) participation is voluntary and that I have the right to withdraw from this study at any time by contacting the principal investigators;

(14) I will withdraw from the project if I need to begin taking medication for my health during this study;

(15) this research is beneficial to the public in that the risk cardiovascular disease increases among women after menopausal

(16) I may contact Dr Janice Hermann or Dr Andrea Arquitt or Dr Barbara Stoecker at (405) 744-5040 should I wish further information. I may also contact the office of University Research Services, 001 Life Sciences East, Oklahoma State University, Stillwater, OK 74078 telephone (405) 744-5700.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date_____ Time_____

Signed_____

I certify that I have personally explained all elements of this form to the subject before requesting the subject to sign it.

Signed _____
(project director or her authorized representative)

APPENDIX D

HEALTH QUESTIONNAIRE

APPENDIX D

HEALTH QUESTIONNAIRE

Health Questionnaire

1. **Subject Number** _____

2. **Date of Birth**_____

3. **Do you have or have you had any of the following conditions?**

Conditions	NO	YES	Specify
Allergies			
Anemia			
Sickle Cell Anemia			
Blood Clotting Disease			
Cancer			
Diabetes			
Heart Disease			
Intestinal Disorder			
Liver Disease			
Osteoporosis			

4. **Do you currently take any medications on a regular basis**? No_____ Yes___

Specify all medications taken on a regular basis:

Name _____ How often _____ per day Or _____ per week

Name _____ How often _____ per day Or _____ per week

Name _____ How often _____ per day Or _____ per week

Name _____ How often _____ per day Or _____ per week

Name _____ How often _____ per day Or _____ per week

Name _____ How often _____ per day Or _____ per week

Name _____ How often _____ per day Or _____ per week

5. **What types of exercise do you do on a regular basis?**

How many times a week do you exercise?_____

How many minutes would you estimate you exercise in a week?_____

6. **Height** _____ **cm** **Weight** _____

APPENDIX E

FOOD FREQUENCY QUESTIONNAIRE

Code Number_____ **Date**_____

Vitamin and Mineral Supplement

1. Do you take any vitamin or mineral supplement(s)? Yes ___No ___
2. If **Yes**, please, list all **names** of vitamin or mineral supplements, and **how often** do you take the supplement(s)?

Name _____ How often ____ per day Or ____ per week
Name _____ How often ____ per day Or ____ per week
Name _____ How often ____ per day Or ____ per week
Name _____ How often ____ per day Or ____ per week
Name _____ How often ____ per day Or ____ per week

Seven Day Food Frequency Questionnaire

This questionnaire asks you about your consumption of foods and beverages over the past week. The "How Often" columns are for day, week, or rarely/never. We want you to think back over the past week and tell us how many times (per day or per week) you consumed each item. A medium serving is in parentheses.

EXAMPLES:

Ate 1/2 grapefruit about twice last week.
Ate 1 large hamburger four times last week.
Drank 2 cups of whole milk each day.

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
Grapefruit (1/2)		2			X	
Hamburger, regular (1 patty, 3 oz)		4				X
Whole milk (1 cup, 8 oz)	2				X	

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
DAIRY FOODS						
Whole milk (1 cup, 8 oz)						
2% milk (1cup, 8 oz)						
Skim milk (1 cup, 8 oz)						
Cream, whipped (1 Tbsp)						
Sour cream (1 Tbsp)						
Coffee cream (1 Tbsp)						
Ice cream (½ cup)						
Low fat ice cream (½ cup)						
Frozen yogurt (½ cup)						
Yogurt (1 cup)						
Low fat yogurt (1 cup)						
Cottage cheese (½ cup)						
Cream cheese (1 oz)						
Low fat cream cheese (1 oz)						
Other cheese (1 slice or 1 oz)						
Low fat cheese (1 slice or 1 oz)						
Margarine (1 tsp)						
Butter (1 tsp)						
Reduced fat margarine (1 tsp)						

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
FRUITS, FRUIT JUICES						
Raisins (1 oz or 1 sm box)						
Grapes (20)						
Prunes (½ cup)						
Bananas						
Cantaloupe (¼ melon)						
Watermelon (1 slice)						
Apples, applesauce or pears (1 fresh, ½ cup)						
Apple juice (½ cup)						
Oranges						
Orange juice (½ cup)						
Grapefruit (½ cup)						
Grapefruit juice (½ cup)						
Other fruit juices (½ cup)						
Strawberries—fresh, frozen, or canned (½ cup)						
Blueberries—fresh, frozen, or canned (½ cup)						
Peaches (1 fresh, ½ cup canned)						
Apricots (1 fresh, ½ cup canned)						
Plums (1 fresh, ½ cup canned)						
Honeydew melon (¼ melon)						

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
VEGETABLES, VEGETABLE JUICE						
Tomatoes (1)						
Tomato juice (½ cup)						
Tomato sauce (½ cup)						
Spaghetti sauce (½ cup)						
Red chili sauce, taco sauce, or salsa (1 Tbsp)						
Tofu or soybeans (3-4 oz)						
String beans, green beans (½ cup)						
Broccoli (½ cup)						
Cabbage (½ cup)						
Cole slaw (½ cup)						
Cauliflower (½ cup)						
Brussels sprouts (½ cup)						
Carrots, raw (½ carrot or 2-4 sticks)						
Carrots, cooked (½ cup)						
Corn (1 ear or ½ cup frozen or canned)						
Peas (½ cup fresh, frozen or canned)						
Lima beans (½ cup frozen, or canned)						
Mixed vegetables (½ cup)						
Beans or lentils, baked or dried (½ cup)						
Summer or yellow squash (½cup)						
Winter squash (½ cup)						
Zucchini (½ cup)						
Yam or sweet potato (½ cup)						
Spinach, (cooked ½ cup, raw 1						

cup)						
Iceberg lettuce, romaine or leaf (1 cup)						
Celery (4" stick)						
Beets (½ cup)						
Alfalfa sprouts (½ cup)						
Kale, mustard, or chard greens (½ cup)						
Vegetable, vegetable beef, minestrone or tomato soup (1 cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
EGGS, MEAT, ECT.						
Eggs (2)						
Chicken or turkey, roasted or broiled with skin (3-4 oz)						
Chicken or turkey, roasted or broiled skinless (3-4 oz)						
Chicken, fried with skin (3-4oz)						
Bacon (2 slices)						
Hot dogs (2)						
Low fat hot dogs (2)						
Sausage (2 patties or 2 links)						
Bologna (1 slice)						
Other processed luncheon meat (1 slice)						
Liver, chicken or beef (3-4 oz)						
Hamburger, regular (1 patty, 3-4 oz)						
Hamburger, lean (1 patty, 3-4 oz)						
Meat loaf (3-4 oz)						
Pork, chops, roasts (3-4 oz)						
Lamb (3-4 oz)						
Beef, roast, steak (3-4 oz)						
Beef stew with vegetables (1 cup)						
Ham (3-4 oz)						
Tuna fish (3-4 oz)						
Tuna salad (½ cup)						

Fish, baked or broiled (3-4 oz)						
Fish, fried or fish sandwich (3-4 oz)						
Shrimp, Lobster, Scallops						
Pizza (2 slices)						
Mixed dishes with cheese(1cup)						
Lasagna or meat pasta dishes (1 cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
BREADS, CEREALS, STARCHES						
Cold breakfast cereal (1 cup)						
Cold breakfast cereal—fortified (1 cup)						
Cooked oatmeal (1 cup)						
Other cooked breakfast cereal (1 cup)						
White bread (1 slice)						
Pita bread (1 piece)						
Dark bread (1 slice)						
English muffin (1)						
Bagel (1)						
Dinner roll (1)						
Hamburger or hotdog bun (1)						
Muffin (1)						
Biscuit (1)						
Corn bread, corn muffin (1)						
Brown rice (1cup)						
White rice (1cup)						
Spaghetti noodles (1 cup)						
Macaroni noodles (1 cup)						
Other pasta noodles (1 cup)						

Bulgar, kasha, couscous (1 cup)						
Pancakes or waffles (2)						
Potatoes, french fries or fried (½ cup)						
Potatoes, baked or boiled (1)						
Mashed potatoes (1 cup)						
Potato chips or corn chips (small bag or 1 oz)						
Saltine crackers (5)						
Saltine crackers, low sodium (5)						
Saltine crackers, fat free (5)						
Other crackers (5)						
Other crackers, low fat (5)						

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
BEVERAGES						
Regular soft drink (1)						
Diet soft drink (1)						
Caffeine free soft drink (1)						
Caffeine free, Diet soft drink (1)						
Lemonade or other non-carbonated drink (1 glass, bottle, or can)						
Coffee (1 cup)						
Decaffeinated coffee (1 cup)						
Tea (1 cup)						
Herbal tea (1 cup)						
Beer (1 glass, bottle, or can)						
Red wine (4 oz glass)						
White wine (4 oz glass)						
Whiskey, gin, or other liquor (1 drink or shot)						

Type of Food (Medium Serving)	How Often			Size		
	Day	**Week**	**Rarely/ Never**	**S**	**M**	**L**
SWEETS, BAKED GOODS, MISC.						
Chocolate (1 small bar or 1 oz)						
Candy bar (1 small bar)						
Candy without chocolate (1 oz)						
Cookies, home baked (2)						
Cookies, ready made (2)						
Brownies (2)						
Doughnuts (2)						
Cake, home baked (1 slice)						
Cake, ready made (1 slice)						
Sweet roll, coffee cake, or other pastry ready made (1 serving)						
Sweet roll, coffee cake, or other pastry home baked (1 serving)						
Pie, homemade (1 slice)						
Pie, ready made (1 slice)						
Jam, jelly, preserves, syrup, or Honey (1 Tbsp)						
Peanut butter (1 Tbsp)						
Popcorn (1 cup)						
Popcorn, air popped (1 cup)						
Nuts (small packet or 1 oz)						
Bran, added to food (1 Tbsp)						
Wheat germ (1 Tbsp)						
Chowder or cream soup (1 cup)						
Oil and vinegar dressing (1 Tbsp)						
Mayonnaise or other creamy salad dressing (1 Tbsp)						
Mustard, dry or prepared (1 tsp)						
Salt (1 shake)						
Pepper (1 shake)						

APPENDIX F

FOLLOW-UP HEALTH QUESTIONNAIRE

Monthly Questionnaire

Subject Number_____

1. Have you had a cold in the last month? No_____ Yes_____

IF YES, when?

how long did it last?

did you have a fever?

2. Have you had the flu in the last month? No_____ Yes_____

IF YES, when?

how long did it last?

did you have a fever?

3. Have you had any other illness in the last month? No_____ Yes_____

IF YES, when?

how long did it last?

did you have a fever?

4. If YES TO ANY OF QUESTIONS 1-3, did you continue to take your supplement during the illness? No_____ Yes_____

5. Did your exercise pattern change in the last month? No_____ Yes_____

IF YES TO QUESTION 5, in way way did your exercise pattern change:

VITA

Jeanette Marie Daniel Korte Kniess

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF COPPER SUPPLEMENTATION ON SERUM LIPIDS, SERUM GLUCOSE AND SERUM INSULIN IN POSTMENOPAUSAL WOMEN

Major Field: Nutritional Sciences

Biographical:

Personal Data: Born in Pueblo, Colorado, on July 15, 1974, the daughter of Melvin and Marie Korte.

Education: Graduated from Bowling Green High School, Bowling Green, Missouri in May 1992; received Bachelor of Science degree in Foods and Nutrition from Southeast Missouri State University, Cape Girardeau, Missouri in May 1997. Completed the requirements for the Master of Science degree with a major in Nutritional Science at Oklahoma State University in May 1999.

Professional Experience: Dietitian Assistant, Southeast Hospital, Cape Girardeau, Missouri, Dietetic Intern, Oklahoma State University, Integris Baptist Hospital, Oklahoma City, Oklahoma and United Postal Service Training Center-Marriott, Norman, Oklahoma, August 1997-May 1998; Registered Dietitian, 1998; Graduate Research and Teaching Assitant, Department of Nutrition, Oklahoma State University, 1997-1999.

Professional Organizations: American Dietetic Association, Oklahoma Dietetic Association, Kappa Omicron Nu,