EFFECT OF CHROMIUM SUPPLEMENTATION IN REDUCING CARDIOVASCULAR DISEASE RISK FACTORS IN POSTMENOPAUSAL WOMEN: EFFECT ON SERUM LIPIDS, GLUCOSE AND INSULIN

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CHAPTER I

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death among women in the United States (Sowers, 1998). The incidence of CVD is not as common in premenopausal women as it is in men. However, the incidence increases among women at the onset of menopause, probably due to reduced levels of female sex hormones (Sowers, 1998). Menopause is a natural biological phenomena marked by estrogen withdrawal, which is regarded as a risk factor for coronary heart disease. Statistics show that CVD risk doubles in women entering spontaneous menopause at the usual age, and quadruples in women following early menopause, such as those undergoing ovariectomy (Sullivan, 1996). As a consequence, one third of women between 50 and 75 years of age have CVD, which accounts for more than 50% of all deaths among females each year (Giardina, 1998).

Estrogen seems to have a protective effect against the risk of CVD (Ottesen & Sorensen, 1997). Estrogen withdrawal affects lipid metabolism in many ways including decreased HDL-cholesterol, and increased total and LDL-cholesterol (Sullivan, 1996). In postmenopausal women, LDL-cholesterol concentration tends to be higher than age-matched men (Sullivan, 1996). The net result is a change in HDL-cholesterol and LDL-cholesterol concentrations, leading to an associated increase in CVD risk. Currently, estrogen replacement therapy is prescribed for many postmenopausal women. However, hormone replacement therapy is suspected to increase the risk of cancer development, and is not recommended for all postmenopausal women (Barrett-Connor & Grady, 1998).

Chromium status may also affect CVD risk among postmenopausal women (Davies et al., 1997). Generally, poor mineral status including chromium has been reported in older adults (Wood et al., 1995). Low chromium status may be due in part to poor dietary intake (Wood et al., 1995) and to age related tissue loss (Wallach & Verch, 1986).

Poor chromium status can result in a decline in many physiological functions (Chapman et al., 1996). Insufficient dietary chromium intake has been reported to result in symptoms similar to those seen in CVD and diabetes (Anderson, 1997). In humans, some studies reported that chromium supplementation significantly reduced total cholesterol and LDL-cholesterol concentrations, and increased HDL-cholesterol concentration (Mertz, 1993; Riales & Albrink, 1981). However, other studies found insignificant or only partial effects of chromium on serum lipids (Potter et al., 1985; Uusitupa, 1983).

A significant reduction in serum triglycerides (TG) concentration was reported in non-insulin dependent diabetes mellitus (NIDDM) patients supplemented with chromium (Lee & Reasner, 1994). Rats fed chromium also showed an insulin hyperresponsiveness with insulin secretory response nearly two fold (Striffler et al., 1995). Hyperglycemia is associated with hypertriglyceridemia and this association may increase postmenopausal CVD risk (Stout, 1990). Since chromium deficiency may increase glucose intolerance, chromium deficiency may explain the association between age-related glucose intolerance and the increase in the risk for CVD development (Davies et al., 1997), and thus poor chromium status may be a risk factor for increased CVD in postmenopausal women.

The mechanism of chromium's action on glucose and lipid metabolism is not clear (Mertz, 1993). Chromium helps control blood glucose by increasing insulin action through binding to insulin receptors in peripheral tissue. Chromium deficiency has been reported to decrease cyclic adenosine monophosphate-dependent phosphodiesterase activity (cAMP-PDE) which is associated with increased tissue insulin resistance (Striffler et al., 1995). Controlling glucose metabolism may be the key for chromium action on lipid metabolism (Anderson et al., 1997).

Purpose

The purpose of this study was to investigate the effects of twelve-week chromium supplementation at a level of 200 μg/day on serum lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol and TG), apolipoproteins (apo A-I and apo-B), estradiol, glucose and insulin concentrations in postmenopausal women with elevated serum cholesterol, who were not using estrogen replacement therapy.

Hypothesis

H_0 1: there will be no significant effect due to twelve-week supplementation with 200 μg/day of chromium on serum lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, and TG) and apolipoproteins (apo A-I, apo B).

H_0 2: there will be no significant effect due to twelve-week supplementation with 200 μg/day of chromium on serum insulin and serum fasting glucose.

H_0 3: there will be no significant effect due to twelve-week supplementation with 200 μg/day of chromium on serum estradiol.

Assumptions

It was assumed that:

1: the subjects accurately reported their use of medications, vitamin and mineral supplements;

2: the subjects accurately reported their food frequency questionnaires;

3: the subjects took their supplements as directed;

4: the subjects fasted twelve hours before each blood collection;

5: the subjects did not change their eating patterns during the course of the study;

6: the chromium supplements did not interact chemically with the lactose filling in the supplements, and consequently remained biologically available;

7: all the antropometric measurements and sample analyses were accurate and reliable.

Limitations

The limitations of this study included:

1: the low number of participants in the study due to the difficulty in finding postmenopausal women with elevated cholesterol who were not on estrogen replacement therapy in the city of Stillwater, OK and surrounding area;

2: the noncompliance of some participants in taking the supplements as directed, following the overnight fasting instructions, and water consumption prior to blood collection;

3: the loss of blood samples due to poor blood collection.

CHAPTER II

LITERATURE REVIEW

Incidence of Cardiovascular Disease at Menopause

Since the beginning of this century women's life expectancy has increased from 62 to almost 80 years of age (Ottesen & Sorensen, 1997). On the other hand, the average age of menopause has remained around 51 years of age (Ottesen & Sorensen, 1997). As a consequence, women are now spending a longer period of life in the state of estrogen deficiency (Ottesen & Sorensen, 1997). By the year 2030 it is expected that about 1.2 billion women worldwide will be postmenopausal (Ottesen & Sorensen, 1997).

Cardiovascular disease (CVD) is the leading cause of death in postmenopausal women (Ottesen & Sorensen, 1997). One third of the women between 50 and 75 years of age have some forms of CVD. This accounts for more than 50% of all annual deaths among women (Giardina, 1998). The impact of CVD on postmenopausal women's life is that it is more lethal and less aggressively treated in women than men (Giardina, 1998). This is shown by the fact that twice as many women die from heart disease as from all forms of cancer combined (Giardina, 1998).

Biochemical Changes at Menopause

Description of Estrogen Loss at Menopause

The period between puberty and menopause is marked with a continuous variation in the level of serum estrogen throughout the menstrual cycle (Barrett-Connor & Bush, 1991). During the follicular phase, estrogen concentration is higher than progesterone. During the luteal phase, both estrogen and progesterone levels become

high without any dominance for either (Barrett-Connor & Bush, 1991). At the perimenopausal period serum estrogen level begins to decline (Barrett-Connor & Bush, 1991). Due to ovarian failure at menopause, estradiol is replaced by estrone, a less active form of estrogen produced in the adipose tissue (Barrett-Connor & Bush, 1991). With advancing age, estrogen continues its gradual withdrawal, but in a slower manner (Barrett-Connor & Bush, 1991).

These marked changes have many effects on postmenopausal women's health, especially on the lipid metabolism, which in turn affects the cardiovascular system (Barrett-Conor & Bush, 1991). Some changes in lipids observed in postmenopausal women are increased serum TG, total cholesterol, LDL-cholesterol, and apo B, and decreased HDL-cholesterol, and apo A, increased insulin concentration and resistance, and decreased insulin peripheral utilization (Nasr & Breckwoldt, 1998).

Changes in Total and LDL-Cholesterol at Menopause

Cholesterol is a fat-like substance involved in the synthesis of cell membranes, hormones and other compounds. In the circulation, cholesterol is carried in particles containing lipids and proteins known as lipoproteins among which are the low density lipoprotein (LDL) and the high density lipoprotein (HDL). The LDL is the major atherogenic class, containing 60% to 70% of the total serum cholesterol. LDL contains apolipoprotein B (apo B), which possesses an atherogenic property (Knopp et al., 1994). Cholesterol concentration is known to increase with age. However, major gender differences exist in the age-related changes. In men, circulating cholesterol increases in concentration until 50 years of age where it starts to decline. In contrary to what is seen

in men, cholesterol concentration continues to rise in women even after menopause (Corti et al., 1997).

For the general population, total serum cholesterol concentration below 200 mg/dl is classified as desirable. A total serum cholesterol concentration of 200 to 239 mg/dl is classified as being borderline to high risk. A total serum cholesterol concentration above 240 mg/dl is classified as high, and the risk of CVD rises sharply above this level (The Expert Panel, 1988). Elevated total serum cholesterol is an important risk factor of CVD, and a 10% to 15% reduction in elevated total serum cholesterol may reduce CVD risk by 20% to 30% (The Expert Panel, 1988).

Since LDL contains the major fraction of serum total cholesterol, it is expected to be the major component that enhances CVD risk (Acra et al., 1994). An LDL-cholesterol concentration of 160 mg/dl or greater is classified as high risk, and this LDL-cholesterol concentration corresponds to a total serum cholesterol concentration of 240 mg/dl (The Expert Panel, 1988). Estrogen lowers LDL concentration by increasing its clearance from plasma (Nabulsi et al., 1993). Estradiol withdrawal decreases the rate of LDL catabolism (Corti et al., 1997), but has no effect on LDL synthesis (Walsh et al., 1991). Estrogen withdrawal results in higher LDL concentration (Walsh et al., 1991). The decrease in LDL catabolic rate is not due to a reduced binding affinity of LDL particles to receptors. Rather, decreased LDL catabolism seems to be mediated via a decrease in the expression of LDL-cholesterol hepatic receptors (Corti et al., 1997). The net effect of estrogen withdrawal is an increase in the circulating LDL-cholesterol concentration, which results in an increased cholesterol influx into the arterial wall (Nasr & Breckwoldt, 1998).

Changes in HDL-Cholesterol, Apolipoprotein A-I and B at Menopause

The HDL contains 20% to 30% of total cholesterol (The Expert Panel, 1988). The cholesterol content of HDL is inversely related to CVD incidence (Schaefer et al., 1982). HDL also contains apolipoprotein A-I (apo A-I) which comprises almost 90% of the HDL protein mass, and to which the anti-atherogenic property of HDL-cholesterol is attributed (Schaefer et al., 1982). Within the general population, an HDL-cholesterol concentration below 35 mg/dl is classified as low. Such a concentration or below is considered to be a risk factor for CVD (The Expert Panel, 1988). It was reported that the cardioprotective role of HDL is about two fold stronger than the atherogenic role of LDL. An increase of 1 mg/dl of circulating HDL-cholesterol is associated with a decrease of 5% in CVD risk factor (Kannel, 1987).

The role of HDL is to remove excess tissue cholesterol via transfer from the cell membrane to the liver, which implies that HDL concentration is inversely related to the risk of CVD development (Wakatsuki & Sagara, 1995). Estrogen withdrawal results in a decreased circulating HDL concentration (Barrett-Connor & Bush, 1991). This may be mediated through the effect of estrogen on plasma hepatic triglyceride lipase. The absence of estrogen increases plasma hepatic triglyceride lipase concentration, which reduces the circulating concentration of HDL (Wakatsuki & Sagara, 1995). Plasma hepatic triglyceride lipase decreases circulating HDL by causing the removal of HDL particles from the circulation into the liver (Wakatsuki & Sagara, 1995).

Low HDL-cholesterol concentration was reported to be associated with high plasma TG concentration and high hepatic triglyceride lipase concentration (Brinton et al., 1991), a condition appearing in the absence of estrogen (Wakatsuki & Sagara, 1995).

It has been reported that postmenopausal women have higher TG (Wu et al., 1990). During hypertriglyceridemia, TG concentration increases in the HDL core. TG is readily hydrolyzed by hepatic triglyceride lipase, which causes the catabolism and removal of HDL (Brinton et al., 1991).

Apo A-I is a major constituent of HDL. As much as 90% of apo A-I is found in HDL (Schaefer et al., 1982). As a part of HDL, apo A-I is reported to activate lecithin:cholesterol acyltransferase (LCAT), which is the enzyme responsible for cholesterol esterification in plasma (Schaefer et al., 1982). LCAT activates HDL to transport cholesterol from peripheral tissues to the liver for subsequent excretion (Schernthaner et al., 1983). The net result of a high apo A-I is an antiatherogenic effect (Schaefer et al., 1982).

Estrogen loss affects apo A-I by more than one mechanism. Estrogen loss increases hepatic triglyceride lipase concentration, which catabolizes apo A-I (Brinton et al., 1991). This would decrease apo A-I circulating level (Schaefer et al., 1982). On the other hand, it was reported that estrogen increases apo A-I circulating concentration by increasing its production (Walsh et al., 1994).

Apo B is an apolipoprotein found in LDL. An elevation of apo B content in LDL precursor is associated with increasing atherosclerosis (Knopp et al. 1994), and reflects a risk factor for CVD (Lamarche et al., 1998). Estrogen was reported to decrease circulating concentration of apo B by increasing LDL receptors that recognize apo B (Knopp et al., 1994).

Changes in Triglycerides at Menopause

Hypertriglyceridemia is divided into two categories: mild to moderate for TG concentrations of 250 to 700 mg/dl, and moderate to severe for TG concentrations of 700 to 1000 mg/dl (Kuczmarski, 1998). Although some studies have found the relationship between plasma TG and CVD controversial (The Expert Panel, 1988), the Framingham study reported an increased risk of CVD with increasing plasma TG concentration. This relationship is stronger in women than in men (Davignon & Cohn, 1996), and especially in postmenopausal women (Wu et al., 1990).

Even for those studies denying any direct relationship of TG concentration to CVD, high plasma TG concentration is associated to lipoprotein abnormalities, such as low HDL-cholesterol, apo A-I and high apo B (The Expert Panel, 1988). In other studies, high serum TG concentration was reported to be a strong predictor of CVD, independent of lipoprotein changes (Davignon & Cohn, 1996). The atherogenic property of TG is related in part to the type of lipoprotein carrying the TG (Davignon & Cohn, 1996).

Hypertriglyceridemia and hyperglycemia are closely associated. TG formation is in part due to de novo synthesis of fatty acids from blood glucose via the lipogenesis pathway (Aarsland et al., 1996). In hypertriglyceridemia, acetyl coenzyme A-carboxylase, which is the rate-limiting enzyme in fatty acid synthesis from glucose via pyruvate, is highly activated. This activation increases the influx of synthesized fatty acids into the liver to form TG (Davignon & Cohn, 1996). However, it was reported that hepatic TG formation mainly results from the re-esterification of fatty acids cleared

from plasma and delivered to the liver, rather than from de novo synthesized fatty acids (Aarsland et al., 1996).

Insulin resistance is common in elderly women (Sowers, 1998). Insulin resistance could increase the concentration of TG since high insulin concentration was reported to inhibit lipolysis (Coppack et al., 1994). Insulin was also reported to promote hepatic TG release (Berthezene, 1992).

Diabetes as a Risk Factor for CVD

Diabetes was reported to increase the risk of CVD three fold in women and thus places them at the same risk for CVD as men of the same age (Kannel, 1987). The Framingham study reported that, for individuals 50 to 80 years of age, diabetes is a greater risk factor for CVD in women than in men. Risk factors for CVD in women with diabetes, including dyslipidemia, were reported to be higher than in non-diabetic women (Sowers, 1998). In addition, preexisting CVD risk factors become more aggravated at the onset of diabetes (Kannel, 1987). An epidemiological study on women with CVD reported that the mortality rate in women with diabetes (mostly type 2) is significantly higher than the rate without diabetes (Sowers, 1998). The primary cause of death from CVD among the NIDDM subject is not fully understood. However, atherosclerosis is the major factor (Litwak et al., 1998).

Definition of Non-Insulin Dependent Diabetes Mellitus (NIDDM type 2)

Non-insulin dependent diabetes mellitus (NIDDM), or type 2 diabetes, is a maturity onset type of diabetes. It occurs after 40 years of age and it is associated with obesity. NIDDM individuals are characterized with high fasting (Stout, 1990) and postprandial concentrations of insulin (Howard, 1987). They are also characterized with

high serum glucose concentration. The reason for the high glucose-high insulin phenomena is the fact that insulin is unable to induce adequate glucose disposal due to insulin resistance, which results from a defect at the cellular level in peripheral tissues (Howard, 1987).

Lipoprotein Metabolism in NIDDM

Diabetic dyslipidemia may be a major risk factor for CVD in NIDDM (Lee & Reasner, 1994). Hyperinsulinemia may enhance the risk of CVD by adversely affecting the concentration and composition of lipoproteins (Stout, 1990). The catabolic rate of LDL in NIDDM is decreased. High glucose concentration could lead to a nonenzymatic glycosylation of apo B which affects the interaction of LDL with its receptor, leading to a lower LDL catabolism. In addition, in NIDDM, the transfer of cholesteryl ester to LDL is inhibited. The inhibition of cholesteryl ester transfer to LDL leads to an increase in cholesterol content in LDL (Howard, 1987).

There is a decreased rate of HDL synthesis in NIDDM. HDL composition is also altered. A decrease in the insulin sensitive lipoprotein lipase activity causes a decrease in the rate of HDL_2 formation (King et al., 1991). As a result, HDL-cholesterol concentration is lower in NIDDM subjects (Stout, 1990). Besides, since apo A-I concentration in HDL is correlated with the HDL-cholesterol concentration, the circulating level of apo A-I is also diminished. On the other hand, high TG in HDL is observed in NIDDM. Hepatic triglyceride lipase activity is increased (Stout, 1990), and this may contribute to lowering the HDL-cholesterol concentration (Howard, 1987). In conclusion, the changes in lipoproteins metabolism during NIDDM would increase the probability that diabetes is a risk factor for CVD development.

Role of Insulin in Abnormal Lipoprotein Metabolism

There is a negative correlation between HDL and insulin concentration and a positive correlation between LDL and insulin concentrations. A positive correlation also exists between TG and insulin concentrations. Insulin is important in lipoprotein metabolism, but only within limited ranges. Insulin in minimum amounts is needed for normal lipoprotein (HDL and LDL) metabolism, lipoprotein lipase production, and for the hydrolysis of triglycerides by lipoprotein lipase (Stout, 1990). Nevertheless, hyperinsulinemia leads to metabolic alteration in liver, adipocytes, and muscles, which influences plasma lipoproteins (HDL and LDL) concentrations and composition (Howard, 1987).

It seems that there are two different kinds of insulin receptors in muscle tissues, receptors for metabolic functions and others for cell proliferation. Due to excess insulin secretion, insulin resistance may develop, and the metabolic activity of insulin becomes blunt, causing abnormal lipoprotein (HDL and LDL) metabolism, which is a risk factor for CVD. In the same time the proliferative activity of insulin remains active, causing arterial resistance which also increases the risk for CVD development (Stout, 1990).

Effect of Hyperglycemia on CVD

Hyperglycemia is related to abnormal low density and high density lipoprotein metabolism, which is associated with atherogenesis (Lehto et al., 1997). Hyperglycemia leads to excessive formation of malonyl CoA, which in turn decreases the transport of fatty acid to mitochondria in muscle tissue. The result is an elevation of plasma TG and a subsequent accumulation in liver leading to abnormal lipoprotein metabolism, which is considered a risk factor for the development of CVD (Laybutt et al., 1997). On the other

hand, hyperglycemia leads to de novo synthesis of fatty acid, and eventually to TG formation, as mentioned earlier (Aarsland et al., 1996).

Hyperglycemia may have other negative effects on the development of CVD. It was reported that hyperglycemia alters the production of endothelial cell matrix which contributes to the thickening of the basement membrane (Stout, 1990). This delays cell replication and increases cell death by enhancing oxidation and glycation, leading to an increased plaque formation, and consequently higher CVD incidence (Sowers, 1998; Litwak et al., 1998).

Chromium in Elderly Nutrition

Nutritional Status and Mineral Requirements for Elderly

Nutrition and health status are interrelated, especially when it comes to the theory that malnutrition may lead to chronic diseases (Wood et al., 1995). Undernutrition is well documented in elderly, whether they are apparently healthy, or in nursing homes. Consumption of less than two thirds of the RDA is common among the elderly who are 70 years of age and older (Mowe et al., 1994). This is very important since the elderly population is a rapidly growing segment of the US population. It is estimated that by the year 2030, 25% of the US population will be elderly (Wood et al., 1995). Living alone and low income status are some of the factors that explain in part the poor nutritional status of the elderly. However, what is more important is their limited capability of consuming food. Elderly report that they enjoy food less than before (Mowe et al., 1994), which potentially increases the risk of mineral deficiency (Wood et al., 1995). This could be due to an alteration of appetite following medication, or age-related change in smell and taste. Poor food intake could be also due to poor

denture condition, oral health problem, or difficulty in swallowing (Wood et al., 1995).

Sources of Dietary Chromium

The typical American diet is generally low in chromium (Anderson & Kozlovsky, 1985). No food is known to be an outstanding source of chromium. Chromium distribution is almost similar among fruits, vegetables, dairy products, meat and beverages. Some examples of relatively high chromium containing food are: high bran breakfast cereals, broccoli, green beans, mushrooms, brewer's yeast, black pepper, prunes, raisins, nuts and milk colostrum (Anderson, 1988). Foods high in simple sugars enhances chromium loss from the body and are at the same time poor sources of chromium (Anderson, 1997).

Chromium Intake in Elderly

The ESADDI of chromium is between 50 to 200 μg/day (Anderson, 1997), and an adult who consumes 50 μg chromium absorbs and retains around 200-500 ng (Wallach, 1985). Chromium exists in the biological tissues in many forms. These forms are ionic, trivalent, and as a part of glucose tolerance factor complex (Wallach, 1985). Chromium intake in the elderly population was reported to be below the recommended range (Wood et al., 1995). This may lead to chromium deficiency and depletion of body stores among elderly (Wallach, 1985). In addition, it seems that elderly cannot get adequate chromium intake from diet alone (Wood et al., 1995). For a typical adult intake, 3000 kcal are needed to provide an amount of 50 μg of chromium per day (Wood et al., 1995; Anderson, 1988). This caloric intake seems to be difficult for the elderly to achieve, since the average daily caloric intake of elderly is approximately 1871 kcal for men and 1468 kcal for women (Wood et al., 1995). Adult women consume an average

of 25 μg of chromium a day (Anderson, 1997). Processing may contribute to low chromium content in food. It is estimated that the chromium concentration of institutional diet is 16 μg per every 1000 kcal (Anderson, 1988), ranging from 5 to 24 μg per 1000 kcal (Anderson, 1997).

Mineral Loss at Menopause

All tissues show an age related decrease in chromium concentration except lungs (Davies et al., 1997). Premenopausal women have a higher chromium tissue content, probably due to female sex hormones (Davies et al., 1997). Age related chromium loss may be due to low intake, defect in absorption, excessive urinary loss, or a sudden decrease in the ability of tissue to retain chromium. Concerning tissue retention, aging alters chromium distribution in the body by decreasing cellular chromium transport. The most remarkable decrease in tissue chromium is observed in bone (Wallach & Verch, 1986). Althought there is no accurate established standard to measure total body chromium (Lee & Reasner, 1994), one of the largest chromium long term storage compartments in the body is bone (Thomann et al., 1994).

Effect of Chromium on Lipid and Glucose Metabolism, and on Weight Reduction

The effect of chromium on blood lipids and glucose metabolism is controversial. Chromium, in some studies, was reported to improve several risk factors of CVD including total, LDL and HDL cholesterol, TG, apo B, apo A-I, glucose level and insulin output in humans and animals. In other studies no or insignificant changes in these parameters were obtained. Negative results have been attributed to numerous factors including inadequate amount of chromium supplementation, body mineral status of the subjects, experimental design and others.

One randomized study on atherosclerotic patients (average age of 63 years), some of them having NIDDM, and consuming 250 μg/day of chromium for eleven months, reported no significant changes in mean fasting glucose concentration, total cholesterol, HDL and total TG in both diabetic and non-diabetic subjects. In this study, a nonsignificant increase in mean fasting glucose concentration was reported among the diabetic subjects in the chromium treated group. The mean HDL cholesterol serum concentration significantly increased among the chromium treated group compared to their initial values (Abraham et al., 1992).

One similar study was done on men with an average age of 57 years using adrenergic blockers to lower their blood pressure. Beta blockers are known to decrease the serum concentration of HDL cholesterol and to increase the concentration of TG. In this study the experimental group consumed an amount of 600 μg/day of chromium for two months. Chromium intake increased significantly the HDL cholesterol concentration compared to the control whereas no significant changes were seen in other plasma lipids or lipoproteins (Roeback et al., 1991).

Another randomized double blind trial done on healthy young adults consuming 200 μg/day of chromium for three months, reported no significant changes in fasting blood glucose, LDL, HDL, TG and blood insulin between chromium treated and placebo groups. Within the chromium group, those who had a relatively higher insulin concentration showed a significant decrease in insulin concentration whereas no significant changes were seen in fasting glucose concentration or in serum lipids (Wilson & Gondy, 1995).

A similar double blind crossover study, done on healthy volunteers aging between the age of 25 to 80 years consuming 200 μg/day of chromium for a period of 42 days, was reported to significantly decrease total and LDL-cholesterol compared to the control. Apo B decreased significantly and apo A-I showed a significant elevation in concentration, whereas HDL cholesterol and TG concentrations showed an insignificant elevation (Press et al., 1990).

Another 16 week crossover double blind study conducted on healthy and NIDDM subjects with ages around 45 years, consuming an amount of 200 μg/day of chromium for a period of 8 weeks, reported no significant effect for chromium on glucose tolerance and plasma lipid concentrations. Chromium supplementation tended to lower total and LDL cholesterol, TG and glucose and to raise HDL cholesterol concentrations in the NIDDM individuals (Thomas & Gropper, 1996).

A double blind crossover study involving NIDDM patients between 32 and 65 years of age consuming 200 μg/day of chromium for a period of 2 months showed no effect of chromium on glucose control, LDL and HDL cholesterol. The only significant effect of chromium was observed on plasma TG (Lee & Reasner, 1994).

Another study involved a supplementation of 10 g/day of chromium-containing substance, in the form of either brewer's yeast (providing 7 μg/day chromium) or torula yeast (providing 0.3 μg/day chromium). Supplements were randomly assigned to healthy adults having an average age of 51 years, for a period of 12 weeks. The study reported a significant decrease in glucose concentration following an oral glocose tolerance test within the brewer's group, but a significant increase within the torula group. Insulin concentration following the tolerance test did not decrease significantly

within both groups. However, brewer's yeast significantly decreased plasma TG concentration but not other serum lipids (Li, 1994).

Two groups of normolipidemic and hyperlipipdemic subjects with an average age of 54 years consuming 20 g of brewer's yeast providing 40 μg chromium daily for 8 weeks were compared to each other. The results showed an insignificant decrease in serum TG concentration among the hyperlipidemic subjects and an insignificant increase among the normolipidemic subjects. Total cholesterol concentration decreased significantly in both groups, whereas HDL cholesterol concentration significantly increased in the hyperlipidemic group to the same value of the normolipidemic group (Elwood et al., 1982).

NIDDM and non diabetic elderly subjects aging 78 years and older, randomly consumed 9g/day of either brewer's yeast (10.8 μg chromium) or torula yeast (0.45 μg chromium) for 8 weeks. The study showed a significant decrease in glucose concentration and in insulin secretory response to glucose within the brewer's group (diabetic and non diabetic), and an insignificant change in the same parameters within the torula group. Also total cholesterol decreased significantly within the brewer's group, whereas the TG did not show any significant change in either group (Offenbacher & Pi-Sunyer, 1980).

A pre-test post-test study on elderly subjects with an average age of 66 years, having glucose intolerance and supplemented with 200 μg chromium daily for 12 weeks reported no significant change in plasma glucose and insulin concentrations compared to pre-test. Lipid parameters did not show any significant change; however, TG tended to increase and total, HDL and LDL cholesterol tended to decrease (Potter et al., 1985).

One double blind crossover study done on NIDDM patients aging between 37 and 68 years and consuming 200 μg chromium daily or a placebo reported no significant change in fasting and 2 hour postprandial blood glucose concentrations in the chromium group compared to placebo. There were also no significant changes reported in the fasting insulin, total cholesterol, HDL, LDL and serum TG concentrations in the same group (Uusitupa et al., 1983).

Uusitupa et al. also investigated the effects of chromium supplementation on elderly people with glucose intolerance, between 65 and 74 years of age. Subjects were supplemented with 160 μg chromium daily for a period of six months or a placebo. The study reported no significant change in the fasting and postprandial blood glucose and insulin concentrations in either groups, even though insulin concentration tended to be lower within the chromium group. There were also no significant changes in total, LDL and HDL cholesterol, TG, apo A-I and apo B (Uusitupa et al., 1992).

On the contrary, a double blind study on healthy men between 31 and 60 years of age receiving either 100 μg/day chromium or a placebo for a period of 12 weeks reported a significant increase in HDL cholesterol concentration, a significant decrease in TG concentration and no significant change in LDL cholesterol concentration. Fasting and postprandial plasma glucose concentrations were significantly lower at 6 weeks and only fasting glucose concentration was lower at 12 weeks within the chromium group. Insulin concentration also decreased in the chromium supplemented group at 12 weeks (Riales & Albrink, 1981).

The effect of different dosages of chromium supplementation on NIDDM subjects was also tested. Subjects between 35 and 65 years of age were randomly

assigned to either a placebo, 200 μg/day, or 1000 μg/day of chromium for 4 months. Fasting and postprandial plasma glucose were significantly lower after 2 and 4 months in the group supplemented with 200 μg chromium per day. Fasting insulin was also significantly lower in both chromium groups after two months. Chromium supplementation also significantly decreased the total cholesterol at 4 months in the high chromium group. There was no effect of chromium on HDL cholesterol, TG, or body weight (Anderson et al., 1997).

Animal studies show the same inconsistency in their results concerning the effect of chromium supplementation on glucose metabolism and CVD risk factors. A 90 day randomized trial using rabbits reported a significant reduction in the aortic plaque in the chromium injected group. Total cholesterol and TG concentrations tended to be lower in these groups but not in a significant manner. Also chromium increased HDL and decreased LDL but the differences were not significant (Abraham et al., 1982).

A study on rats fed either a low or high chromium diet for 18 months revealed no significant change in the fasting plasma glucose concentration between the low and the high chromium group diet. Postprandial plasma glucose concentration was lower in the high chromium group at 4 and 8 months but not at 12 months. No significant differences were also seen in total cholesterol and TG between the two groups. The study also reported that there was no significant change in the distribution of cholesterol and TG in LDL and HDL (Donaldson et al., 1985).

Another trial using pigs fed either chromium or placebo for 21 days reported an increase in plasma cholesterol within the chromium group, whereas plasma glucose was

not affected. Serum TG and fasting plasma insulin were also reported to decrease in the chromium group (Amoikon et al., 1995).

Male rats randomly assigned to a 24 week study and supplemented with either chromium deficient or chromium diet beside a glucose intolerance-inducing diet, showed a significant decrease in insulin concentration within the chromium supplemented group but a lower blood glucose concentration was not significant (Striffler et al., 1995). The same author reported a fasting hyperinsulinemia in the low chromium diet group after 16 weeks in a similar experimental protocol, compared to the high chromium diet group. Plasma TG concentration tended to be lower within the high chromium group, but without any statistical significance (Striffler et al., 1998).

During hyperinsulinemia, chromium may have an indirect effect on body weight. Weight gain can occur in hyperinsulinemic subjects, and the magnitude of chromium action on insulin is proportional to the body fat content. On the other hand, chromium supplementation resulted in an increase in lean body mass and these changes tended to be greater in older subjects (Anderson, 1998). However, chromium action in improving glucose tolerance may be limited to those subjects with chromium deficiency (Anderson, 1988). Besides, effective action of chromium on glucose tolerance and CVD risk factors depends also on appropriate timing and dosage (Mertz, 1993).

Mechanism of Chromium's action on Insulin

The mechanism of chromium's effect on lipid metabolism is not clear (Mertz, 1993; Abraham et al., 1992). It was hypothesized that chromium helps control blood glucose concentration by increasing insulin action (Anderson et al., 1997). Chromium was reported to minimize the amount of insulin needed by tissues for glucose utilization

and to decrease insulin secretion in NIDDM patients (Offenbacher & Pi-Sunyer, 1980). It was hypothesized that chromium has a greater effect on individuals who are glucose intolerant (Riales & Albrink, 1981)

Decreased sensitivity of pancreatic β cells to circulating glucose leads to insulin overproduction, which in turn down regulates the sensitivity of the peripheral tissue to insulin. Chromium was reported to reverse these compensatory changes in pancreatic β cells, by increasing the sensitivity of the peripheral tissue to insulin and thus decreasing insulin requirements (Striffler et al., 1995). It is suspected that chromium binds to insulin receptors in peripheral tissues to improve insulin action (Mertz, 1993). On the other hand, chromium was also reported to increase the sensitivity of pancreatic β cells to circulating glucose level. The result is a decrease in glucose tolerance and in the risk factors for CVD (Anderson et al., 1997). Chromium was also reported to enhance the insulin hepatic clearance, which helps reduce the circulating insulin concentration (Striffler et al., 1995). Consequently, through its effect on glucose metabolism, chromium may have some effect on serum lipids (Anderson et al., 1997).

In case of adequate chromium stores, chromium would be released from stores following a glucose load challenge to facilitate insulin action on peripheral tissues. This explains the increase in circulating chromium concentration following a glucose challenge. In case of chromium deficiency, a decrease in circulating chromium is reported to compensate for the poor storage. This is done to keep the physiological action of insulin on peripheral tissues. However, when circulating chromium is not adequate for normal insulin function, insulin resistance may appear (Mertz, 1993). It was reported that some of the changes seen after chromium supplementation are

transitory and that extended supplementation over a relatively longer time will revert those changes to the pretreatment period (Abraham et al., 1992).

Chromium has been hypothesized to bind to calmodulin at the intracellular level, and the presence of chromium bound calmodulin may be required for the activation of cAMP-dependent phosphodiesterase (cAMP-DPE). As a consequence, activated cAMP-DPE inhibits the action of pancreatic cAMP to secrete insulin. The result is a decrease in the hyperactivity of pancreatic β cells, which reduces insulin secretion (Striffler et al., 1995).

Due to the associated risk of cancer development (Barrett-Connor & Grady, 1998) chromium may be an alternative to hormonal replacement therapy. The low cost and the lack of side effects make it a preferred lipid lowering agent over other therapies (Lee & Reasner, 1994). However, the lack of standardized tests to assess body chromium status makes it difficult to determine the healthy young adults who may suffer from subclinical chromium deficiency, and whose chromium intake may help reduce the risk of insulin resistance and risk of CVD (Wilson & Gondy, 1995).

CHAPTER III

MATERIALS AND METHODS

Institutional Review and Investigators Training

This study was approved by the Institutional Review Board (IRB) for human subjects at Oklahoma State University (Appendix A). In addition, the principal investigator and the research assistants involved in this study attended training sessions on laboratory safety, including biohazard and radioactive material.

Subjects Solicitation

After approval of Oklahoma State University, volunteers were recruited by announcements through University mailings, local newspapers, and contact with churches, senior citizen organizations, and local stores (Appendix B). Solicited women subjects were postmenopausal, with elevated total cholesterol, and not on estrogen replacement therapy.

Supplement Preparation

Supplements for this study were prepared in the Department of Nutritional Sciences laboratory at Oklahoma State University. Number two gelatin capsules (Apothcary Product, Inc., Minneapolis, MN) were filled using a gelatin capsule filler machine (Quanterron, Inc,. Burnsville, MN).

Placebo capsules contained approximately 0.1970 g U.S.P. grade lactose (Spectrum Quality Product, Inc. Gardene, CA). The chromium supplementation consisted of 2.653 g U.S.P. grade $CrCl_3.6H_2O$ (Professional Compounding Centers of America, Inc. Houston, TX) in 982.437 g U.S.P. grade lactose, so that 0.1970 g of chromium supplement mixture was calculated to contain 100 μg chromium as

$CrCl_3.6H_2O$. The lactose and $CrCl_3.6H_2O$ were mixed together for 8 hours in a ball mixture to obtain a homogeneous distribution of chromium in lactose.

The chromium and placebo supplements were analyzed for chromium content using atomic absorption spectrophotometer, Model 5100 PC (Perkin-Elmer Corp., Norwalk, CT). Ten 0.1 g randomly chosen samples from the placebo and chromium supplement mixtures were wet and dry ashed using a modification of the Hill et al. method (Hill et al., 1986). The average analyzed chromium content of the placebo was -2.1 μg/g lactose. The average analyzed chromium content of the chromium supplement was 288.72 μg/g of chromium supplement mixture. Since the analyzed chromium concentration was lower than calculated values, the filled weight of the chromium supplement mixture was adjusted to 0.346 g per capsule in order to provide 100 μg chromium per capsule.

Experimental Design

A pre-test, experimental treatment, post-test design was used for this study (Ott, 1977a). Volunteer subjects were screened for serum total cholesterol concentration three weeks prior to starting the study to ensure they had an elevated serum cholesterol concentration. Pre and post-data collections were performed at the beginning and the end of twelve-week supplementation.

Data Collection

Screen

At the screen, subjects signed an informed consent (Appendix C) after it was verbally explained to them by the principal investigator. Subjects also completed a Health Questionnaire to assess their health status and medication use (Appendix D).

Subjects were trained by the principal investigator on how to complete a one week food frequency questionnaire (Appendix E).

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

Pre-data Collection

At the pre-data collection, subjects brought in a complete one week food frequency questionnaire for their food intake the week before the pre-data collection. Subjects came in following a twelve-hour fast, but were instructed to consume water. Subject's weight was measured to the nearest pound. A licensed phlebotomist drew a fasting blood sample in two separate 7.5 ml serum tubes (Monovette, Germany), after which the subjects were asked to sit for 20-30 minutes, where they were provided with nutritional support in the form of orange juice, muffins and coffee.

The subjects were provided with a one-month supply of the randomly assigned supplement and instructed to take two capsules each day, one in the morning and one in the evening with meals. The second and the third month's supply of supplements were delivered to the subjects individually. Volunteer subjects received $50 for their participation in the study.

Post-data collection

At the end of the twelve-week supplementation period, subjects returned for the post-data collection following a twelve-hour fast period. Subjects brought in a second

completed one-week food frequency questionnaire for their food intake the week prior to the post-data collection. Subject's weight was measured to the nearest pound. A licensed phlebotomist drew a fasting blood sample in two separate 7.5 ml serum tubes. After the blood collection subjects were asked to sit for a period of 20 to 30 minutes for nutritional support.

Blood Analysis

The same blood handling techniques were followed in both the pre and the post-data collection. After drawing the blood, the serum tubes were allowed to sit in an ice bath for 30 minutes. The tubes were centrifuged for 20 minutes at a speed of 10,000 rpm to separate the serum. The serum was transferred to individual 0.5 ml storage tubes, covered with parafilm and stored at -20 °C for future analysis of total cholesterol, HDL-cholesterol, TG, apo A-I, apo B, glucose, insulin and estradiol. LDL-cholesterol concentration was calculated.

Total cholesterol, HDL-cholesterol, TG, apo A-I, apo B and glucose were determined using enzymatic methods adapted for the COBAS-FARA clinical analyzer (COBAS FARA, Roche. Diagnostic System, Inc. Montclair, NJ). Insulin and estradiol were analyzed using radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) counted on the Packard Cobra II gamma counter.

Total cholesterol was determined using Roche method kit number 44307. The principle of this technique is based on the release of cholesterol from its esters by the enzymatic action of an ester hydrolase, then by oxidizing the free cholesterol with oxidase and producing hydrogen peroxide. The hydrogen peroxide, under the action of aminoantipyrine and phenol, forms a chromophore, quinoneimine in a proportional

amount to the cholesterol concentration. Quinoneimine is determined photometrically at 500 nm.

HDL-cholesterol was determined using Roche method kit number 44136. The principle is based on the precipitation of chylomicrons, VLDL and LDL by the action of phosphotungstic acid and magnesium and a subsequent centrifugation, which keeps the HDL in the supernatant. HDL cholesterol is then collected and determined by a cholesterol reagent similar to that of total cholesterol determination.

TG was determined using Roche method kit number 44119. The principle is based on the hydrolysis of TG by lipoprotein lipase to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol 3-phosphate, which is then oxidized to form dihydroxiaceton phosphate and hydrogen peroxide. Hydrogen peroxide reacts with aminophenazone and chlorophenol to form quinoneimine in a proportional quantity to TG concentration. Quinoneimine is determined photometrically at 490-500 nm.

Apo A-I was determined using Roche method kit number 44162. The method is based on the precipitation of apo A-I using sheep antiserum. The precipitate is then measured at 340 nm.

Apo B was determined using Roche method kit number 44161. The method is based on the precipitation of apo B with a rabbit antiserum. The precipitate is then measured at 340 nm.

Glucose was determined using Roche method kit number 47382. The method is based on the phosphorylation of glucose to glucose 6-phosphate. In the presence of NAD, glucose 6-phosphate is oxidized to 6-phosphogluconate, while NAD is reduced to

NADH. NADH production is proportional to the glucose concentration and can be read at 340 nm.

LDL cholesterol was calculated using the following formula:

LDL cholesterol = Total cholesterol - (HDL + TG/5) (Friedewald et al., 1972).

Insulin was determined using a radioimmunoassay competitive Coat-A-Count method (Diagnostic Products Corporation, Los Angeles, CA). The method is based on competition for an antibody, for a fixed time, between ^{125}I-labeled insulin and sample insulin. The antibody is immobilized on the wall of an assay tube. After incubation, the labeled insulin fraction bound to the tube wall is isolated by decanting the supernatant. A Packard Cobra II gamma counter and a calibration curve then determine the sample insulin.

Estradiol was determined using a radioimmunoassay competitive Coat-A-Count method (Diagnostic Products Corporation, Los Angeles, CA). The method is based on a competition for an antibody, for a fixed time, between ^{125}I-labeled estradiol and sample estradiol. The antibody is immobilized on the wall of the tube. After incubation, the labeled estradiol fraction bound to the tube wall is isolated by decanting the supernatant. A Packard Cobra II gamma counter is used to determine the amount of labeled estradiol, which is inversely proportional to the estradiol concentration in the sample.

Dietary Record Analysis

A seven day food frequency questionnaire was used to assess the subjects' dietary intake (Eck et al., 1991). The questionnaire by Eck et al., was a modification of Willett's one year food frequency questionnaire, which was tested for reliability and

validity (Eck et al., 1991). The subjects' seven day food frequency questionnaires were analyzed and averaged using the Food Processor Plus Program (version 7.11, ESHA Research, Salem OR). The subjects' seven day food frequencies were analyzed and averaged for daily intake of kilocalories, protein(g) carbohydrate(g), fiber(g), fat(g) and cholesterol(mg), and the percent of calories from carbohydrate, protein, total and saturated fat.

Data Analysis

Age, weight, BMI, average dietary intake, serum lipids, apolipoproteins, glucose, insulin and estradiol concentrations were compared between groups at baseline to see if there were any differences between the groups at baseline. Changes in serum lipid concentrations and apolipoproteins (total cholesterol, LDL-cholesterol, HDL-cholesterol, apo A-I, apo B, and TG), serum glucose, serum estradiol and serum insulin concentrations after twelve weeks of supplementation were compared to changes in the placebo group after twelve weeks supplementation. Data were analyzed using the Statistical Analysis System (SAS) general linear model procedure (GLM). Average pre and post dietary intakes were compared within each group to determine if there were any significant changes in dietary intakes within supplement groups during the twelve weeks supplementation, using the SAS repeated measures procedure. Significance level was set at $p \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

The aim of this study was to investigate the effects of twelve-week chromium supplementation on serum lipids, apolipoproteins, glucose, insulin, and estradiol among hyperlipidemic postmenopausal women not using estrogen replacement therapy. Nineteen subjects initially volunteered for the study. One subject was dropped because she did not meet the study criteria, one subject was dropped due to poor compliance taking the supplement, one subject was dropped because of a poor blood draw, and two subjects were dropped because they were diabetic. Thus the data represented fourteen subjects, seven in the placebo group, and seven in the chromium supplemented group.

Subjects' Age and Anthropometric Measurements

Subjects' mean age and anthropometric measurements by supplement group are presented in Table I. The mean age of the placebo group was 58 years. The mean age of the chromium group was 60 years. There was no significant difference at baseline between the two groups in age. The placebo group had a mean height of 166 cm while the chromium group had a mean height of 161 cm. There was also no significant difference in height between the two groups at baseline.

At baseline, the placebo group had a mean body weight of 79.0 kg, and a mean body mass index (BMI) of 28.6 kg/m^2. The chromium group had a mean body weight of 84.5 kg, and a mean BMI of 32.4 kg/m^2. After twelve weeks of supplementation, the placebo group had a mean body weight of 80.8 kg and a BMI of 29.3 kg/m^2, while the chromium group had a mean weight of 79.9 kg and a BMI of 30.7 kg/m^2. Mean body

TABLE I

Subject's Age and Anthropometric Measurements[1]

	Placebo	Chromium
Age (years)		
0 Weeks (Baseline)	58 ± 4	60 ± 5
Height (cm)		
0 Weeks (Baseline)	166 ± 1	161 ± 2
Body Weight (kg)		
0 Weeks (Baseline)	79.0 ± 3.9	84.5 ± 6.7
After 12 Weeks Supplementation	80.8 ± 3.5	79.9 ± 2.2
BMI (kg/m^2)		
0 Weeks (Baseline)	28.6 ± 1.6	32.4 ± 2.3
After 12 Weeks Supplementation	29.3 ± 1.5	30.7 ± 0.8

[1]Means ± standard error.

weights of both groups were above average compared to height in both groups (Metropolitan Height-Weight Tables, 1994). Also both groups are considered overweight also because their BMIs were above 27.3 kg/m^2 (Kuczmarski, 1998). There were no differences between the two groups in body weight and BMI at baseline. In addition there were no significant changes within each group in pre and post body weight and BMI.

Subjects' Seven Day of Dietary Intake

Subjects' mean pre and post seven-day dietary intakes are reported in Table II. There were no significant differences in mean dietary intakes between the two groups at baseline. In addition, there were no significant changes within each group between pre and post mean dietary intakes.

Energy

The mean kilocaloric intake of the placebo group at was 1835 kcal and that of the chromium group was 1614 kcal. There was no significant difference in the kilocaloric intake at baseline between the two groups. After supplementation the mean caloric intake of the placebo group was 1632 kcal and that of the chromium group was 1558 kcal. There was also no significant change within each group between the pre and post mean kilocaloric intake. The recommended energy intake for women with an age of 51 years and older is 1900 kcal (Ausman & Russell, 1994). The mean caloric intake of both groups at baseline was below the recommendation, and the mean caloric intake of both groups after supplementation was also below the recommendation. The anthropometric data showed that both groups had high mean BMIs before and after

TABLE II

Subjects' Average Seven Day Dietary Intake[1]

	Placebo	Chromium
Kilocalories		
0 Weeks (Baseline)	1835 ± 257	1614 ± 151
After 12 Weeks Supplementation	1632 ± 269	1558 ± 182
Carbohydrates (g)		
0 Weeks (Baseline)	213 ± 30	189 ± 24
After 12 Weeks Supplementation	201 ± 29	186 ± 24
Protein (g)		
0 Weeks (Baseline)	79 ± 11	70 ± 7
After 12 Weeks Supplementation	71 ± 12	71 ± 8
Total Fat (g)		
0 Weeks (Baseline)	67 ± 15	65 ± 6
After 12 Weeks Supplementation	61 ± 14	59 ± 7
Saturated Fat (g)		
0 Weeks (Baseline)	24 ± 3	20 ± 2
After 12 Weeks Supplementation	20 ± 4	20 ± 3
Cholesterol (mg)		
0 Weeks (Baseline)	214 ± 39	187 ± 38
After 12 Weeks Supplementation	196 ± 31	217 ± 31

[1]Means ± standard error.

TABLE II

Subject's Average Seven Day Dietary Intake[1] (Continued)

	Placebo	Chromium
Fiber (g)		
0 Weeks (Baseline)	17 ± 3	14 ± 2
After 12 Weeks Supplementation	14 ± 3	15 ± 3
% Calories from Carbohydrate		
0 Weeks (Baseline)	48 ± 2	46 ± 3
After 12 Weeks Supplementation	51 ± 2	48 ± 2
% Calories from Protein		
0 Weeks (Baseline)	19 ± 1	18 ± 1
After 12 Weeks Supplementation	17 ± 1	18 ± 1
% Calories from Total Fat		
0 Weeks (Baseline)	33 ± 5	36 ± 2
After 12 Weeks Supplementation	32 ± 3	34 ± 2
% Calories from Saturated Fat		
0 Weeks (Baseline)	12 ± 1	12 ± 2
After 12 Weeks Supplementation	11 ± 1	12 ± 2

[1]Means ± standard error.

supplementation. However the mean caloric intakes of both groups were below the recommendation. This may be the result of underreporting total energy intake by the subjects due to underrepresentation of some food groups in the questionnaire (Eck et al., 1991).

Carbohydrate

The mean carbohydrate intake for the placebo group at baseline was 213 g and that of the chromium group was 189 g. There was no significant difference between the two groups at baseline. After supplementation the mean carbohydrate intake was 201 g for the placebo group and 186 g for the chromium group. In addition, there was no significant difference in mean carbohydrate intake within each group between pre and post supplementation. There is no Recommended Dietary Intake for carbohydrate (Ausman & Russell, 1994). However the median carbohydrate intake, derived from one day dietary recalls, for white women aging 60 to 69 years is 185 g and that of black women is 180 g for the same age group (Kuczmarski, 1998). The carbohydrate intake for both groups was above the median intake.

Protein

The mean protein intake of the placebo group at baseline was 79 g and the mean protein intake of the chromium group was 70 g. There was no significant difference in mean protein intake between the two groups at baseline. The mean protein intake of the placebo group after supplementation was 71 g and that of the chromium group was 71 g. There was also no significant change in mean protein intake within each group between the pre and post supplementation. The Recommended Dietary Intake for protein is 50 g/day for females aging 51 years and older (Kuczmarski, 1998). The mean protein

intake for both groups at baseline and after supplementation was above the recommendation.

Total and Saturated Fat

At baseline, the mean total fat intake was 67 g and the mean saturated fat intake was 24 g in the placebo group. For the chromium group the mean total fat intake was 65 g and the mean saturated fat intake was 20 g at baseline. There was no significant difference at baseline between the two groups in mean total or saturated fat intake. After supplementation mean total fat intake for the placebo group was 61 g and the mean saturated fat intake was 20 g. For the chromium group, the mean total fat intake was 59 g and the mean saturated fat intake was 20 g after supplementation. There was also no significant change within each group in mean total or saturated fat intake between pre and post supplementation. No Recommended Dietary Allowance is set for total fat intake (Ausman & Russell, 1994). The median of total fat intake, derived from one-day dietary recalls, is 55 g for white women age 60 to 69 years and 45 g for black women. The median saturated fat intake for both categories of women are 18 and 14 g respectively (Kuczmarski, 1998). The mean pre and post total saturated fat intakes for both the placebo and chromium groups were above the median intake.

Percent of Calories From Carbohydrate, Protein and Fat

The percent of calories from carbohydrate, protein and fat are reported in Table II. The percent of calories from carbohydrate at baseline was 48 for the placebo group and 46 for the chromium group. No significant difference in percent of calories from carbohydrate was observed at baseline between the two groups. After supplementation the percent of calories from carbohydrate was 51 for the placebo group and 48 for the

chromium group. There was also no significant change in the percent of calories from carbohydrate within each group between pre and post supplementation.

The percent of calories from protein at baseline was 19 for placebo group and 18 for the chromium group. There was no significant difference in percent of calories from protein between the two groups at baseline. After supplementation the percent of calories from protein was 17 for the placebo group and 18 for the chromium group. There were also no significant changes, within each group in the percent of calories from protein between pre and post supplementation.

At baseline the percent of calories from total fat was 33 for the placebo group and 36 for the chromium group. The percent of calories from saturated fat was 12 for both the placebo and the chromium group at baseline. There were no significant differences between the two groups at baseline in the percent of calories from total or saturated fat. After supplementation, the percent of calories was 32 from total fat and 11 from saturated fat for the placebo group, and 34 from total fat and 12 from saturated fat for the chromium group. There were no significant changes within each group in the percent of calories from total or saturated fat between pre and post supplementation.

The percent of calories from carbohydrate at baseline in both groups was below the recommended percent of calories from carbohydrate, which is 50 to 60 % (National Cholesterol Education Program, 1994). After supplementation, the placebo group was close to the lower end of the recommended intake range, but the chromium group remained below the recommendation range. The percent of calories from protein was adequate in both groups before and after the supplementation, since the recommended intake for protein is 10 to 20 % of calories (National Cholesterol Education Program,

1994). The percent of calories from total fat in both groups before and after the supplementation was above the recommendation of less than 30 % of calories from total fat (National Cholesterol Education Program, 1994). In addition, the percent of calories from saturated fat for both groups at baseline and after supplementation was above the recommendation of less than 10 % of calories from saturated fat (National Cholesterol Education Program, 1994).

Cholesterol

The mean intake of cholesterol at baseline for the placebo group was 214 mg and that of chromium group was 187 mg. There was no significant difference in mean cholesterol intake between the two groups at baseline. After supplementation the mean cholesterol intake for the placebo group was 196 mg and that for the chromium group was 217 mg. There was no significant change in mean cholesterol intake within the two groups between pre and post supplementation. Cholesterol intake in both groups before and after supplementation was within the recommendation, which is less than 300 mg (Kuczmarski, 1998).

Fiber

The mean fiber intake at baseline was 17 g for the placebo group and 14 g for the chromium group. There was no difference at baseline between the two groups in mean fiber intake. After supplementation the mean fiber intake was 14 g for the placebo and 15 g for the chromium group. There was also no significant change in mean fiber intake within the two groups between pre and post supplementation. Fiber is reported to have a hypocholesterolemic effect and an intake above 18 g/day is recommended. Fiber intake above 18 g/day is hypothesized to reduce serum cholesterol by 5 % (Schneeman &

Tietyen, 1994). The mean pre and post fiber intake for both groups was below this recommendation.

Effect of Chromium Supplementation on Serum Lipids and Apolipoproteins

Subjects' serum lipid and apolipoprotein concentrations before and after 12 weeks of supplementation are presented in Table III. At baseline, the placebo group had a total cholesterol concentration of 239 mg/dl. A total cholesterol concentration greater than 240 mg/dl is considered high risk for CVD (Kuczmarski, 1998). Thus, the placebo group appeared to be on the borderline of high risk for CVD. The placebo had an LDL cholesterol concentration of 180 mg/dl at baseline. This value is also considered a high risk for CVD since an LDL cholesterol concentration above 160 mg/dl is considered high risk (Kuczmarski, 1998). The placebo group had an HDL cholesterol concentration at baseline of 29 mg/dl. An HDL cholesterol concentration below 35 mg/dl is considered a high risk for CVD (Kuczmarski, 1998). Thus the mean HDL cholesterol concentration for the placebo indicates a risk factor for CVD. The mean TG concentration for the placebo group at baseline was 148 mg/dl. The TG concentration of the placebo group at baseline can be classified normal since a TG concentration below 200 mg/dl is classified desirable (Feldman, 1994). The baseline concentration of apo A-I for the placebo group was 143 mg/dl. The average value for apo A-I concentration for women aging 60 to 64 is 135 mg/dl (Feldman, 1994), and thus the baseline concentration of apo A-I for the placebo group is close to normal. The apo B concentration for the placebo group at baseline was133 mg/dl. This is considered a risk

TABLE III

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

	Placebo	Chromium
Total Cholesterol (mg/dl)		
0 Weeks (Baseline)	239 ± 11 [a]	299 ± 23 [b]
After 12 Weeks Supplementation	230 ± 11	244 ± 16 *
LDL-cholesterol (mg/dl)		
0 Weeks (Baseline)	180 ± 11	213 ± 22
After 12 Weeks Supplementation	170 ± 12	177 ± 12
HDL-cholesterol (mg/dl)		
0 Weeks (Baseline)	29 ± 2	34 ± 2
After 12 Weeks Supplementation	28 ± 1	30 ± 3
Triglycerides (mg/dl)		
0 Weeks (Baseline)	148 ± 17	260 ± 36
After 12 Weeks Supplementation	161 ± 19	185 ± 30 *
Apolipoprotein A-I (mg/dl)		
0 Weeks (Baseline)	143 ± 13	148 ± 28
After 12 Weeks Supplementation	154 ± 17	155 ± 25
Apolipoprotein B (mg/dl)		
0 Weeks (Baseline)	133 ± 23	155 ± 7
After 12 Weeks Supplementation	119 ± 23	135 ± 21

[1]Means ± standard error.

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

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

factor for CVD since the average apo B concentration for this population segment is 100 mg/dl (Feldman, 1994).

At baseline the chromium group had a total cholesterol concentration of 299 mg/dl. Thus, the chromium group would be considered at high risk for CVD because its total cholesterol concentration was above 240 mg/dl (Feldman, 1994). The chromium group had an LDL cholesterol concentration of 213 mg/dl at baseline. Thus, the chromium group's mean LDL cholesterol concentration would be considered high risk because it was above 160 mg/dl (Feldman, 1994). The mean HDL cholesterol concentration of the chromium group at baseline was 34 mg/dl. The mean HDL-cholesterol concentration for the chromium group was near the recommended concentration for HDL cholesterol, which is 35 mg/dl (Feldman, 1994). The TG concentration of the chromium group at baseline was 260 mg/dl. The range for mild to moderate hypertriglyceridemia is 250 to 700 mg/dl (Feldman, 1994), and thus the chromium group would be classified in the category of mild to moderate hypertriglyceridemia at baseline (Feldman, 1994). The apo A-I concentration of the chromium group at baseline was 148 mg/dl which was above the normal concentration of 135 mg/dl (Feldman, 1994). The apo B concentration of the chromium group at baseline was 155 mg/dl. This apo B concentration is above100 mg/dl, which would be considered as a risk factor for CVD (Feldman, 1994).

At baseline the mean total cholesterol concentration for the chromium group was significantly higher than the mean cholesterol concentration for the placebo group. However, no significant differences were observed at baseline between the two groups

for other serum lipids and apolipoproteins (LDL cholesterol, HDL cholesterol, TG, apo A-I and apo B).

After twelve weeks of supplementation a significant decrease in serum total cholesterol concentration was observed in the chromium group compared to the placebo group. The post supplementation concentration of total serum cholesterol for the chromium group was 244 mg/dl and that of the placebo was 230 mg/dl. It is important to note that the serum total cholesterol concentration of the chromium group was significantly higher than the placebo group at baseline, and this may have affected the results. The results of the current study concerning a significant decrease in total cholesterol are in agreement with many chromium supplementation studies (Anderson et al., 1997; Offenbacher & Pi-Sunyer, 1980; Press et al., 1990; Elwood et al ., 1982).

Twelve-week chromium supplementation resulted in a larger trend to decrease LDL cholesterol concentration compared to the placebo group ($p = 0.07$). The post supplementation concentration of LDL cholesterol for the chromium group was 177 mg/dl and that of the placebo was 170 mg/dl. Press et al. reported a significant decrease in LDL cholesterol after chromium supplementation (Press et al., 1990). However, many studies reported an insignificant decrease in LDL cholesterol following chromium supplementation (Thomas & Gropper, 1996; Potter et al., 1985; Uusituipa et al., 1992; Abraham et al., 1982; Donaldson et al., 1985).

There was also no significant change in serum HDL cholesterol concentration within the two groups after twelve-week supplementation. The post supplementation concentration of the HDL cholesterol for the chromium group was 30 mg/dl and that of the placebo group was 28 mg/dl. However, there was a trend towards a decrease in

HDL cholesterol in both groups. Contrary to the current study, HDL cholesterol was reported to significantly increase in many studies following chromium supplementation (Abraham et al., 1992; Elwood et al., 1982; Riales & Albrink 1981). Even though HDL is reported to be more sensitive to chromium supplementation than total cholesterol (Riales & Albrink 1981), a significant increase in HDL cholesterol was not expected because the mean HDL cholesterol concentration of both groups at baseline was above the recommended range.

Chromium supplementation also resulted in a significant decrease in serum TG compared to the placebo. The TG concentration after chromium supplementation was 185 mg/dl and that of the placebo was 161 mg/dl. For the chromium supplemented group, the mean TG concentration at baseline was in the range of mild to moderate hypertriglyceridemia. After twelve-week chromium supplementation the mean TG concentration was within normal range. The change in TG is also in agreement with other studies that have reported a significant decrease in mean TG concentration with chromium supplementation (Abraham et al., 1992; Lee & Reasner, 1994; Riales & Albrink, 1981).

No significant difference in apo A-I concentration was observed after twelve-week chromium supplementation compared to the placebo group. The apo A-I concentration after supplementation was 155 mg/dl for the chromium group and 154 mg/dl for the placebo group. However, apo A-I concentration tended to increase in both groups. In contrast to the current results, one study reported that apo A-I significantly increased following supplementation with 200 μg/day of chromium (Press et al., 1990). However, in this study, apo A-I at baseline was within normal range and thus a

significant increase was not expected. There was also no significant change observed in apo B concentration within the chromium supplemented group compared to the placebo. The post supplementation concentration of apo B was 135 mg/dl for the chromium group and 119 mg/dl for the placebo group. However, apo B tended to decrease in both groups. One study reported that apo B significantly decreased with chromium supplementation (Press et al., 1990).

Effect of Chromium Supplementation on Serum Glucose, Insulin and Estradiol

Subjects' mean pre and post supplementation serum glucose, insulin and estradiol concentrations are reported in Table IV. For the placebo group, the mean fasting serum glucose concentration was 101 mg/dl at baseline. This is considered within normal range because a fasting plasma glucose below 115 mg/dl is considered normal (Kuczmarski, 1998). The placebo group also had a mean fasting serum insulin concentration of 13 μIU/ml at baseline. This is almost at the upper limit of the normal fasting insulin range, which is 10-12 μIU/ml (Glascow, 1990). The serum estradiol concentration for the placebo group at baseline was 70 pg/ml Serum estradiol is not tightly regulated and variation in circulating concentrations is common (Schiff, 1990). Women with an average age of 48 year were defined as early perimenopausal and reported to have a mean circulating estradiol concentration of 107.3 pg/ml. Women with and average age of 50 years were defined as late perimenopausal reported to have a mean circulating serum estradiol of 48.0 pg/ml. Women with and average age of 51 years were defined as early postmenopausal and reported to have a mean circulating serum estradiol of 32.0 pg/ml. Women with an average age of 53 years were defined as

TABLE IV

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

	Placebo	Chromium
Glucose (mg/dl)		
0 Weeks (Baseline)	101 ± 1	113 ± 5
After 12 Weeks Supplementation	99 ± 3	101 ± 4
Insulin (uIU/ml)		
0 Weeks (Baseline)	13 ± 1	12 ± 1
After 12 Weeks Supplementation	12 ± 2	14 ± 3
Estradiol (pg/ml)		
0 Weeks (Baseline)	70 ± 19	46 ± 11
After 12 Weeks Supplementation	60 ± 9	71 ± 29

[1]Means ± standard error.

late postmenopausal and reported to have a mean circulating serum estradiol of 24.7pg/ml (Slemenda, 1987).

For the chromium group, the mean fasting serum glucose concentration at baseline was 113 mg/dl. Similarly to the placebo group, this concentration is also within the normal range for fasting serum glucose. The mean fasting serum insulin concentration for the chromium group was 12 μIU/ml at baseline. This concentration was also within the normal range. The chromium group had a serum estradiol concentration of 46 pg/ml at baseline. There were no significant differences at baseline between the two groups in serum glucose, insulin and estradiol.

There was a small decrease in serum glucose after twelve week of chromium supplementation compared to the placebo group. However, this decrease was insignificant. The post supplementation concentration of fasting serum glucose was 101 mg/dl for the chromium and 99/mg/dl for the placebo. A significant decrease in the mean fasting serum glucose was not expected because the mean fasting glucose concentration in the chromium group at baseline was within the normal range. Some studies have reported that serum glucose decreased with chromium supplementation (Anderson et al., 1997; Riales & Albrink, 1981; Li, 1994; Offenbacher & PI-Sunyer, 1980). However, other studies have reported no effect on serum glucose following chromium supplementation (Abraham et al., 1992; Wilson & Gondy, 1995). Chromium status at baseline may be a determinant for chromiums' action on glucose tolerance (Lee & Reasner, 1994). As mentioned earlier, the subjects of the current study had a fasting serum glucose within normal range.

In this study insulin concentration did not significantly change after twelve weeks of chromium supplementation compared to the placebo. The post supplementation insulin concentration for the chromium group was 14 μIU/ml and that of the placebo was 12 μIU/ml. Serum insulin has been reported to significantly decrease with chromium supplementation (Wilson & Gondy, 1995; Offenbacher & Pi-Sunyer, 1980; Riales & Albrink, 1981; Anderson et al., 1997). On the other hand, other studies have reported similar results to the current study, observing no significant effect on fasting serum insulin with chromium supplementation (Wilson & Gondy, 1995; Thomas & Gropper, 1996). Subjects with relatively higher fasting serum insulin appeared to benefit more from chromium supplementation (Wilson & Gondy, 1995). In this study serum insulin was not expected to decrease in the chromium group because the baseline serum insulin concentration was within the normal range.

In this study, mean serum estradiol concentration did not significantly change after twelve weeks chromium supplementation compared to the placebo. The mean estradiol concentration after supplementation was 71 pg/ml for the chromium group and 60 pg/ml for the placebo group. Both post supplementation estradiol concentrations were above the concentration of the late postmenopausal group. However, this may be due to the high variation in the observed values.

The absence of the chromium effect on serum glucose, insulin and estradiol may be due to sample size. The sample size in the current study was small. It is a well known fact that a small sample size will decrease statistical power. Loss of power is one reason for not having a statistical significance, since sample size is usually calculated to detect significant differences between treatments, which in this study, are chromium

versus placebo. Therefore in many cases, it is difficult to show significant differences when the planned sample size is not reached (Ott, 1977b). As a result, careful consideration should be given when interpreting the results of the current study due to the small sample size.

CHAPTER V

SUMMARY, RECOMMENDATIONS AND CONCLUSION

The purpose of this study was to investigate the effects of twelve-week chromium supplementation on serum lipids and apolipoproteins (total cholesterol, LDL and HDL cholesterol, TG, apo A-I and apo B), serum glucose, insulin and estradiol, in hypercholesterolemic postmenopausal women. With the approval of the Institutional Review Board, this study was conducted in the spring and summer of 1998, using fourteen postmenopausal women from the community of Stillwater and surrounding area.

Subjects were randomly assigned to one of two groups. The placebo group consumed a lactose placebo for twelve weeks. The chromium group consumed 200 μg per day chromium in the form of $CrCl_3.6H_2O$ in lactose for twelve weeks. All subjects were independently living and consumed self-selected diet throughout the study.

Height, weight, and twelve-hour fasting blood samples were obtained at baseline. Weight and twelve-hour fasting blood samples were also obtained after twelve weeks of supplementation. The subjects also completed a health questionnaire and a seven day food frequency at each data collection.

Changes after twelve weeks in serum lipids, glucose, insulin and estradiol in the placebo group were compared to changes in the chromium group. Seven day food frequencies were used to evaluate dietary intake. Dietary parameters included in the dietary analysis were total calories, protein, total and saturated fat, cholesterol, fiber, and percent of calories from carbohydrates, protein and total and saturated fat.

After twelve weeks chromium supplementation, a significant decrease in serum total cholesterol and triglycerides was observed compared to the placebo. In addition, there was a trend towards a decrease in serum LDL cholesterol concentration after twelve weeks chromium supplementation compared to the placebo.

CONCLUSION

The first null hypothesis H_0 1 stated that there would be no significant effect due to twelve weeks supplementation with 200 μg/day of chromium on serum lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, and TG) and apolipoproteins (apo A-I, apo B). The first null hypothesis was rejected because twelve weeks supplementation with 200 μg of chromium decreased serum total cholesterol and TG concentrations, compared to the placebo. No significant effects were observed on LDL and HDL cholesterol, apo A-I and apo B. However, HDL cholesterol and apo A-I were initially within normal ranges and no changes were expected.

The second null hypothesis H_0 2, stated that there would be no significant effect due to twelve weeks supplementation with 200 μg/day chromium on serum insulin and serum fasting glucose. The second null hypothesis was accepted because there were no significant effect due to twelve weeks supplementation with 200 μg chromium on serum insulin and serum fasting glucose concentrations compared to the placebo.

The third null hypothesis H_0 3 stated that there would be no significant effect due to twelve weeks supplementation with 200 μg/day chromium on serum estradiol. The third hypothesis was accepted because there was no significant effect due to twelve weeks supplementation with 200 μg of chromium on serum estradiol compared to the placebo.

Due to the observed results in this study, it seems that a chromium intake at the ESADDI, from either a dietary source or a supplementary source may help reduce some risk factors for the development of CVD among postmenopausal hypercholesterolemic women. However the mechanism for the effect of chromium on serum lipids is unknown. In this study, the theory that the effect of chromium on serum lipids is mediated through an action on serum insulin was not observed. The absence of any significant effect of chromium on fasting serum glucose, insulin or estradiol may be due to the fact that subjects in this study were not insulin resistant or may be due to the small sample size.

RECOMMENDATIONS

Based on the results of this study, it is recommended that:

1. future studies use a larger sample size;
2. future studies should check the accuracy of the balance before subjects are weighed;
3. future studies be conducted on hyperlipidemic NIDDM patients to investigate the theory that individuals with NIDDM are the most likely to benefit from chromium supplementation;
4. future studies investigate the effect of various amounts and forms of chromium;
5. future studies include an assessment of dietary chromium intake;
6. future studies investigate the effect of chromium supplementation on premenopausal women.

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APPENDIXES

APPENDIX A

THE OKLAHOMA STATE UNIVERSITY'S INSTITUTIONAL REVIEW BOARD APPROVAL FORM FOR HUMAN SUBJECTS

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 08-28-96 **IRB#**: HE-97-001

Proposal title: EFFECTS AND INTERACTIONS OF CHROMIUM AND COPPER ON LIPID METABOLISM IN HYPERCHOELSTEROLEMIC POST MENOPAUSAL WOMEN

Principal Investigator(s): Janice R. Herrman, 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 INSTITUTIUONAL REVIEW BOARD AT NEXT MEETING.
APPROVAL STATUS PERIOD VALID FOR ONE CALANDER YEAR AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Condition for Approval or Reasons for deferral or Disapproval are as follows:

Signature _____ Date: August 28, 1996
Chair of Institutional Review Board

APPENDIX B

THE STUDY RECRUITMENT ADVERTISEMENT FORM

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 high blood cholesterol, over 200 mg/dl
- Not using estrogen replacement therapy
- Not using medication to lower blood cholesterol

This study will investigate the effects and interactions of chromium and copper supplementation on the risk of cardiovascular disease in postmenopausal 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 µg/day chromium, which are the upper level of the safe and adequate intake range for each nutrient, as set by the National Research Council, Food and Nutrition Board.

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
Oklahoma State University
Stillwater, Oklahoma 74078
(405) 744-6824

APPENDIX C

THE INDIVIDUAL CONSENT FORM OF RESEARCH PARTICIPATION

Individual Consent to Participate in Research

Women's Cholesterol Study: Effect 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 id to 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 µg chromium
(d) 1.5 mg copper plus 100 µg 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 the study

(5) a phlebotomist will draw fasting blood samples of 30 ml (about 6 teaspoons) by venipuncture at the beginning and the 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 protocol, 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 the 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 the study at any time by contacting the principal investigators;

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

(15) this research is beneficial to the public in that the risk of cardiovascular disease increase among women after menopause;

(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 Scinces 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

THE HEALTH INFORMATION QUESTIONNAIRE FORM

Health Questionnaire

1. Subject Number _____

2. Date of Birth _____

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

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 exercice? _____

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

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

APPENDIX E

THE SEVEN DAY 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, 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 parenthesis.

EXAMPLES:

Ate ½ 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**
Grape Fruit (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
VEGETABLES, VEGETABLE JUICE						
Tomato (1)						
Tomato juice (1/2 cup)						
Tomato sauce (1/2 cup)						
Spaghetti sauce (1/2 cup)						
Red chilli sauce, taco sauce, or salsa (1 Tbsp)						
Tofu or Soybean (3-4oz)						
String beans, green beans (1/2 cup)						
Broccoli (1/2 cup)						
Cabbage (1/2 cup)						
Cole slaw (1/2 cup)						
Cauliflower (1/2 cup)						
Brussels sprouts (1/2 cup)						
Carrots, raw (1/2 carrot or 2-4 sticks)						
Carrots, cooked (1/2cup)						
Corn (1 ear or1/2 cup frozen or canned)						
Peas (1/2 cup fresh, frozen or canned)						
Lima beans (1/2 cup frozen or canned)						
Mixed vegetables (1/2 cup)						
Beans or lentils, baked or dried (1/2 cup)						
Summer or yellow squash (1/2 cup)						
Winter squash (1/2 cup)						
Zucchini (1/2 cup)						
Yam or sweet potato (1/2 cup)						
Spinach (cooked1/2 cup, raw 1 cup)						
Ice burg lettuce, romaine or leaf (1 cup)						
Celery (4" stick)						
Beets (1/2 cup)						
Alfalfa sprouts (1/2 cup)						
Kale, mustard, or chard greens (1/2 cup)						
Vegetable, vegetable beef, minestrone or tomato soup (cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
BREAD, CEREALS, STARCH						
Cold breakfast cereal (1 cup)						
Cold cereal breakfast-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 (1 cup)						
White rice (1 cup)						
Spaghetti noodles (1 cup)						
Macaroni noodles (1 cup)						
Other pasta noodles (1 cup)						
Bulgar, kasha, couscous (1cup)						
Pancakes or waffles (2)						
Potatoes, frensh fries or fried (1/2 cup)						
Potatoes, backed or boiled (1)						
Mashed potatoes (1cup)						
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 cracker, low fat (5)						

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 (3-4 oz)						
Bacon (2 slices)						
Hotdogs (2)						
Low fat hotdogs (2)						
Sausage (2 patties or 2 linkes)						
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 (1patty, 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 (1/2 cup)						
Fish, backed or broiled (3-4 oz)						
Fish, fried or fish sandwich (3-4 oz)						
Shrimp, Lobster, Scallops						
Pizza (2 slices)						
Mixed dishes with cheese (1 cup)						
Lasagna or meat pasta dishes (1 cup)						

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 (1/2 cup)						
Bananas						
Cantaloupe (1/4 melon)						
Watermelon (1 slice)						
Apples, applesauce or pears (1fresh, ½ cup)						
Apple juice (1/2 cup)						
Oranges						
Orange juice (1/2 cup)						
Grapefruit (1/2 cu						
Grapefruit juice (1/2 cup)						
Other fruit juices (1/2 cup)						
Strawberries-fresh, frozen, or canned (1/2 cup)						
Blueberries-fresh, frozen, or canned (1/2 cup)						
Peaches (1 fresh, ½ cup canned)						
Apricots (1 fresh, ½ cup canned)						
Plums (1fresh, ½ cup canned)						
Honeydew melon (1/4 melon)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
SWEETS, BACKED GOODS, MISC.						
Chocolate (1 small bar or 1 oz)						
Candy bar (1small bar)						
Candy without chocolate (1 oz)						
Cookies, home baked (2)						
Cookies, ready made (2)						
Brownies (2)						
Doughnuts (2)						
Cake, home baked (1slice)						
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 (1slice)						
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)						

VITA[2]

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Candidate for the Degree of

Master of Science

Thesis: EFFECT OF CHROMIUM SUPPLEMENTATION IN REDUCING CARDIOVASCVULAR DISEASE RISK FACTORS IN POSTMENOPAUSAL WOMEN: EFFECT ON SERUM LIPIDS, GLUCOSE AND INSULIN

Major Field: Nutritional Sciences

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Personal Data: Born in Beirut, Lebanon, on August 17, 1967, the son of Dr. Aref and Huda El-Osta.

Education: Received a Bachelor of Science degree in Nutrition and Food Technology from the American University of Beirut, Beirut, Lebanon in July 1991. Awarded a diploma in Food Handling and Safety Inspection from the Royal Institute of Public Health and Hygiene, Portland Place, United Kingdom in March 1993. Awarded a diploma in Quality University Instruction from Oklahoma State University, Stillwater, Oklahoma in December 1998. Completed the requirements for the Master of Science degree with a major in Nutritional Sciences at Oklahoma State University in May 1999.

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