EFFECTS OF CHROMIUM OR COPPER SUPPLEMENTATION ON PLASMA LIPIDS, PLASMA GLUCOSE AND SERUM INSULIN IN ADULTS OVER AGE 50

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CHAPTER I

RESEARCH PROBLEM

Introduction

Atherosclerosis is the leading cause of death in the United States and in most other developed countries (Ranade, 1993). Over 50% of all deaths in the United States each year can be attributed to atherosclerosis (Ranade, 1993). Elevated plasma lipids and abnormal lipoprotein profiles are considered to be major risk factors in the development of atherosclerosis (Ross, 1986; Steinberg, 1988). Atherosclerosis also is the largest single cause of death among diabetic subjects (Wenck et al., 1980). Additionally, disorders in glucose and lipid metabolism have been reported among atherosclerotic subjects (Albrink, 1962; Falsetti et al., 1968). Therefore, current research in nutrition to reduce atherosclerosis has focused on dietary modifications to improve plasma glucose, lipids and lipoprotein profiles.

Chromium (Cr) is a trace mineral essential for glucose and lipid metabolism (Mertz, 1969). Chromium is necessary for insulin action through its role with glucose tolerance factor (Morris et al., 1992; Mertz, 1993). Chromium deficiency in humans and animals impairs glucose tolerance, elevates blood glucose concentrations, and causes hypercholesterolemia and aortic plaque development (Mertz, 1969; Anderson, 1986). Chromium supplementation has improved glucose tolerance, blood lipids and insulin concentrations (Anderson et al., 1991). In a human study, oral administration of chromium as chromium chloride or as brewer's yeast improved glucose tolerance,

decreased insulin concentrations and enhanced insulin effectiveness in elderly and diabetic subjects (Offenbacher and Pi-Sunyer, 1980).

Recent reports indicate that copper (Cu) also is involved in cholesterol and triglyceride metabolism. Copper deficiency increases plasma cholesterol and triglyceride concentrations (Allen and Klevay, 1980; Mazur et al., 1992). Murthy and Petering (1976) demonstrated that serum cholesterol concentration was related inversely to dietary copper intake and serum copper concentration in rats. Allen and Klevay (1980) reported a 25% increase in plasma triglyceride concentrations in copper deficient rats. Low copper intakes also have been implicated with abnormal plasma lipoprotein profiles (Medeiros et al., 1991; Al-Othman et al., 1992). Copper deficiency in rats increases both high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations and alters lipoprotein composition (Allen and Klevay, 1980; Lei, 1983). Copper deficiency in rats decreased the percent of cholesterol associated with high density lipoproteins and increased the percent of cholesterol associated with low density lipoproteins. Furthermore, copper deficiency in rats caused glucose intolerance and elevated fasting insulin concentrations (Klevay et al., 1983). Because of these possible etiological links between chromium and copper deficiencies and the development of coronary heart disease, the effects of chromium and copper supplementation on glucose and lipid metabolism warrant investigation.

Objectives

The purpose of this study was to investigate the effects of chromium or copper supplementation on plasma lipids, apolipoproteins, glucose and serum insulin concentrations in older adults. The objectives of this study were to:

1. Determine the effects of eight weeks of chromium or copper supplementation on plasma total, LDL, HDL-cholesterol and triglyceride concentrations in adults over age 50;
2. Determine the effects of eight weeks of chromium or copper supplementation on plasma apolipoprotein A-I and apolipoprotein B concentrations in adults over age 50; and
3. Determine the effects of eight weeks of chromium or copper supplementation on plasma glucose and serum insulin concentrations in adults over age 50.

Null Hypotheses

The following null hypotheses were developed for this study. There will be no statistically significant effects due to eight weeks of chromium or copper supplementation on:

1. Plasma total, LDL, HDL-cholesterol, or triglyceride concentrations in adults over age 50.
2. Plasma apolipoprotein A-I and apolipoprotein B concentrations in adults over age 50.
3. Plasma glucose and serum insulin concentrations in adults over age 50.

Assumptions

The following assumptions were made for this research;

1. We assumed that the subjects recorded their three-day dietary intakes as instructed.
2. We assumed that the subjects took one supplement capsule two times each day for eight weeks.
3. We assumed that the subjects fasted for 12 hours before each blood collection.

4. We assumed that the subjects did not alter their regular exercise and eating patterns during the course of the study.
5. We assumed that the subjects did not take any lipid altering medication.
6. We assumed that time trends in analytical values of individuals on each treatment paralleled those individuals in the placebo group so that covariance analysis was appropriate.

Limitations

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

Thesis Format

The tables and bibliographic citations in this thesis follow the Journal of Nutrition for the Elderly Format.

CHAPTER II

REVIEW OF LITERATURE

This chapter includes a literature review of glucose, insulin, lipids, chromium and copper. Effects of chromium and copper on glucose, insulin, lipids and apolipoproteins also are reviewed.

Glucose Metabolism

Digestion and Absorption

Chief food sources of carbohydrate are starch, disaccharides, and glucose polymers. Carbohydrates are cleaved in the mouth and intestinal tract by various enzymes such as salivary and pancreatic amylase (Felig, 1980). The first step in carbohydrate digestion begins as food enters the mouth. Salivary amylase is mixed with the food bolus and begins to hydrolyze starch into disaccharides. The most important site of carbohydrate digestion is the small intestine. Pancreatic amylase, released into the small intestine lumen, hydrolyzes dextrins to a specific disaccharide, maltose. The cells along the brush border of the small intestine contain disaccharidases that cleave sucrose, maltose, or lactose to their component monosaccharides; fructose, galactose, and glucose. All the digestive dietary carbohydrates are hydrolyzed in the small intestine to these three monosaccharides. The mixture of monosaccharides resulting from the action of digestive enzymes is absorbed into the blood through mucosal cells lining the intestine. The absorptive process occurs primarily in the jejunum. Monosaccharides are transported through the capillary walls into the portal vein by passive diffusion and active transport.

All three monosaccharides have a high absorption rate, approaching 98 percent of digestible carbohydrates in a diverse American diet. The portal blood transports all monosaccharides to the liver, where fructose and galactose are converted enzymatically to glucose (Kreutler, 1980).

Glucose circulating in the blood provides body cells with an energy source and a substrate for the synthesis of other compounds. In the fasting state, blood glucose concentration normally ranges between 70 and 100 mg/dl. After a meal containing carbohydrates, blood glucose may rise to about 140 mg/dl, but in non-diabetic individuals it returns to its former level within two hours (Kreutler, 1980).

Fate of Glucose

Glucose metabolism may take anabolic or catabolic pathways. In anabolic pathways, glucose is converted to glycogen by the liver or muscles for storage as an immediate energy reserve, or to fat by the liver or adipose tissue. To store glucose as glycogen results in a 5% energy loss as compared to direct glucose oxidation (Horton, 1983). When glucose is converted to fatty acids, the energy cost is approximately 28%; this makes fat less efficient than glycogen for storing energy from glucose (Horton, 1983; MacDonald, 1988). Glucose-6-phosphate, the first step in glucose metabolism, has several fates depending on glucose demand. Glucose-6-phosphate can be converted to glycogen when the body's demand for glucose is low. An increased glucose demand reverses this process, resulting in glucose release from glycogen. Glucose-6-phosphate also can be converted to acetyl-CoA via glycolysis and pyruvate dehydrogenase. Most acetyl-CoA is used for fatty acids, phospholipids, and cholesterol synthesis. Glucose-6-phosphate also can be converted to glucose by glucose-6-phosphatase for transport via the blood stream to the peripheral organs (Voet & Voet, 1990). Glucose also combines

with proteins to form certain essential body compounds, and glucose products are used to form certain nonessential amino acids (Felig, 1980).

By catabolic pathways, glucose is oxidized to release energy. Glucose oxidation in body cells occurs in one of two general ways, depending on available oxygen (Wenck et al., 1980). In sufficient oxygen, glucose is converted into pyruvic acid and two moles of adenosine triphosphate (ATP). Pyruvic acid is further catabolized, first to acetyl CoA, and then to citrate. In the citric acid cycle, citrate produces reducing equivalents and eventually ATP and water via oxidative phosphorylation plus carbon dioxide. In low oxygen, glucose catabolism stops at lactic acid, and only two moles ATP are produced per mole glucose. Lactic acid can be oxidized to carbon dioxide and water when or where oxygen becomes available, but most lactate is resynthesized into glycogen to re-establish muscle glycogen stores (Wenck et al., 1980).

When blood glucose concentration is high, normally immediately after a meal, the polypeptide hormone insulin is released from pancreatic beta cells and glucagon release decreases. Glucose transport rate across many cell membranes increases in respose to insulin (Voet & Voet, 1990). Insulin acts mainly on muscle and adipose tissue cells stimulating glycogen, fat, and protein synthese while inhibiting their catabolism (Voet & Voet, 1990). Insulin increases glucose uptake by most cells with the notable exception of brain and liver cells. When blood glucose concentration is low, pancreatic alpha cells release glucagon into the blood stream. Glucagon receptors on liver cell surfaces activate adenylate cyclase. An increase in cAMP (cyclic Adenosine Monophosphate) triggers glycogen hydrolysis leading to increased intracellular glucose-6-phosphate concentrations. Insulin together with glucagon maintains blood glucose concentration within a narrow range (Voet & Voet, 1990).

Glucose Metabolism in Liver, Muscle, and Adipose Tissue

The liver is the principal site for glucose catabolism. The liver plays a central role in stabilizing blood glucose concentration and adjusting carbohydrate movement into appropriate metabolic channels (Felig, 1980). The hepatocyte membrane has specific glucagon and insulin receptors (Voet & Voet, 1990). However, because liver cells are freely permeable to glucose, insulin plays no direct role on glucose uptake into the liver. In the liver, glucose is phosphorylated by two different enzymes, hexokinase and glucokinase. As plasma glucose concentration increases, glucose phosphorylation increases. Thus, glucose uptake by the liver is proportional to plasma glucose concentration (Felig, 1980).

In the liver, glucose-6-phosphate can enter four different pathways. Glucose can be released from glucose-6-phosphate by glucose-6-phosphatase, which is present in liver but not in other tissues. Because liver cells are freely permeable to glucose, this enzyme permits glucose return to the blood stream. Glucose-6-phosphate also can form glycogen or enter the glycolytic anaerobic pathway, ultimately yielding pyruvic acid, or glucose can be oxidatively degraded via the pentose cycle to form various pentoses; ribulose-5-phosphate, ribose-5-phosphate and xylulose-5-phosphate (Felig, 1980).

Major fuels for muscle cells are glucose, fatty acids, and ketone bodies. Muscle synthesizes glycogen and stores one to two percent of its mass as glycogen. Muscle can not release glucose because it lacks glucose-6-phosphatase (Voet & Voet, 1990). Nevertheless, muscle glycogen serves the body as an energy reserve. During fasting, muscle proteins are degraded to amino acids, many of which are converted to pyruvate. Pyruvate can be transaminated to alanine, which is exported via the blood stream to the liver. In the liver, alanine is transaminated back to pyruvate, and pyruvate is used for glucose synthesis. Muscle cells do not have glucagon receptors; however, muscle possess

epinephrine receptors that control the phosphorylation/dephosphorylation cascade system and regulate glycogen catabolism and synthesis (Voet & Voet, 1990).

Glucose is converted into lipids in both liver and adipose tissue. Glucose is used by adipose tissue to form triacylglycerol. In human adipose cells, 16% or less of glucose is converted into fatty acids and 80% is converted to glycerol (Bjorntorp et al., 1968). Adipose tissue obtains most of its fatty acids from the liver or from the diet. Fatty acids are activated by the formation of corresponding fatty acyl-CoA and esterified with glycerol-3-phosphate to form triglycerides which are stored. Adipose tissues hydrolyze triglycerides to fatty acids and glycerol in response to high glucagon and epinephrine concentrations, and to low insulin concentrations through a hormone-sensitive lipase. This enzyme is more active in adipose cells during fasting. Because hormone-sensitive lipase is the rate-determining enzyme in lipolysis, its activity controls overall lipolysis rate (Hollett and Auditore, 1967; Voet & Voet, 1990). If glycerol-3-phosphate is abundant, fatty acids released are re-esterified to triglycerides. However, if glycerol-3-phosphate is deficient, fatty acids are released into the blood stream. Adipocyte glucose uptake rate, which is regulated by insulin and glucose availability, thereby control triglyceride synthesis and mobilization (Voet & Voet, 1990).

Dietary Carbohydrate and Serum Triglycerides

Dietary carbohydrate can affect serum lipid concentrations in both the long and short term. Following acute ingestion of carbohydrate, serum triglyceride concentration decreases. This effect is considered to be due to an increase in lipoprotein lipase activity and insulin output (Nestel, 1967; Felig, 1980). Lipoprotein lipase, the adipose tissue enzyme that mediates plasma triglyceride hydrolysis, is readily altered by dietary changes. Fasting reduces lipoprotein lipase activity in human adipose tissue. Conversely, glucose ingestion stimulates lipoprotein lipase release into plasma within one hour after glucose

ingestion in normal individuals. This is responsible for the rapid decrease in plasma triglyceride concentration after an acute glucose load. Insulin can lower triglyceride concentration by directly enhancing free fatty acid removal or by suppressing mobilization. Incorporation of plasma free fatty acids into triglycerides is reduced when free fatty acid turnover is suppressed by insulin. An increase in fasting serum triglyceride concentration has been observed after a sudden change to a high-carbohydrate diet (MacDonald, 1988). However, this increase in serum triglyceride concentration seems to be short-lived. A few weeks after switching to a high carbohydrate diet, fasting serum triglyceride concentrations tend to decline (MacDonald, 1988). Therefore, serum triglyceride concentrations measured after a 12-hour fast may be considered endogenous in origin.

Glucose Induced Insulin Secretion

Insulin dramatically reduces plasma glucose concentration. Besides lowering plasma glucose, insulin promotes liver and muscle glycogen synthesis, and liver and adipose tissue fatty acid synthesis. Insulin also stimulates RNA, DNA and protein synthesis and is essential for growth and maturation (Voet & Voet, 1990).

Insulin is a protein composed of two polypeptide chains, designated A and B, connected by two disulfide bridges. An additional disulfide bridge is found between the sixth and eleventh amino acid residues of the A chain (Bondy & Rosenberg, 1980). Insulin is synthesized by pancreatic beta cells as a single chain precursor, proinsulin. Proinsulin consists of a spiral molecule in which the A and B chains are joined by a connecting peptide (C-peptide). Within the golgi complex, a membrane-bound protease cleaves proinsulin into equimolar amounts of insulin and C-peptide. Insulin along with zinc accumulates within the central core of the maturing secretory granule. Release of the mature secretory granules contents involves progressive migration of the granules to

the cell plasma membrane followed by insulin and C-peptide extrusion (Bondy & Rosenberg, 1980).

Glucose is the predominant metabolite that stimulates insulin secretion. The precise mechanism whereby glucose acts on the beta cells to release insulin has not been clarified. Two alternative theories have been proposed, one involving the interaction of glucose with a membrane receptor (glucoreceptor) in which glucose is recognized by activation of a membrane bound receptor, thereby triggering insulin release (Gerich et al., 1976). A major stimulus to insulin secretion occurs as a result of glucose interacting with receptors within the gastrointestinal tract. Thus, plasma insulin concentration is higher after administration of oral glucose than glucose given intravenously. Administered into the intestinal tract, glucose stimulates insulin release due to release of additional hormones by cells in the stomach or upper small intestine. The microvillous surface of endocrine cells receive signals from the lumen by the presence of food in the gut. In this connection, gastric inhibitory polypeptide (GIP) secreting cells characteristically respond only to carbohydrate and produce GIP whose major physiological function is to stimulate pancreatic insulin release (Holst, 1986). In addition, sugar and sugar derivatives, which are metabolized in the pancreatic islets of Langerhans, elevate cAMP concentration. An increase in cAMP is believed to act primarily as a positive modulator of glucose-sensitive secretion (Hedeskov, 1980). An increase in cAMP is not, however, sufficient to stimulate insulin secretion and has little stimulatory action on insulin secretion unless glucose is present. An increase in intracellular calcium is believed to be the final triggering mechanism whereby glucose or other stimuli cause insulin to be released from beta cells. Mobilization of stored intracellular calcium is enhanced by cAMP, and addition of calcium to beta cells results in a burst of insulin secretion (Hedeskov, 1980).

One theory of insulin action concerns migration of glucose transporters. In the basal state, body cells are thought to store most of their glucose transporters in internal membranous vesicles. Upon insulin stimulation, these vesicles fuse with the plasma

membrane in a process known as exocytosis. The increase in cell-surface glucose transporters proportionally increases glucose uptake rate. Upon insulin withdrawal, the process is reversed through plasma membrane-embedded glucose transporters endocytosis. Thus, insulin may increase basal glucose transporter vesicle exocytosis rate and slow endocytosis rate (Voet & Voet, 1990).

Lipid Metabolism

Digestion and Absorption

Triacylglycerol consists of glycerol esterified with three fatty acids. Triglycerol constitutes over 90% of dietary lipids and is the major form of energy storage in humans. Lipid digestion and absorption take place mainly in the small intestine. Pancreatic lipase catalyzes triacylglycerol hydrolysis at the first and third positions to sequentially form 1,2-diacylglycerols and 2-acylglycerols. The mixture of fatty acids, mono-, and diacylglycerols produced by lipid digestion is absorbed by cells lining the small intestine. Digestion is facilitated by bile acids synthesized by the liver and secreted via the gallbladder into the small intestine. Micelles formed by bile acids take up nonpolar lipid degradation products and permit their transport across the intestinal wall's unstirred aqueous boundary layer. Glycerol and fatty acids less than 12 carbons long immediately enter the portal blood and are carried to the liver. Lipid digestion products absorbed by the intestinal mucosa are converted by intestinal tissues back to triacylglycerols and packaged into lipoprotein particles called chylomicrons (Voet & Voet, 1990).

Cholesterol is a structural component of cell membranes and a precursor to other steroids. Cholesterol is used for bile acid production and hormone synthesis. Although all tissues have the ability to synthesize cholesterol, over 90% of total body cholesterol comes from intestinal and hepatic synthesis (Kris-Etherton et al., 1988).

Cholesterol is poorly absorbed; in contrast over 98% of dietary fat is absorbed. Some 30 to 40% of dietary cholesterol was absorbed by men with intakes ranging between 40 mg/day to more than 2 g/day (Quintao et al., 1971; Connor and Lin, 1974;). In normal human subjects the maximal capacity for absorbing dietary cholesterol is 300 to 500 mg/day (Wilson and Lindsey, 1965). Unabsorbed dietary cholesterol or unabsorbed bile acids secreted into the intestine are excreted in feces.

Cholesterol occurring free in foods requires no digestion, but most cholesterol exists in foods as a cholesterol ester, with one fatty acid attached to each cholesterol molecule. Cholesterol esterase, secreted by the pancreas, hydrolyze the bond between cholesterol and fatty acids, freeing cholesterol for absorption. Bile salts emulsify digestion products, and help them travel through the intestinal lumens' watery medium to reach intestinal mucosal cells. Upon reaching mucosal cells, bile salts release lipid substances which diffuse passively across the cell membrane, mostly as free cholesterol. Cholesterol is re-esterified in the mucosa and incorporated into chylomicrons. Chylomicrons enter the lymph through enterocyte lateral leaky cell membranes. Cholesterol is transported by chylomicrons in two forms; as free cholesterol forming part of the chylomicron hydrophilic outer layer and as esterified cholesterol in the chylomicron hydrophobic core. Chylomicrons containing apolipoprotein B-48, A-I, and A-IV pass from mucosal cells into small lymph vessels; lymph empties into the thoracic duct and enters the blood at the subclavian vein of the neck (Kris-Etherton et al., 1988).

Lipoprotein and Apolipoprotein Metabolism

Lipoproteins are water-soluble macro-molecule complexes containing lipids and one or more specific proteins. Plasma lipoproteins primary function is to transport lipids to various tissues throughout the body for metabolism. The hydrophobic lipids - triglycerides and cholesterol esters - are located in the spherical plasma lipoprotein core,

whereas the hydrophilic lipids - free cholesterol, phospholipids and apolipoproteins - are located on the lipoprotein surface (Kris-Etherton et al., 1988).

Lipoprotein fractions are separated according to their size (by gel filtration), density (by ultra-centrifugation), net surface charge (by electrophoresis), or other surface properties (precipitation and absorption techniques) (Ernest and Levy, 1988). Plasma lipoproteins commonly are divided into five major classes: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoprotein (HDL) (Kris-Etherton et al., 1988).

Chylomicrons are the lightest and largest lipoprotein, having a density of less than 0.95 g/ml and a diameter greater than 100 nm (Kris-Etherton et al., 1988). Chylomicrons consist of approximately 90% triglyceride, with small amounts of phospholipids, cholesterol and protein. They are synthesized mainly in the jejunal mucosa with apolipoprotein B-48 as the major apolipoprotein. The primary function of chylomicrons is to transport dietary triglycerides and cholesterol from the site of intestinal absorption to different cells in the body (Kris-Etherton, et al., 1988). Chylomicrons acquire apolipoprotein C and E in the blood from HDL. Apolipoprotein C-II is a required cofactor for lipoprotein lipase, an enzyme on the capillary endothelium that hydrolyses chylomicron triglycerides (Kris-Etherton et al., 1988). Lipoprotein lipase releases approximately 90% of the triglycerides contained in the chylomicron; the resulting product is a chylomicron remnant. Chylomicron remnants are about half the size of the parent chylomicron and are rich in cholesterol and cholesterol ester. Apolipoprotein E is transferred from HDL to chylomicron remnants, which then are rapidly cleared by the liver through apolipoprotein E receptor-mediated endocytosis.

VLDL is the second lightest and second largest lipoprotein, having a density of 0.950 to 1.006 g/ml and a diameter of 30 to 90 nm. VLDL is produced in either the liver or the intestinal mucosa (Kris-Etherton et al., 1988). VLDL mainly functions to transport endogenously synthesized triglycerides and cholesterol from the liver for redistribution to

various tissues. Compared to chylomicrons, VLDLs' have higher cholesterol and lower triglyceride content. Triglycerides comprise approximately 50 to 60% of the VLDL. The major apolipoproteins associated with VLDL are 25% apolipoprotein B-100, 35 to 50% apolipoprotein C (Apo C), and 7 to 10% apolipoprotein E (Apo E). Lipoprotein lipase hydrolyzes most VLDL triglycerides for release to extra-hepatic tissues, predominantly heart muscle and adipose tissue. Increased concentrations of VLDL and chylomicron produce a mixed type of hyperlipidemia commonly associated with insulin-dependent diabetes mellitus and characterized by premature vascular disease.

Within the plasma, VLDL triglycerides are hydrolyzed by endothelial lipoprotein lipase in muscle capillary beds. During this hydrolysis Apo C leaves the VLDL, and the resulting triglyceride-poor particles are called intermediate density lipoprotein. The IDL is an intermediate in the catabolism of VLDL to LDL. The size and density of IDL falls between the VLDL and LDL. The IDL contains 95% lipid, primarily cholesterol esters. Triglycerides comprise approximately 25% of the IDL. IDL is characterized by apolipoprotein E enrichment. Surface material from IDL is transferred to HDL. Unesterified cholesterol is esterified in HDL via the enzyme lecithine cholesterol acyltransferase (LCAT). The esterified cholesterol is transferred back to IDL, leading to a cholesterol ester enrichment of IDL. Some IDL is cleared from plasma by hepatic LDL receptors that bind apolipoprotein E. The remainder undergo rapid continued lipolysis, probably by hepatic triglyceride lipolysis; all apolipoproteins except B-100 are transferred to other lipoproteins. This results in the formation of LDL with apolipoprotein B-100 on its surface.

Low density lipoprotein is the final product of VLDL catabolism. Cholesterol, both free and esterified, makes up about 50% of the LDL, phospholipids make up 20% and triglycerides 10% (Kris-Etherton et al., 1988). Approximately two-thirds of the LDL are metabolized after binding to Apo B and E receptors located on the liver and other body cells which recognize LDL Apo B-100. The remaining LDL is modified in the

plasma and removed by scavenger receptors on macrophages and endothelial cells (Kris-Etherton et al., 1988). Plasma LDL concentrations are influenced by LDL receptors, which in turn are regulated by the cells' need for cholesterol. When need is low, cells make fewer receptors and remove LDL from the blood at a reduced rate. Elevated blood LDL concentration accelerates the rate of atherogenesis (Kris-Etherton, 1988).

High density lipoprotein has the greatest density. It carries about half as much cholesterol as LDL. HDL is comprised of approximately 50% protein, 30% phospholipids, and 20% cholesterol. HDL seems to be synthesized by both the liver and intestine (Kris-Etherton et al., 1988). HDL is called the "beneficial" lipoprotein because it plays a role in reverse cholesterol transport, which involves the delivery of cholesterol from peripheral tissues back to the liver for excretion via the bile (Kris-Etherton et al., 1988).

The protein moiety of HDL is composed of two apolipoprotein families: A and C. Ninety percent of total HDL protein consists of Apo A-I and A-II with a ratio of 3:1 in both HDL subclasses. Apo A-I is believed to activat the enzyme LCAT which esterifies free cholesterol in HDL (Rubinstein and Rubinstein, 1972). HDL often is divided into two subclasses based on density. The HDL_2 subdivision has a density of 1.063 to 1.120 g/ml. The HDL_3 contains relatively more protein and less neutral lipid than HDL_2. The Apo C class is present in HDL in small amounts, approximately 5 to 10% of HDL_2 protein and 1 to 2% of HDL_3 protein (Brunzell et al., 1973).

Plasma total cholesterol, LDL-cholesterol, and HDL-cholesterol are related to coronary heart disease risk. Plasma total cholesterol and LDL-cholesterol are positively whereas HDL-cholesterol is negatively related to coronary heart disease risk. HDL_2 is a better predictor of coronary heart disease risk than total, HDL-cholesterol or HDL_3-cholesterol (Miller et al., 1981). The National Cholesterol Education Program (Report of National Cholesterol Education Program Expert Panel, 1988) recommends that plasma total cholesterol be assayed first. If it is higher than 240 mg/dl, then a lipoprotein

cholesterol assay is indicated. An LDL-cholesterol concentration of 130 to 159 mg/dl places an individual at risk for coronary artery disease, and a concentration greater than 160 mg/dl is an indicator of high risk. A HDL-cholesterol concentration less than 35 mg/dl also is a risk factor for coronary artery disease (Kris-Etherton et al., 1988).

Serum apolipoproteins may be better predictors of coronary artery disease risk than plasma lipid and lipoprotein concentrations (Kris-Etherton et al., 1988). Apolipoprotein A-I and B often are used to assess coronary artery disease risk. Apo A-1 is the major apolipoprotein in HDL-cholesterol, representing approximately 60% of HDL protein; and Apo B is the major apolipoprotein in LDL-cholesterol, comprising approximately 90% of LDL protein (Ortola et al., 1992). Some investigators have suggested that apolipoprotein A-1 and apolipoprotein B concentrations are better predictors of coronary artery disease than total, LDL, or HDL-cholesterol concentrations (Kwiterovich and Sniderman, 1983; Brunzell et al., 1984). The apolipoprotein A-I to apolipoprotein B ratio appears to be the most consistent coronary artery disease risk indicator. Apolipoprotein E has been reported to be higher in myocardial infarction survivors. In addition, serum apolipoprotein A-I and A-II concentration are lower, and apolipoprotein B levels are higher in patients with coronary artery disease than in control subjects (Kris-Etherton et al., 1988).

Pathogenesis of Atherosclerosis

Atherosclerosis is an artery disorder, characterized by lipid accumulation in cells lining the inner walls of medium and large arteries (Woolf, 1983). Atheromatous plaques begin as soft, mushy lipid accumulations in blood vessels' intima. These plaques consist of lipids deponsited in blood vessel wall connective tissue. These lipids include free cholesterol, cholesterol esters, and triglycerides in proportions similar to circulating blood lipids (Robinson et al., 1986). Atherosclerosis begins in the first decade of life and is

almost universally present in people who live in developed countries. It develops gradually resulting in thickening of the arterial wall, loss of elasticity, and lumen narrowing. Finally, some event brings about vessel occlusion and affected tissue ischemia (Robinson et al., 1986).

Atherosclerosis is asymptomatic in its early stages; people generally do not know they have atherosclerosis until they suffer a heart attack or stroke. In some instances, atheroma ulcerates and hemorrhages into the lumen results in clot formation. The anatomic location of the atheroma, the extent of lumen narrowing, changes in the blood lipid clearance, and decreased fibrinolytic activity also are involved in clot formation (Robinson et al., 1986).

One major problem in the etiology of atherosclerosis is that many factors contribute to its development. Cigarette smoking (McGil, 1990), hypertension (Burke & Motulsky, 1992), hypertriglyceridemia (Doolittle et al., 1992), hypercholesterolemia (Kris-Etherton et al., 1988), abnormal lipoprotein profiles (Kris-Etherton et al., 1988), various genetic disorders and numerous other factors (Kaner & Hajjar, 1992) have been linked to the development of this disease. Recently, attention has been directed to arterial wall pathology and the key role of smooth muscle cell proliferation in lesion formation.

All arteries consist of three basic distinct layers-the intima, the media and the adventitia. The layer adjacent to the arterial lumen is the intima; it consists of a narrow region bounded on the luminal side by a single continuous layer of endothelial cells. The middle layer, or media, consists of diagonally oriented smooth muscle cells surrounded by collagen in variable amounts, small elastic fibers and mucopolysaccharides. Media morphology does not change with age. Lastly, the outermost layer or adventitia consists primarily of fibroblasts intermixed with collagen bundles and surrounded by mucopolysaccharides (Woolf, 1983).

Three different types of lesions are classically recognized - the fatty streak, the fibrous plaque and the complicated lesion (Woolf, 1983). The fatty streak, found

commonly in young persons, is characterized by a focal accumulation of a relatively small number of intimal smooth muscle cells surrounded by lipid deposits. Lipid deposits in the smooth muscle cells gives the fatty streak a yellow color. Most deposited lipid is free cholesterol and cholesterol esters. Presumably, plasma lipids are hydrolyzed and re-esterified once they have been taken up by smooth muscle cells (Scott & Hurley, 1970).

The second type of lesion, the fibrous plaque, is the most characteristic lesion of advanced atherosclerosis. Grossly white in appearance, it is elevated so that it protrudes into the arterial lumen. It is composed primarily of intimal lipid and is rich in smooth-muscle cells. Presumably, fatty streaks are precursors to fibrous plaque because they occur first (Woolf, 1983).

The third type of lesion is called the complicated lesion. It consists of fibrous plaque that has become altered due to hemorrhage, calcification, cell necrosis and thrombosis. One distinctive feature of this lesion is its calcification. This type of lesion is associated with occlusive disease (Woolf, 1983).

A key question which researchers have tried to answer is: "What causes lipid accumulation in arteries?" Among a number of possible causes, the most widely accepted is uptake of cholesterol and other fatty substances from the blood (Berliner & Gerrity, 1992). The endothelial barrier, which allows controlled passage of blood constituents into the artery wall, may be injured by hyperlipidemia, hormone dysfunction or hypertension. Once the endothelial barrier has been disrupted, substances normally screened out by the endothelium can reach smooth muscle cells in the media.

Lipoproteins, specifically LDL cholesterol, is believed to supply lipids to developing plaque (Kris-Etherton et al., 1988). LDL cholesterol is believed to accumulate in smooth muscle cell mucopolysaccharides (Harberland & Steinbrecher, 1992). Platelets adhere to newly exposed subendothelial collagen, aggregate and release their granules contents which stimulate cell proliferation (Territo et al., 1992). Subsequently, smooth muscle cells, normally present in the media, begin to proliferate

and migrate to the intimal layer where injury occurs. The proliferation of smooth muscle cells is accompanied by large quantities of connective tissue proteins and other macromolecules (Ross & Glomet, 1976). Smooth muscle cells in injured animal arteries continue to proliferate and accumulate both connective tissue and lipids if injuries are sustained. These lesions resemble advanced atherosclerosis in humans (Ross & Glomet, 1976).

Chromium

Chemistry

Chromium (Cr) is a shiny, hard, white metal in the first transition series, m.p. (melting point) 1903° C, atomic number 24 and atomic weight 51.996 g/mol (Borel and Anderson, 1984; Ducros, 1992). Four stable isotopes of chromium exist with mass numbers 50 (4.31%), 52 (83.76%), 53 (9.55%), and 54 (2.38%). Five radioisotopes can be produced, but only ^{51}Cr, with a half-life of 27.8 days, is commercially available and used for radiotracer studies. Other chromium radioisotopes have half-lives less than one day.

Chromium can occur in oxidation states from -2 to +6; the most common oxidation states are 0,+2, +3, and +6. Divalent chromium compounds are readily oxidized to the trivalent state with air exposure; thus, chromium^{+2} compounds are unlikely to occur in biological systems (Mertz, 1969). Trivalent chromium (+3) is the most stable oxidation state existing in biological systems. It has a strong tendency to form coordination compounds, complexes, and chelates (Ducros, 1992). Trivalent chromiums' coordination number is 6, and it can bind to ligands to form hexacoordinates or octahedrals. Ligand exchange rate of such compounds is very slow: ligand-displacement reactions have half-times in the range of several hours. Thus, chromium is unlikely to be involved as a metal catalyst at an enzyme active site where the exchange

rate would need to be rapid. However, chromium complexes have a slow ligand exchange rate and could function as cofactors to bind hormones to receptors or to stabilize proteins (Mertz, 1967).

In aqueous solution, chromium is coordinated to water and exists as an octahedral hexaquo ion $[Cr(H_2O)_6]^{3+}$. Free chromic ions do not exist in aqueous solution; they are always coordinated, either with water or with other ligands in solution. Below pH 4, chromium complexes are quite stable and may retain their visible spectrum over a long period of time. At neutral pH, as in biological tissues, hydrolysis of the coordinated water of hexaquo chromium occurs, leading to the formation of bridges between hydroxyl groups, a process called olation. This results in the formation of polynucleated chromium complexes that ultimately precipitate and are biologically inert. Olation is enhanced by alkali and temperatures greater than 120^{o}C. Strong ligands, such as oxalate ions, can prevent and even reverse olation, but weak ligands can only prevent the reaction (Mertz 1967,1969). In biological systems, chromium can function because it is held in solution by weak organic and inorganic ligands.

Function

Chromiums' primary role in a biologically active complex is to potentiate insulin action; thereby, playing an important role in regulating cellular glucose uptake and maintaning normal plasma lipid concentrations (Evans et al., 1973; Borel & Anderson, 1984; Yamamoto et al., 1989). Chromium's role in carbohydrate metabolism was first observed in rats when impaired glucose tolerance was restored by a postulated "glucose tolerance factor" (GTF) in brewer's yeast (Schwarz & Mertz, 1957). The "factor" subsequently was isolated from brewer's yeast and determined to contain trivalent chromium (Mertz, 1975).

In a study by Mertz (1969), a difference in epididymal adipose tissue glucose uptake was reporte between low-chromium rats and chromium-supplemented controls. When insulin was absence, glucose uptake was not significantly different between low-chromium rats and chromium-supplemented controls. However, when insulin was added, glucose uptake was much greater in chromium-supplemented rats. Thus, although chromium alone did not influence glucose uptake, chromium enhanced insulin effectiveness. These results suggest that higher doses of insulin are required by chromium-deficient systems to maintain the same insulin response as chromium-sufficient groups.

When insulin and chromium are in solution together, before being added to adipose tissue, glucose uptake is considerably less than when insulin is alone. This suggests that chromium reacts directly activates insulin (Mertz, 1967). In a study by Christian and coworkers (1963), insulin added to the mitochondria produced a shift in the mitochondrial sulfhydryl wave; this indicates an interaction between insulin and sulfhydryl groups. Such groups are part of the mitochondrial membrane. Chromium added to this system increased the magnitude of the shift to approximately three times the sum of the shifts caused by insulin or chromium alone. These findings suggest that chromium may facilitate the formation of a ternary complex between membrane sulfhydryls and the insulin intrachain disulfide.

Chromium's action with insulin also affects protein metabolism. Chromium-supplemented animals have enhanced insulin-mediated amino acid transport into tissues and greater glycine, serine and methionine incorporation rates into heart protein (Roginski & Mertz, 1969). Chromium also may affect certain enzyme systems. Glycerokinase and lipoprotein lipase were influenced by chromium status through chromium's effect on insulin action (O'Flaherty and McCarty, 1978; Mma and Serfass, 1981). Chromium's effect on lipid metabolism maybe via chromium's action on insulin and lipoprotein lipase. Chromium deficient rats were less able to re-esterify free fatty

acids and hence had greater free fatty acid release than chromium-supplemented rats. Mma and Serfass (1981) also reported lower lipoprotein lipase activity in chromium-deficient rats compared to chromium-sufficient rats.

Absorption, Distribution, and Excretion

Chromium absorption appears to take place in the jejunum with some absorption from the ileum and duodenum (Anderson, 1987). Intestinal uptake of hexavalent chromium is six times greater than trivalent chromium because hexavalent chromium has greater ability to cross membranes (Donaldson and Barreras, 1966). The stomach acid pH reduces conversion of hexavalent chromium to trivalent chromium (Donaldson and Barreras, 1966). Chromium absorption from nutritionally adequate diets fed ad libitum has been reported to be 0.4% (Anderson et al., 1983a), 0.5% (Anderson and Kozlovsky, 1985), 0.8% (Bunker et al., 1984), and 1.8% (Offenbacher et al., 1986). In one study, human absorption of orally administered ^{51}Cr 0.69%. Chromium absorption was not different among eldery maturity-onset diabetics; however, in another study chromium absorption among insulin-requiring diabetics was almost three-fold higher (Doisy, et al., 1976). Low chromium intake may increase chromium absorption. From a daily dose of 10 ug, almost 2% of the chromium was absorbed; at a daily dose of 40 ug, only 0.4% was absorbed (Anderson & Kozlovsky, 1985).

Chromium absorption also is influenced by chelating agents. Phytates decreased chromium absorption in rats, both in vivo and in vitro (Chen et al., 1973) whereas oxalate increased absorption. Other chelators, such as citrate and EDTA had no effect (Chen et al., 1973). Ligands that increase chromium absorption may function by preventing chromium olation and precipitation in the intestinal neutral milieu.

Chromium absorption and metabolism also are affected by interactions with other minerals, especially zinc and iron. Hahn and Evans (1975) reported that intestinal wall

and whole body chromium concentrations following an oral dose of radioactive chromium were greater in zinc-deficient than control rats. Both zinc and chromium are eluted in the same low-molecular weight fraction when mucosal supernatant extracts were separated by gel filtration; this suggests that similar ligands bind both minerals in the intestine. Chromium and iron also may share a common gastrointestinal transport mechanism, because iron-deficient animals absorb more chromium than iron-supplemented controls. Oral iron administration to chromium deficient animals inhibited chromium absorption (Hopkins and Schwarz, 1964).

Chromium absorption is increased by certain amino acids, such as histidine, which chelates chromium in the intestine. These amino acids prevent chromium precipitation in the intestines' basic pH, and thereby increase chromium absorption (Mertz and Roginski, 1971). Carbohydrate also influences chromium absorption; chromium absorption is facilitated by starch more than glucose, fructose, or sucrose (Seaborn and Stoecker, 1989).

After absorption, trivalent chromium binds to serum beta-globulin fractions, specifically to transferrin (Hopkins and Schwatz, 1964). Transferrin possesses two binding sites, A and B, with different affinities for iron. Chromium binds exclusively to site B. Iron readily binds to transferrin; thus, there is antagonism between Cr^{3+} and Fe^{3+} at high iron concentrations. Chromium can bind nonspecifically to other plasma proteins, such as gamma- and beta-globulin fractions and lipoproteins (Hopkins and Schwarz, 1964).

Blood chromium concentration is not a good indicator of chromium nutritional status, since blood chromium is not in equilibrium with body stores. Reported serum chromium concentrations have decreased in recent years because of improved analytical techniques and increased awareness of problems associated with chromium contamination. The lowest and probably most reliable reported fasting serum chromium

concentration for normal healthy subjects is about 0.10 to 0.14 ug/l, determined by atomic absorption spectrophotometry (Kayne et al., 1978; Chappuis et al., 1992).

Distribution of chromium^{+3} in the body has been studied in animals and humans by following injected ^{51}chromium disapearance from the blood and body tissues (Hopkins, 1966; Onkelinx, 1977, Jain et al., 1981, Lim et al., 1983). Lim et al. (1983) confirmed earlier animal studies' findings that chromium^{+3} is localized in human blood, liver, spleen, kidney, soft tissues, and bone. Lim et al. (1983) further reported that 50% of plasma chromium was absorbed by tissues and organs within hours after intravenous injection.

Investigators have hypothesized that biologically active chromium exists in a metabolic pool from which it is mobilized as needed (Lim et al.,1983; Wallach, 1985). Lim et al. (1983) described a functional model in which the plasma pool is in equilibrium with three compartments with fast ($t_{1/2}$ = 0.5 to 12 hours), medium (t 1/2 = 1 to 14 days and slow ($t_{1/2}$ = 3 to 12 months) exchange rates. Each functional compartment was found to be present to some extent in each organ measured (spleen, liver, kidney, muscle, adipose tissue, and bone). The fast exchange compartment was estimated to contain 0.13 ug chromium, the medium exchange compartment 0.8 ug, and the slow exchange compartment 25 ug chromium. Long term chromium storage occurred mostly in the liver with some storage in the spleen.

In humans, absorbed chromium is excreted primarily in the urine, with small amounts lost in hair, perspiration, and bile (Ducros, 1992). Eighty percent of injected chromium is excreted via urine. Since absorption of orally ingested chromium is less than 1%, the rest is excreted via feces. Urinary chromium excretion can be expressed in relationship to creatinine excretion. Daily urinary output of endogenous creatinine is correlated with muscle mass and varies little from day to day in healthy individuals. Gurson and Saner (1978) reported that chromium excretion, expressed as the ratio of ng chromium to mg creatinine (Cr/Creatinine), eliminated diurnal variation in chromium

excretion and corrected for urine volume variation. They observed no significant difference between 24-hour chromium excretion calculated from four-hour samples and that measured in a 24-hour sample.

Dietary Intake and Requirements

Chromium consumption in typical western diets ranges between 11 ug chromium/day to 200 ug chromium/day, with most intakes below 100 ug/day. Schroeder et al. (1962) reported 70 ug/day chromium intake in a typical institutional diet. In West Germany, reported dietary chromium intake averaged 62 ug/day, ranging from 11 to 195 ug/day. However, it is difficult to evaluate these data because suitable standard reference materials for chromium were not available at the time of these analyses (Kumpulainen et al., 1979). Recent chromium intake estimates in the United States are quite low, with a group mean of 33.3 ug, ranging from 22-48 ug/day (Anderson & Kozlovosky, 1985; Offenbacher, et al., 1986).

It has been suggested that chromium deficiency contributes to impaired glucose tolerance in elderly subjects. Two studies using elderly subjects reported mean chromium intakes of 25 and 37 ug chromium/day (Bunker et al., 1984; Offenbacher et al., 1985). If elderly subjects are chromium deficient, their chromium intakes may be lower than they need, or their need for chromium may be increased by changes in chromium metabolism such as increased urinary chromium excretion. However, age may not be a factor causing chromium deficiency. The Estimated Safe and Adequate Daily Intake (ESADDI) for chromium is 50-200 ug/day (Food and Nutrition Board of the National Research Council, 1989).

Toxicity

Trivalent chromium, the form of chromium found most commonly in foods, is poorly absorbed; therefore, exceedingly high oral intakes are necessary to attain toxic levels. Chromium compounds have a wide variety of industrial uses; thus, a potential occupational exposure through inhalation exists for many industrial workers. Toxicity to kidney, liver, nervous system, and blood are the major causes of death due to excessive chromium inhalation (Langard, 1980). In a study by Randall and Gibson (1987), workers chronically exposed to trivalent chromium showed elevated body chromium concentrations. Tannery workers had a mean serum chromium concentration of 9.4 nmol/L compared to 2.9 nmol/L in control subjects. In addition, urinary chromium was fourfold higher in tannery workers than in control subjects. Chronic occupational exposure to chromates has caused skin allergies and ulceration, perforation of nasal septum, and bronchial asthma (Langard, 1980). Toxic levels of orally administered trivalent chromium compounds have not been determined for humans.

Chromium and Glucose Metabolism

Glucose intolerance usually is one of the first measurable signs of both chromium deficiency and diabetes. Early experiments by Schwarz and Mertz (1957, 1959) revealed that chromium was an essential nutrient for preventing and reversing glucose intolerance. Supplementing rats fed low chromium diets with chromium led to rapid improvements in glucose tolerance. Further studies confirmed earlier reports that chromium was involved in glucose metabolism (Mertz et al., 1961; Schroeder, 1966).

Studies by Jeejeebhoy et al. (1977) and Brown et al. (1986) provided evidence that chromium plays a role in glucose metabolism in humans. In these studies, patients on total parental nutrition supplemented with chromium had improvements in several diabetic-like symptoms including glucose intolerance. Improvements in diabetic-like

symptoms with chromium supplementation also have been reported among protein calorie malnourished children (Gurson & Saner, 1971), diabetics (Glinsmann & Mertz, 1966; Mossop, 1983), elderly (Levine et al., 1968), individuals with marginally impaired glucose tolerance (Anderson et al., 1983b), and hypoglycemic patients.

The majority of chromium supplementation studies have focused on diabetic subjects due to chromiums' role in insulin resistance and impaired glucose tolerance (Anderson, 1981). Several investigators have reported that supplemental chromium, as inorganic chromium chloride ($CrCl_3$), improved glucose tolerance and decreased serum insulin concentrations among diabetics (Freiberg et al., 1975; Doisy et al., 1976; Anderson et al., 1991). Similarly, supplementing diabetic subjects with brewer's yeast reduced insulin requirements and improved glucose tolerance (Offenbacher & Pi-Sunyer, 1980; Saner et al., 1983). Other researchers, however, observed no effect on glucose tolerance among diabetic subjects supplemented with either inorganic chromium (Rabinowitz et al., 1983; Uusitupa et al., 1983; Abraham et al., 1992) or chromium-rich brewer's yeast (Rabinowitz et al., 1983).

Studies also have been conducted to evaluate chromium supplementation among non-diabetic individuals. Riales and Albrink (1981) reported that supplementation with 200 ug chromium as $CrCl_36H_2O_3$ daily for 12 weeks resulted in slightly improved glucose tolerance and a trend towards decreased serum insulin concentrations in supplemented subjects compared to controls. However, in other studies (Anderson et al., 1983b; Offenbacher et al., 1985) chromium supplementation did not affect blood glucose concentration in subjects with normal glucose tolerance. In contrast, plasma glucose concentrations increased with chromium supplementation in subjects who had marginally depressed glucose concentrations. In a follow-up study (Anderson et al., 1987), involving hypoglycemic subjects, a significant improvement in glucose concentrations was observed with chromium supplementation. These results suggest that hypoglycemics also may benefit from supplemental chromium (Anderson et al., 1987). Therefore,

supplemental chromium appears to not only decrease blood glucose concentrations in subjects with elevated serum glucose following a glucose load, but also to increase blood glucose concentrations in hypoglycemic subjects.

Chromium functions by increasing insulin activity and, thereby, reducing the amount of insulin required to regulate blood glucose and related processes (Anderson, 1992). As insulin efficiency declines, the body responds by producing more insulin to regulate blood glucose. In a human study by Morris et al. (1992), plasma chromium concentration was correlated negatively with increased plasma insulin concentration rather than with changes in plasma glucose concentration. This change in plasma chromium could be explained by chromium binding to insulin at insulin sensitive tissues, resulting in decreased plasma chromium concentrations.

Insulin-dependent diabetics absorb significantly more chromium than normal, elderly, or non-insulin-dependent diabetics (Anderson, 1992). However, once chromium is absorbed, it is rapidly excreted in the urine. Plasma chromium concentration among insulin dependent diabetes is lower (60% of normal) and urinary chromium excretion is higher (nearly three times normal) than in healthy subjects (Morris et al., 1988). Urinary chromium losses may be related to carbohydrate insulinogenic properties. Therefore, a chronically elevated insulin concentration could deplete the biologically-active chromium pool. In addition, insulin concentration must be kept low to prevent secondary signs of diabetes. Plaque formation is an insulin-sensitive process, and increased circulating insulin stimulates increased plaque formation leading to atherosclerosis (Stout, 1977).

It has been reported that diabetic mice lose their ability to convert inorganic chromium to a usable form (Tuman, et al., 1978). No effect was observed among diabetic mice supplemented with inorganic chromium, whereas mice supplemented with chromium in a biologically active form had reduced plasma glucose concentrations. Chromium supplementation studies are conflicting regarding chromium's role in glucose metabolism. It is unclear whether much of chromium's influence on insulin and glucose

regulation is due to chromium itself, or due to chromium complexes such as glucose tolerance factor.

Chromium and Lipid Metabolism

Chromium deficiency has been related to atherosclerosis, perhaps through an effect on lipid metabolism (Tipton et al., 1965; Simonoff, 1984). Rats fed low chromium diets had increased serum cholesterol, aortic lipids, and plaque formation (Schroeder & Balassa, 1965). Rabbits fed a cholesterol-enriched diet with added chromium had reduced aortic plaque and aortic cholesterol concentration (Abraham et al. 1991). Chromium in conjunction with insulin, increases glucose uptake and incorporation of glucose carbons into epidymal fat (Mertz et al., 1961). Glucose uptake by adipocytes from chromium deficient rats did not differ from controls in the absence of insulin. However, addition of chromium in vitro and in vivo to animals increases tissue response to exogenous insulin. Using labelled acetate, Cupo and Donaldson (1987) reported that chromium enhanced incorporation of acetate into hepatic fatty acids in supplemented chicks compared to controls. In obese mice, hepatic lipid concentrations decreased in chromium supplemented animals (Li & Stoecker, 1986). Hepatic lipids were positively correlated with circulating insulin concentrations in these mice. In a recent study, Evans and Bowman (1992) investigated the effects of chromium supplementation on insulin-receptor internalization and glucose uptake in rat skeletal muscle cells. The quantity of insulin initially bound to receptors and insulin internalization rate were markedly elevated in cells cultured with chromium as chromium picolinate. In addition, glucose uptake was increased in cells cultured in a medium containing chromium as chromium picolinate. However, Further research is needed to explain lipid response among chromium deficient animals.

In a human study, Schroeder and co-workers (1962) observed that aortas from persons who died of heart disease had lower chromium concentrations than aortas from healthy accident victims. Also, the authors reported marked differences in tissue chromium distribution, suggesting a negative correlation between chromium status and cardiovascular disease risk. Moreover, the relationship between serum chromium concentration and coronary artery disease was investigated in two independent studies (Newman et al., 1978; Simonoff et al., 1984) using a total of 122 patients, of whom 82 were diagnosed as having coronary artery disease. Although reported serum chromium concentrations were high by present standards, they differed significantly between subjects with coronary involvement and normal subjects. Mean serum chromium concentrations were 41% (Newman et al., 1978) and 12% (Simonoff et al., 1984) lower among patients with coronary disease than healthy controls. In a recent study, Press and associates (1990) investigated chromium supplementation as chromium tripicolinate on serum cholesterol, LDL-cholesterol, apolipoprotein B, and apolipoprotein A-I concentrations in 28 healthy adults. Subjects were supplemented with 200 ug of chromium as chromium picolinate for 42 days. Total cholesterol, LDL-cholesterol, and apolipoprotein B concentrations decreased significantly with chromium supplementation. Apolipoprotein A-I concentrations also increased with chromium supplementation, and HDL-cholesterol concentrations were elevated slightly but not significantly.

In another study involving non-insulin-dependent diabetics, 250 ug of supplemented chromium as $CrCl_3$ for 7 to 16 months resulted in a significant increase in HDL-cholesterol, as well as a decrease in serum triglyceride concentration. However, no change in serum cholesterol or serum glucose concentrations were observed (Abraham et al., 1992). Uusitupa and associates (1983) reported no significant improvement in glucose tolerance or serum lipid concentrations in non-insulin-dependent diabetics after supplementation with 200 ug trivalent chromium as chromium chloride for six weeks. However, they did report a significant decrease in serum insulin concentration compared

to placebo-treated diabetics, indicating that less insulin was required with chromium supplementation. The effects of chromium on serum lipids are difficult to predict. Subjects with elevated lipids usually improve the most. Elevated serum lipids are due to many factors other than dietary chromium, therefore, improved chromium nutrition may only lead to improvements in individuals whose elevated serum lipids are due to marginal dietary chromium.

Copper

Chemistry

Copper is a tough, reddish metal of the first transition series, m.p. 1083°C, atomic number 29, and atomic weight 63.54 g/mol (Cotton & Wilkinson, 1988). It has two naturally occurring isotopes: ^{63}Cu (69.1%) and ^{65}Cu (30.9%). Two radioisotopes of copper, ^{64}Cu with a half-life of 12.7 hours, and ^{67}Cu with 61.9 hours, are commercially available and used for radioisotope studies (Cotton & Wilkinson, 1988). Copper ions exist in two oxidation states; Cu(I), which is cuprous, and Cu(II), which is cupric. Most Cu(I) compounds are fairly readily oxidized to Cu(II) compounds, but further oxidation to Cu(III) is more difficult. The cupric ion is a potent oxidizing agent. It becomes dull when exposed to air. In moist air it gradually becomes coated with green basic carbonate. The relative stabilities of Cu(I) and Cu(II) in aqueous solutions depend very strongly on the nature of anions or other ligands present. Cu(II) is isomorphous with Zn^{+2}, Mg^{+2}, and Fe^{+2}. Copper ions complex with greater or lesser affinity to many chelation agents known to bind zinc (Solomons, 1988).

Function

Copper primarily functions as a component of copper metalloenzymes. Common cuproenzymes include ceruloplasmin, tyrosinase, cytochrome c oxidase, monoamine

oxidase, dopamine-beta-hydroxylase, and Zn-Cu-superoxide dismutase. All of these cuproenzymes are involved in reactions which molecular oxygen or related radicals (Solomons, 1988).

Most severe copper deficiency symptoms can be explained by deficiency of one or more of cuproenzymes. Tyrosinase is important in the formation of melanin and dipigmentation is easily explained by tyrosinase deficiency. Cytochrome c oxidase is the terminal oxidase in the mitochondrial electron transport system, catalyzing electron transfer from cytochrome c to oxygen. Reduced cytochrome c oxidase activity is explained by copper deficiency. Copper deficiency usually has a greater effect on liver cytochrome c oxidase concentrations than brain (Prasad, 1978).

Amine oxidase catalyzes amine oxidative deamination. An amine oxidase of specific importance is lysyl oxidase, a key enzyme in collagen and elastin cross-linking reactions in supportive connective tissues (Solomons, 1988). Collagen and elastin constitute 30% of total body protein, and their maturation is dependent upon the copper dependent enzyme lysyl oxidase (O'Dell, 1990). Copper deficiency results in low lysyl oxidase activity and consequently decreased elastin and collagen cross linking. Osteoporosis and bone abnormalities have been reported in copper deficient dogs, rabbits, pigs, and children. Collagen in copper deficient bone is more soluble than control bone, indicating impaired crosslinking (Davis & Mertz, 1986). Lung tissue contains both collagen and elastin, and impaired crosslinking in copper-deficient animals causes emphysema (Soskel et al., 1982).

Superoxide dismutase along with glutathione peroxidase constitute the primary catalytic defense against metabolically generated free radicles (Taylor et al., 1988). Copper also plays a key role in iron metabolism and thereby in hemoglobin biosynthesis. Serum iron concentration tends to be lower with copper deficiency, whereas liver and intestinal mucosa iron concentrations are elevated, suggesting a decrease in iron mobilization (O'Dell, 1990). It appears that ceruloplasmin plays an important role in iron

transfer from reticuloendothelial cells to plasma. Ferroxidation plays a role in supporting erythrocytes. Ceruloplasmin is capable of catalyzing the oxidation of ferrous ions to ferric ions (Osaki, 1966). Ceruloplasmin oxidase activity is greater for iron than any other substrate, and it has been proposed that ceruloplasmin be renamed "ferroxidase" (Osaki, 1966). According to this hypothesis, iron is presented on the cell surface as ferrous iron and must be oxidized to the ferric form in order to be bound by transferrin.

In addition, serum lipoproteins and arterial tissue cholesterol are influenced by copper, adding to the importance of copper nutriture in the development of atherosclerotic coronary heart disease (Carr & Lei, 1990; Koo et al., 1990).

Absorption, Distribution and Excretion

Copper is absorbed throughout the gastrointestinal tract including the stomach and large intestine. Although the site of maximal absorption may vary slightly, the duodenum appears to be the major site for copper absorption among all species. Up to 32% of orally administered copper is absorbed in normal human subjects (O'Dell, 1990). The mechanism for copper absorption is not clear, but a molecular pathway for copper absorption in mammals has been proposed by Evans (1973). Copper binds to ligands in the intestinal lumen before it enters the intestinal epithelial cells. Copper absorption and retention depend on the chemical form in which the mineral is ingested. In addition, the dietary intake of several other minerals and organic substances and pH of the intestinal contents in the absorptive area may affect copper absorption. High dietary calcium carbonate and ferrous sulfide intakes depress copper absorption. The former presumably reduces copper absorption by raising intestinal pH; the latter, by forming insoluble copper sulfide. Zinc, molybdenum, and cadmium also adversely affect copper availability (Johnson, 1989; Kies & Harms, 1989). These observations suggest a competition among various cations for similar binding sites at the intestinal level. The antagonistic effect of

zinc on copper metabolism appears to be mediated primarily at the intestinal mucosal level via metallothionein (Cousins, 1985). Suzuki et al. (1989) suggested that zinc induces metallothionein synthesis. Because metallothionein binds copper to a greater extent than zinc, copper is bound in a nonabsorbable form.

Copper absorption is also depressed by excessive phytate and fiber (Kim & Vanderstoep, 1989). Ascorbic acid was observed to increase the severity of copper deficiency in chicks (Hunt et al., 1970) and rats (Campen & Gross, 1968). Hunt et al. (1970) reported a significant reduction in hepatic copper from chicks fed a control diet supplemented with ascorbic acid, suggesting that ascorbic acid affects either copper absorption, copper utilization or both. Campen and Gross (1968) reported that ascorbic acid significantly decreased ^{64}Cu absorption in rats when ascorbic acid was put into a ligated intestinal segment along with radioactive copper. Ascorbic acid had little or no effect on intraperitoneally administered ^{64}Cu excretion, indicating that ascorbic acid affected copper metabolism by depressing intestinal copper absorption.

Absorbed copper first enters the intestinal mucosa and then the blood. Evans and LeBlanc (1976) reported that copper's passage through intestinal cells to the blood is apparently regulated by a low-molecular-weight copper-binding protein, the synthesis of which is induced by copper. After passing through the intestinal cells' epithelium, copper is transported through the portal blood as a histidine-copper-albumin complex to the liver (Lau & Sarker, 1971). From the liver, some copper enters the bile, some is used for production of internally required liver proteins, and much of it is incorporated into ceruloplasmin and erythrocuprien. Ceruloplasmin is probably the main source of nonhepatic tissue copper, delivering copper via specific receptors (Linder, 1991). Ceruloplasmin is an alpha-globulin with a molecular weight of 151,000 containing eight atoms of copper per molecule. Ceruloplasmin is released into the blood and constitutes 90% of the plasma copper pool, 15.7 umol/L. Copper is tightly bound to ceruloplasmin, whereas it is loosely bound to albumin and some free amino acids such as histidine

(O'Dell, 1990). Ceruloplasmin donates copper to nonhepatic tissues for synthesis of cuproenzymes such as cytochrome c oxidase, superoxide dismutase, and lysyl oxidase. In human subjects, plasma copper concentration did not increase following meals, nor did it decrease during fasting (Prasad, 1978). Plasma copper concentration has been reported to increase from 0.5 to 1.5 ug/ml during pregnancy (Smith et al., 1991), and with oral contraceptive and estradiol use (Prasad, 1978). In addition, reported plasma copper concentrations are higher in women than men (Solomons, 1988).

Copper is distributed throughout the body and, although the there is variation among species in tissue and organ copper concentration, certain organs have consistently higher copper concentrations than others. The liver, brain, heart, and kidneys, in decreasing order, contain the highest concentrations. Intermediate copper concentrations are reported in lung, intestine, and spleen, while endocrine glands, muscle, and bone have the lowest reported copper concentrations. Because of the large mass of muscle and bone, these tissues contain approximately 50% of total body copper; hepatic copper accounts for about 10% of total body copper (Evans, 1973). The adult human body contains approximately 80 mg copper (Cartwright and Wintrobe, 1964). Cartwright and Wintrobe (1964) reported a total of 23 mg copper in the liver, heart, spleen, kidneys, brain, and blood of normal subjects. Of this total, 8 mg was present in the liver and, surprisingly, 8 mg in the brain.

Copper is excreted primarily via the gastrointestinal tract with less than 3% of absorbed copper appearing in the urine (Mason, 1979). Because most circulating blood copper is bound to ceruloplasmin or confined within the erythrocytes, very little copper permeates the glomerular capillaries and urinary copper excretion is negligible under normal conditions (Evans, 1973).

Bile is the major pathway for copper excretion from the mammalian body. The body contains low-molecular-weight copper-binding components as well as macromolecular binding species. The low-molecular-weight components are more

prominent in hepatic bile and the high-molecular weight fraction predominates in bile. Examination of copper enterohepatic circulation demonstrates that biliary copper reabsorption is negligible, but dependent on the degree of protein binding (Mistilis and Farrer, 1968). In this rat study, copper from bile collected during early secretion intervals was absorbed to a much greater extent than that equal doses collected during later intervals. These results suggest that during the initial phase of biliary copper excretion, copper enters the bile in the low-molecular-weight complex. With increasing time, the proportion of copper complexed with macromolecules increases due to both increased association of ionic copper with biliary proteins and increased secretion of copper binding proteins and enzymes into the bile (Farrer & Mistilis, 1967).

Dietary Intake and Requirements

There is no Recommended Dietary Allowance for copper, but a Estimated Safe and Adequate Daily Dietary Intake was established in 1989 (Food and Nutrition Board, 1989). The recommended intake range, 1.5 to 3 mg/day for adults, was based on the amount estimated to maintain copper balance with an added margin of safety (Food and Nutrition Board, 1989). Copper requirements of young men were estimated to be 1.3 mg/day, plus an added increment to replace surface copper losses (Klevay et al., 1980). Typical American diets often contain less than 2 mg/day (Turnlund, 1988). In a 1986 report, the average adult copper intake was estimated to be 0.93 mg/day for women and 1.24 mg/day for men (Rennington, et al., 1986). This report agrees with another study (Holden et al., 1979) in which adult copper intake was estimated to be approximately one mg/day. One study reported that dietary copper intake in institutionalized elderly individuals was 1.0-1.5 ug/day (Dreosti et al., 1984). However, another study reported an average copper intake of 1.74 mg/day for elderly females and 1.9 mg/day for elderly males (Horwath, 1989). Low dietary copper intake was negatively correlated with

plasma triglyceride concentration among elderly people (Hermann et al., 1993). In view of the data from copper absorption, balance, and status, the dietary intake recommendations for copper needs further evaluation.

Toxicity

Copper toxicity can occur in all animal species. In humans, acute copper toxicity is usually associated with accidental consumption of copper sulfate, usually by children (Davis & Mertz, 1986). A lethal dose of copper compounds in humans is thought to be 3.5 to 35 g (Solomons, 1988). Vomiting and diarrhea can result from acute oral consumption of 10 to 15 mg/day. Much larger amounts can produce intravascular hemolysis and be fatal (Solomons, 1988). Chronic copper poisoning can occur when the livers' mechanism for sequesting copper is exceeded (Davis & Mertz, 1986). Amounts of intravenous copper in excess of 0.5 mg/day can be retained. This amount could be deposited in liver tissue causing hepatic overload and scarring (Solomons, 1988).

Copper and Glucose Metabolism

Copper participates in glucose homeostasis both in vivo and in vitro by stimulating insulin binding, hexose transport and lipogenesis (Fields et al., 1983; Koh, 1990). Impaired glucose tolerance has been reported in copper deficient animals and humans compared to copper adequate groups (Hassel et al., 1982; Klevay et al., 1983). Fields et al.(1983) evaluated the effect of copper 2-deoxyglucose transport and insulin binding in adipocytes isolated from rat epididymal fat pads. Maximum 2-deoxy [^{14}C]glucose transport was reported at higher copper concentrations (0.45 mM $CuCl_2H_2O$), compared to controls. In addition, copper increased insulin binding to fat cells and also increased the number of insulin receptor sites. If copper stimulates insulin

binding, hexose transport, and lipogenesis in vitro, it would be expected that glucose homeostasis would be impaired with copper deficiency.

Impaired glucose tolerance in copper deficient animals and humans has been reported compared to copper adequate groups (Hassel et al., 1982; Klevay et al., 1983). Klevay and coworkers (1984) fed two healthy men a diet low in copper (0.5 mg/day) for 30 days, a diet of zero copper for 150 or 120 days, and then a diet supplemented with 3.5 mg of copper/day for 30 days. Average glucose concentrations increased by 38 mg/dl during depletion and then decreased by 24 mg/dl during the repletion period. Glucose clearance increased during the repletion period, although insulin concentration was lower. These data may provide important insights into coppers' role in the etiology of diabetes mellitus.

Copper and Lipid Metabolism

An increase in plasma cholesterol concentration is a principal risk factor in the development of coronary heart disease (Ranade, 1993). Recent reports have suggested copper may have a role in cholesterol metabolism. Hypercholesterolemia and changes in lipoprotein profiles have been reported in copper deficient rats (Yount et al., 1990; Al-Othman et al., 1992) and humans (Klevay et al., 1984; He et al., 1992). However, the mechanism by which copper deficiency induces increased plasma cholesterol concentrations and abnormal plasma lipoprotein profiles is still unknown.

Lei (1977,1978) suggested that hypercholesterolemia occurring with copper deficiency may be due to a shift in cholesterol from the liver pool to the plasma pool. Allen and Klevay (1978) also suggested that hypercholesterolemia reported among copper deficient rats was due to an increased rate of cholesterol clearance from the liver pool to the plasma pool, with this cholesterol being unavailable for excretion as biliary steroids. Subsequently, Shao and Lei (1980) demonstrated that cholesterol esters, newly

synthesized from [2-^{14}C]mevalonate, cleared the liver faster in copper-deficient rats compared to controls. Lei and Lin (1981) also reported marked increases in the size and half-life of the rapidly exchangeable cholesterol pool in copper-deficient rats. They reported that cholesterol in serum and tissues, such as red blood cells and liver cells, equilibrated rapidly. In addition, copper deficiency decreased cholesterol transport rate from the rapidly exchangeable pool to the slowly exchangeable pool, such as skeletal muscle that equilibrates slowly with serum cholesterol. Furthermore, a prolonged half-life of free cholesterol and HDL cholesterol was reported in copper-deficient rats (Lei, 1978).

In an in vivo study hepatic cholesterol synthesis increased two-fold with copper deficiency due to an increase in hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase activity (Yount et al., 1991). However, in an in vitro study, hepatic cholesterogenesis from acetate 1-14C in liver slices decreased in copper deficient rats (Lei, 1977). Lipoprotein lipase (LPL) is responsible for the uptake of cholesterol and triglycerides from lipoproteins to tissue. Lau and Klevay (1982) reported a 40-47% reduction in lipoprotein lipase activity in copper-deficient rats. Copper may be required for the formation of a lipoprotein lipase activator complex. Decreased lipoprotein lipase activity may be responsible for copper deficiency induced hypertriglyceridemia. Thus, decreased cholesterol removal from the circulation, and resulting hypercholesterolemia and hypertriglyceridemia, may partially be due to impaired tissue cholesterol uptake.

Copper deficiency induced hypercholesterolemia in both animals and humans is characterized by an increase in plasma HDL cholesterol concentration (Al-Othman et al., 1990; Hing & Lei, 1991; He et al., 1992) with no change (He et al., 1992), or an elevation in VLDL and LDL cholesterol concentration (Hing & Lei, 1991; Medeiros et al., 1991). In a population-based study of 399 men in South China, serum copper was inversely related to HDL-cholesterol; however, no significant association between serum copper and LDL-cholesterol was reported (He et al., 1992). Al-Othman et al. (1990) reported a marked increase in HDL and LDL protein and cholesterol content, and

triglyceride concentrations in copper-deficient rats. However, in a human study with adult males, three mg copper supplementation daily for six weeks resulted in increased LDL-cholesterol, and decreased VLDL-cholesterol and total serum cholesterol (Medeiros et al., 1991).

Apo B is an obligatory component of lipoproteins synthesized by the liver and functions as a recognition marker for LDL uptake by Apo B,-E receptors (Gibbons et al., 1990). Hing and Lei (1991) reported a significant increase in Apo B concentration in rabbits fed copper-deficient diets for ten weeks. Nassir et al. (1993) reported increased Apo B-100 liver synthesis in copper-deficient rats. An increase in Apo B synthesis is expected to accompany elevated cholesterol synthesis in order to enhance cholesterol secretion. However, at present there is no direct evidence available to support the proposed increase in apolipoprotein synthesis by copper deficiency. HDL is believed to play a key role in removing peripheral cholesterol. HDL apolipoprotein make-up is important in regluating plasma cholesterol clearance. Thus, compositional changes in plasma total, HDL and LDL subfractions have been examined in copper-deficient rats (Lee & Koo, 1988; Lefevre et al., 1986). Lee & Koo (1988) examined the effects of copper deficiency on compositional alterations in three subclasses of plasma HDL. They separated rat plasma HDL by heparin-affinity chromatography into three compositionally distinct subclasses: (1) HDL containing no apo E but high in Apo A-I; (2) HDL with a moderate level of Apo E and A-I; and (3) HDL highly enriched with Apo E but low in Apo A-I. Copper deficiency did not alter the percent distribution of Apo A-I in HDL containing no Apo E, but lowered Apo A-I content in HDL with an intermediated Apo E level and HDL highly enriched in Apo E but low in Apo A-I, with an increase in Apo E in these subclasses. However, total plasma Apo A-I concentration was significantly elevated in copper-deficient rats, which was attributable to an increase in the total number of circulating HDL particles. These results indicate distinct changes in HDL subclasses' lipid and apolipoprotein composition in copper deficient rats, and suggest

possible impairment in plasma clearance of HDL subclass devoid of Apo E or in its metabolic conversion to the Apo E-containing subclasses. However, data from other studies are conflicting with regard to apolipoprotein distribution, particularly, Apo A-I and Apo E in these subfractions (Lefevre et al., 1986; Croswell and Lei, 1985).

CHAPTER III

MATERIALS AND METHODS

Research Design

This study followed a randomized block experimental design consisting of three treatment groups, and four data collections. The three treatment groups included eight weeks of supplementation with either a lactose placebo, 241.2 ug chromium as chromium chloride in lactose, or 3.2 mg copper as copper carbonate in lactose. The four data collections included baseline (week zero), after four weeks supplementation, after eight weeks supplementation and four weeks after supplementation ended.

Subjects

This study was approved by the Oklahoma State University Institutional Review Board for human subject research (Appendix A). Subjects who volunteered to participate signed an approved informed consent (Appendix B). Twenty-nine subjects over the age of 50 who were free of chronic disease, not taking medications to lower blood cholesterol, and who were maintaining a constant body weight volunteered for this study. Subjects were solicited by announcements through local physicians, college and university mailings, newspaper advertisements and personal contacts with senior citizen organizations (Appendix C). Subjects were interviewed individually prior to the onset of the study to complete a medical history form (Appendix D) and were trained individually by a registered/licensed dietitian to keep three-day dietary records (Appendix E).

Treatments

Subjects were assigned randomly to one of three treatments, lactose placebo, chromium supplement, or copper supplement. There were ten volunteers per experimental group, however five subjects dropped out before the study was completed. Eight, subjects remained in the placebo, chromium, and copper supplement groups, respectively. Twice each day during the eight weeks of supplementation, subjects swallowed either a gelatin capsule containing lactose, chromium as chromium chloride in lactose, or copper as copper carbonate in lactose. Subjects were asked to take one capsule each morning and one each evening with meals, and to return any omitted capsules at the next measurement period. The intended level of copper and chromium supplementation were 3 mg and 200 ug each day, respectively. These supplementation levels were equal to the upper safe and adequate intake range for each mineral (Food and Nutrition Board of the National Research Council, 1989).

Preparation of Supplements

Number two sized gelatin capsules (Apothecary Products, Burnsville, MN) were used in this study. The placebo capsule contained 0.25 g lactose. The initial calculations for the compiled chromium supplement consisted of 0.861 g chromium chloride in 420 g lactose, so that 0.25 of the chromium chloride-lactose mixture would contain 100 ug chromium. However, the initial analyzed chromium content was only 70.2 ug chromium. Therefore, the chromium supplement was re-mixed. The second compiled chromium supplement was prepared by adding 1.2587 g $CrCl_3 6H_2O$ to 426 g lactose. The lactose and chromium chloride were mixed together for seven hours in a ball mill to ensure even chromium chloride distribution in the lactose. The capsules were filled using a gelatin capsule filling machine (Quanterron Inc., Burnsville, MN.) so that each capsule contained about 0.25 g of the chromium chloride-lactose mixture.

In order to evaluate the supplements' consistency, four capsules were selected at random for chromium content analysis. Samples and capsules were wet and dry ashed using a modification of the Hill et al. (1986) method, and analyzed for chromium concentration using an atomic absorption spectrophotometer (5100 PC, Perkin-Elmer Corp., Norwalk, CO). The analyzed chromium content of the second chromium chloride-lactose mixture averaged 120.6 ug/capsule. Thus, daily supplementation was 241.2 ug chromium.

The copper supplement was prepared in the same manner as the chromium supplement. The copper capsule contained 0.358 g of copper carbonate-lactose mixture. The copper capsule contained a higher amount of mixture because initially supplements were filled by hand, and supplement mixtures were calculated based on individual techniques. Later a capsule filling machine was purchased and capsules were filled to the initial hand tapped amounts. The copper supplement consisted of 7.2551 g $Cu_2(CO_3)_3$ in 701.5 g lactose, so that 0.358 g of the copper carbonate-lactose mixture would contain 1.5 mg copper as copper carbonate. The lactose and copper carbonate were mixed together for seven hours in a ball mill. The average analyzed copper concentration of four randomly selected capsules was 1.6 mg copper per capsule. Thus, daily supplementation was 3.2 mg copper.

Blood Collection

Twelve 12-hour fasting blood collections were performed at all four data collections; baseline, after four and eight weeks supplementation and four weeks after supplementation ended. Subjects' fasting blood collections were performed between 0730 and 0930 for consistency since blood concentrations can vary with time of day.

Thirty milliliters of blood were collected by a phlebotomist using butterfly needles and trace mineral-free syringes. Twenty milliliters of blood were collected for

plasma analysis; half was placed in each of two 10-milliliter neutral tubes which had been treated with 100 ul of trisodium citrate solution (300 g trisodium citrate/l) as an anticoagulant. In addition, 10-milliliters of blood for serum analysis were collected in a 10-milliliter tube for serum. Plasma tubes were placed on a rotator and the serum tube was placed in an ice chest immediately after blood collection.

Plasma was separated by a TJ-6R Tabletop centrifuge (Beckman Instruments, Inc., Palo, CA) at 1520 g at 4°C for 25 minutes within 30 minutes of blood collection. The plasma was pipetted into separate plastic storage tubes for individual assays. Total cholesterol, HDL-cholesterol, and glucose were analyzed the day blood was collected. Plasma for triglycerides, apolipoprotein A-I, and apolipoprotein B assays was frozen in separate mineral-free Falcon^{R} tubes at -20°C until analyzed.

The serum tube was allowed to stand on ice for two hours to allow clot formation and centrifuged at 1520 g for five minutes at 4°C. The clear top layer of the serum was removed carefully with a transfer pipette into separate mineral-free Falcon^{R} tubes for insulin assay. Serum was frozen in these mineral-free Falcon^{R} tubes at -20°C until analyzed.

Anthropometric Measures

At each of the four data collections, subjects participated in routine anthropometric measurements. At the first data collection (week zero) subjects' height was measured without shoes. Each subject stood erect with heels together against a wall. Height was read from a scaled rule attached to the wall. Subjects' weight was measured to the nearest pound at all four data collections using a calibrated body weight healthometer (Continental Corp., Chicago, IL). Body Mass Index (BMI) was calculated based on the equation BMI = body weight (kg)/height (m^2) (Cronk & Roche, 1982).

Dietary Intake Records

Prior to beginning the study subjects were trained by a registered dietitian to keep dietary records. Subjects were provided with non-biasing bean bag models for one cup, 1/2 cup, and 1/4 cup. Subjects kept dietary records for three week days prior to each of the four data collections. The Food Processor Plus Nutrition & Fitness program (ESHA Research, Salem, OR) was used to analyze three-day dietary records nutrient content. However, this program does not include chromium content of foods.

Health Background

At each data collection, subjects completed a monthly health questionnaire (Appendix F). The health questionnaire contained questions pertaining to any changes in the participants' health and exercise pattern over the last four weeks.

Biochemical Analysis

Total Cholesterol

Plasma total cholesterol concentrations were determined (Allain et al., 1974) using an enzyme kit (Sigma Chemical Company, St. Louis, MO). To 10 microliters of plasma, one milliliter of cholesterol reagent was added. This mixture was vortexed and incubated for five minutes at 37^{o}C. Total cholesterol concentration was determined by measuring absorbance at 500 nm using the Gilford Response spectrophotometer (Ciba Corning Diagnostic Corp., Chicago). The standard used for the assay was 203 mg/dl. Each plasma sample was measured in duplicate. If the difference between duplicates exceeded 5%, the assay was repeated.

Lipoprotein Cholesterol

Plasma HDL-cholesterol concentrations were determined (Allain et al., 1974) using an enzyme kit (Sigma Chemical Company, St. Louis, MO). This assay involves precipitation of the plasma sample and using the supernatant fluid for HDL-cholesterol analysis.

Twenty microliters of HDL-cholesterol precipitating reagent were added to 200 microliters of plasma, and the mixture was vortexed. The mixture was allowed to stand for five minutes at room temperature and then centrifuged for 10 minutes at 1520 g. To 50 microliters of this supernatant fluid, one microliter of cholesterol reagent was added. The mixture again was vortexed and incubated for 10 minutes at 37°C. HDL-cholesterol concentration was determined by measuring absorbance at 500 nm using the Gilford Response spectrophotometer. The standard used was 50 mg/dl. Each plasma sample was measured in duplicate. If the difference between duplicates exceeded 5% the assay was repeated.

LDL-cholesterol was calculated using the equation LDL-cholesterol = total cholesterol - [(HDL-cholesterol + (Triglyceride/5)] (Friedewald, 1972).

Triglycerides

Plasma triglyceride concentrations were determined colorimetrically (Walker et al., 1982) using an enzyme kit (Sigma Chemical Company, St. Louis, MO). Thawed plasma samples were used to determine triglyceride concentration. One milliliter of triglyceride reagent was added to 10 microliters of plasma and vortexed. The mixture was incubated at 37°C for five minutes. Triglyceride concentration was determined by measuring absorption at 540 nm using the Gilford Response spectrophotometer. The standard used was 250 mg/dl. For each plasma sample triglycerides were measured in

duplicate for each sample. The analysis was repeated if the difference between duplicates exceeded 5%.

Apolipoprotein A-I

Plasma apolipoprotein A-I concentration was measured immunoturbidimetrically (Rofal and King, 1986) using a kit (Sigma Chemical Company, St. Louis, MO). Plasma apolipoprotein A-I combines with a specific antibody present in the reagent and forms an insoluble complex resulting in assay mixture turbidity. The degree of turbidity formed is proportional to plasma apolipoprotein A-I concentration.

Five microliters of plasma were added to one ml of working antibody reagent and incubated at room temperature for 15 minutes. The degree of turbidity was measured at 340 nm using the Gilford Response spectrophotometer. A blank absorbance value was subtracted from each respective test value. Plasma apolipoprotein A-I concentrations were determined from a calibration curve obtained using 102 mg/dl and 155 mg/dl apolipoprotein A-I calibrators. Each plasma sample was analyzed in duplicate. The analysis was repeated if the difference between duplicates exceeded 5%.

Apolipoprotein B

Plasma apolipoprotein B concentration also was measured immunoturbidimetrically (Rofal & King, 1986) using a kit (Sigma Chemical Company, St. Louis, MO). The turbidity formed is proportional to plasma apolipoprotein B concentration. Ten microliters of plasma were added to one ml of working antibody reagent and incubated at room temperature for five minutes. The degree of turbidity formed was measured at 340 nm using the Gilford spectrophotometer. Blank absorbance was subtracted from each respective test value. Plasma apolipoprotein B concentration was determined from a calibration curve obtained using 53 mg/dl and 96

mg/dl apolipoprotein B calibrators. Each plasma sample was analyzed in duplicate. The analysis was repeated if the difference between duplicates exceeded 5%.

Glucose

Plasma glucose concentration was determined microenzymatrically (Washko & Rice, 1961) using an enzyme kit (Sigma Chemical Company, St. Louis, MO). To 1.8 ml of water, 0.2 ml of plasma was added and vortexed. Five milliliters of combined enzyme-color reagent solution was added to each mixture, vortexed, and incubated at room temperature for 45 minutes. Glucose concentrations were determined by measuring absorbance at 450 nm using the Gilford spectrophotometer. A standard of 100 mg/dl was used. Each plasma sample was measured in duplicate. If duplicates exceeded 5% the assay was repeated.

Insulin

Serum insulin concentrations were measured with a double radioimmunoassay antibody technique (Hales & Randle, 1963) using a kit (Equate RIA, South Portland, ME). Insulin determination is based on the ability of a limited quantity of antibody to bind a fixed amount of radiolabeled antigen. Frozen serum samples were thawed and kept on ice for analysis. Thawed samples were vortexed for five seconds prior to sample analysis. All tests were run in duplicate except non-specific binding tubes (NSB) which were run in quadruplicate. If duplicates were not within 5% of each other the sample was reanalyzed. The Equate option one procedure was followed. Two hundred microliters of assay buffer were added to NSB tubes. One hundred microliters of each calibrator (A-F) were added to calibrator tubes and 100 ml of insulin tracer were added to all tubes. One hundred microliters of antiserum were added to each tube except for total count (TC) and NSB tubes. All tubes were vortexed, covered with parafilm, and

incubated at room temperature for 10 minutes. All tubes were centrifuged at 2°C to 8°C at 1500 g for 10 minutes. The supernatant fluid from each tube was decanted using a blotting technique, so that only the pellet remained. The amount of radioactivity in each tube was counted for one minute using a gamma counter (Packard Cobra II, Packard Instrument Co., Meriden, CT).

Albumin

Serum albumin was measured by a quantitative, colorimetric determination method using the bromocresol purple method (Sigma Diagnostics No. 625, St. Louis, MO). Absorbance was read using a visible lamp at 600 nm.

Ferritin

Serum ferritin was measured quantitatively using an antibody RIA method (Diagnostic Products Corp., Los Angeles, CA). Sample were counted for one minute using a gamma counter (Packard Cobra II, Auto Gamma, Packard Instrument Co., Meriden, CT).

Data Analysis

Data obtained from this study were analyzed using the Statistical Analysis System (SAS Inst. Inc., Cary, NC, 1987) version 6.06. Subjects' anthropometric measures, blood parameters and dietary intakes at baseline were compared between supplement groups and between supplement groups by gender using the SAS General Linear Model and Least Squared Means procedures. Correlation coefficients were determined between baseline dietary intakes and blood parameters. Changes in blood parameters after eight weeks of supplementation, and four weeks after supplementation ended were

compared to changes in the placebo using the General Linear Model and Least Squared Means procedures. Significance level was set at 0.05.

CHAPTER IV

RESULTS AND DISCUSSION

Description of the Subjects

Sample Size

The subjects in this study were free-living volunteers from the community of Stillwater, Oklahoma. Initially, 29 subjects participated in the study. However, three subjects dropped out of the study before completion; their data were not included in the analysis. Two other subjects completed the study but were not included in the analysis because one did not fit into the age range specified for the study, and the other had excessively elevated blood triglyceride concentrations, above 900 mg/dl. As a result, data from the remaining 24 subjects were used in the data analysis. Of these subjects, eight were in each treatment group (placebo, chromium, and copper supplemented). Two subjects, one in the placebo and one in the chromium group missed the second data collection. Two subjects in the copper group missed the fourth data collection.

Gender and Age

Of the subjects completing the study, eleven were males and thirteen were females. Mean overall age was 66 years with an age range of 51 to 82 years. In the placebo group, five were males and three were females; mean age was 63 years (Table I). In the chromium group two were males and six were females; mean age was 68 years (Table I). In the copper group, four were males and four were females; mean age was

TABLE I

Subjects' Age and Anthropometric Measures

	Placebo*	Chromium*	Copper*
Age (yrs)			
Total	63.1 ± 3.7^{a} (n=8)	68.2 ± 3.6^{b} (n=8)	68.4 ± 3.1^{b} (n=8)
Male	58.0 ± 1.7^{a} (n=5)	69.5 ± 2.6^{b} (n=2)	62.2 ± 1.9^{a} (n=4)
Female	71.7 ± 2.3^{ab} (n=3)	67.8 ± 1.6^{a} (n=6)	74.5 ± 2.0b (n=4)
Body Weight (lb)			
Total	163.9 ± 12.7	158.6 ± 14.2	155.7 ± 10.8
Male	169.2 ± 13.6^{a}	194.0 ± 13.0^{b}	176.7 ± 10.2^{ab}
Female	155.0 ± 28.3	146.8 ± 16.0	134.7 ± 12.2
Height (in)			
Total	68.0 ± 1.5^{a}	64.0 ± 1.2^{b}	66.0 ± 1.6^{c}
Male	70.4 ± 1.2^{a}	68.5 ± 0.5^{b}	70.0 ± 0.4^{ab}
Female	64.0 ± 1.7^{a}	62.5 ± 0.8^{b}	62.0 ± 0.8^{b}
BMI (Kg/m^2)			
Total	24.8 ± 1.5^{a}	26.9 ± 1.8^{b}	25.0 ± 1.3^{ab}
Male	23.9 ± 1.3^{a}	29.1 ± 1.5^{b}	25.4 ± 1.4^{a}
Female	23.6 ± 3.6	26.2 ± 2.3	24.6 ± 2.3

* means ± standard error.
Values with different superscript letters in a row are significantly different at ($p < 0.05$).

68 years (Table I). The placebo group mean age was significantly younger than the chromium and copper groups (Table I). Males mean age in the chromium group was significantly older than males in the placebo and copper group (Table I). Females mean age in the copper group was significantly older than females in the chromium group (Table I).

Anthropometric Measurements

Baseline anthropometric measurements including body weight, height, and BMI are presented in Table I. There was no significant difference in body weight between supplement groups; however, males in the chromium group had significantly higher mean body weight than males in the placebo group. Mean body weights were similar to values reported for older adults of 167 lbs and 150 lbs for males and females, respectively (Deurenberg et al., 1990)

Significant differences did occur in mean height between supplement groups. The chromium group was significantly taller than the chromium and copper groups, and the chromium group was significantly taller than the copper group. Males' mean height in the placebo group was significantly taller than males in the chromium group; and females' mean height in the placebo group was significantly taller than females in the chromium and copper groups. However, mean heights were similar to values reported for older adults of 69 inches and 65 inches for males and females, respectively (Deurenberg et al., 1990).

Mean BMI for the chromium group was significantly larger than the placebo group. In addition, males mean BMI in the chromium group was significantly larger than the placebo and copper groups; however, there was no significant difference in females' mean BMI between supplement groups. Subjects' mean BMI were similar to those

reported in other studies for older adults of 25 and 26 kg/m^2 , for males and females, respectively (Deurenberg, et al., 1990; Lamon-Fava et al., 1994).

Subjects' Diet and Blood Parameters at Baseline

Baseline Blood Lipids and Apolipoprotein Concentrations

Baseline mean plasma lipids, and apolipoproteins concentrations by supplement groups and gender within supplement groups are presented in Table II. There was no significant difference at baseline in mean plasma total cholesterol concentration between supplement groups (Table II). Additionally there was no significant difference in mean plasma total cholesterol concentration between supplement groups by gender (Table II). Mean plasma total cholesterol concentrations in this study were lower than the 6.5 mmol/l (252 mg/dl) among nondiabetic elderly subjects reported by Offenbacher and Pi-Sunyer (1980) and the 6.2 mmol/l reported for elderly subjects with persistent impaired glucose tolerance (Uusitupa et al., 1992).

There was no significant difference in plasma HDL cholesterol concentration between supplement groups (Table II). However, males in the chromium group had significantly higher mean HDL cholesterol concentration than males in the copper and placebo groups; and females in the copper group had significantly higher mean HDL cholesterol concentration than females in the placebo group. (Table II). Mean HDL cholesterol concentrations in this study were similar to the 0.9 mmol/l (35 mg/dl) reported for adult men aged 31 to 60 years (Riales and Albrink, 1981). However, mean HDL cholesterol concentrations in this study for each group were higher than the 0.72 mmol/l (28 mg/dl) reported for elderly subjects aged 65 to 74 years (Uusitupa et al., 1992), but lower than the 1.34 mmol/l (52 mg/dl) observed among adults with total cholesterol concentrations between 5.69 mmol/l (220 mg/dl) and 8.27 mmol/l (320 mg/dl) (Press et al., 1990).

TABLE II

Subjects' Initial Plasma Lipid and Apolipoprotein Concentrations

	Placebo*	Chromium*	Copper*
Total cholesterol (mmol/L)			
Total	5.35 ± 0.33	5.63 ± 0.39	5.76 ± 0.34
Male	5.15 ± 0.44	5.97 ± 1.57	5.53 ± 0.27
Female	5.68 ± 0.52	5.51 ± 0.79	5.99 ± 0.65
HDL cholesterol (mmol/L)			
Total	0.94 ± 0.08	1.04 ± 0.11	0.99 ± 0.11
Male	0.96 ± 0.07^{b}	1.05 ± 0.33^{a}	0.78 ± 0.11^{b}
Female	0.91 ± 0.21^{a}	1.04 ± 0.13^{ab}	1.18 ± 0.12^{b}
LDL cholesterol (mmol/L)			
Total	3.60 ± 0.30^{a}	3.41 ± 0.38^{a}	4.12 ± 0.34^{b}
Male	3.52 ± 0.43^{ab}	3.11 ± 1.65^{a}	4.04 ± 0.28^{b}
Female	3.73 ± 0.48^{ab}	3.51 ± 0.28^{a}	4.19 ± 0.68^{b}
Triglyceride (mmol/L)			
Total	1.76 ± 0.38^{a}	2.56 ± 0.46^{b}	1.42 ± 0.15^{a}
Male	1.46 ± 0.45^{a}	3.94 ± 0.57^{b}	1.52 ± 0.24^{a}
Female	2.26 ± 0.70^{b}	2.10 ± 1.12^{a}	1.33 ± 0.19^{b}

TABLE II Continued

	Placebo*	Chromium*	Copper*
Apo A-I (g/L)			
Total	1.11 ± 0.04^{a}	1.39 ± 0.12^{b}	1.21 ± 0.11^{a}
Male	1.10 ± 0.04^{a}	1.31 ± 0.26^{b}	1.04 ± 0.08^{a}
Female	1.12 ± 0.01^{a}	1.41 ± 0.14^{b}	1.39 ± 0.17^{b}
Apo B (g/L)			
Total	0.89 ± 0.11	1.00 ± 0.08	0.98 ± 0.07
Male	0.77 ± 0.11^{a}	0.93 ± 0.04^{b}	0.99 ± 0.03^{b}
Female	1.10 ± 0.21	1.02 ± 0.10	0.97 ± 0.16

* means ± standard error.
Values with different superscript letters in a row are significantly different at ($p < 0.05$).

The copper group had significantly higher mean plasma LDL cholesterol concentration than the placebo and chromium groups (Table II). In addition, both males and females in the copper group had significantly lower LDL cholesterol concentration than the chromium group. (Table II). Mean LDL cholesterol concentrations in this study were lower than the 4.7 mmol/l (181 mg/dl) reported for healthy adult men aged 31 to 60 years (Riales and Albrink, 1981) and 5.2 mmol/l (200 mg/dl) reported for adults aged 25 to 80 years with total cholesterol concentrations between 5.69 mmol/l (220 mg/dl) to 5.43 mmol/l (210 mg/dl) (Press et al., 1990).

Mean plasma triglyceride concentration for the chromium group was significantly higher than the placebo and copper groups (Table II). Additionally, males in the chromium group had significantly higher mean plasma triglyceride concentration than males in the placebo and chromium groups; and females in the copper group had significantly lower mean plasma triglyceride concentration than females in the chromium and placebo groups (Table II). Mean plasma triglyceride concentrations for the placebo and copper groups were lower than the 1.91 mmol/l (169 mg/dl) reported by Offenbacher and Pi-Sunyer (1980), and the 2.22 mmol/l (196.9 mg/dl) reported by Offenbacher and Pi-Sunyer (1980). All three treatment groups mean plasma triglyceride concentrations were lower than the 3.04 mmol/l (270.0 mg/dl) reported by Uusitupa et al. (1992).

The chromium group had significantly higher mean plasma Apo A-I concentration than the placebo and copper groups (Table II). Additionally, males in the chromium group had significantly higher mean Apo A-I concentration than males in the placebo and copper groups; and females in the placebo group had significantly lower mean Apo A-I concentration than females in the chromium and copper groups (Table II). However, mean Apo A-I concentrations for all three groups were lower than the 1.47 g/l (147 mg/dl) reported for adults aged 25 to 80 years (Press et al., 1990).

There was no significant difference in mean plasma Apo B concentration between supplement groups (Table II). However, mean plasma Apo B concentration for males in the chromium group was significantly higher than males in the placebo and copper groups (Table II). Mean plasma Apo B concentrations in this study were lower than the 1.2 g/L reported by Uusitupa et al. (1992). The lower mean Apo B concentrations compared to other studies coincides with the lower mean LDL cholesterol concentrations, because Apo B is the major apolipoprotein in the LDL fraction.

Baseline mean plasma glucose, serum insulin, serum ferritin, and serum albumin concentrations by supplement groups and gender within supplement groups are presented in Table III. Mean plasma glucose concentration for the chromium group was significantly higher than the placebo and copper groups (Table III). In addition, males in the chromium group had significantly higher mean plasma glucose concentration than males in the placebo and copper groups, and males in the copper group had significantly higher mean plasma glucose concentration than males in the placebo group (Table III). Additionally, females in the chromium group had significantly higher mean plasma glucose concentration than females in the copper group (Table III). Mean plasma glucose concentrations in this study were similar to the 5.3 mmol/l (97 mg/dl) reported by Offenbacher and Pi-Sunyer (1980) among 24 combined diabetic and nondiabetic subjects with a mean age of 78 years.

The chromium group had significantly higher mean serum insulin concentration compared to the copper group (Table III). In addition, males had significantly higher mean serum insulin concentration than males in the placebo and copper groups (Table III). However, females in the placebo group had significantly higher mean serum insulin concentration than females in the chromium and copper groups (Table III). Serum insulin concentrations in this study were higher than the 79 pmol/l reported by Offenbacher and Pi-Sunyer (1980) and lower than the 194 pmol/l reported by Lui and Morris (1978).

TABLE III

Subjects' Initial Plasma Glucose, and Serum Insulin, Ferritin, and Albumin Concentrations

	Placebo*	Chromium*	Copper*
Glucose (mmol/L)			
Total	5.1 ± 0.1^{a}	5.8 ± 0.4^{b}	5.4 ± 0.3^{a}
Male	5.0 ± 0.1^{a}	7.0 ± 0.3^{b}	5.8 ± 0.4^{c}
Female	5.3 ± 0.2^{ab}	5.5 ± 0.3^{a}	5.0 ± 0.3^{b}
Insulin (pmol/L)			
Total	158 ± 19^{ab}	174 ± 14^{a}	149 ± 11^{b}
Male	136 ± 16^{a}	199 ± 9^{b}	139 ± 15^{a}
Female	194 ± 37^{a}	166 ± 17^{b}	160 ± 17^{b}
Albumin (g/L)			
Total	48 ± 1	47 ± 2	48 ± 2
Male	49 ± 1	52 ± 1	50 ± 3
Female	47 ± 2	45 ± 2	47 ± 3
Ferritin (μg/L)			
Total	133 ± 36	121 ± 49	94 ± 32
Male	155 ± 58^{a}	316 ± 122^{b}	152 ± 49^{a}
Female	97 ± 16^{a}	57 ± 11^{b}	35 ± 10^{c}

*means ± standard error.
Values with different superscript letters in a row are significantly different at ($p < 0.05$).

There were no significant differences in mean serum albumin concentrations by supplement groups or by gender within supplement groups. Mean serum albumin concentrations were within normal parameters for males and females.

There were no significant differences in mean serum ferritin concentrations by supplement groups (Table III). However, there were significant differences in males and females between supplement groups. Males in the chromium group had significantly higher mean serum ferritin than males in the placebo and copper groups. This difference is because the two males in the chromium supplement group had elevated ferritin concentrations. Females in the placebo group had significantly higher mean ferritin concentration than females in the chromium and copper groups; and females in the chromium group had significantly higher mean ferritin concentration than females in the copper group. (Table III). It has been reported that iron overload may be more common in patients with non-insulin-dependent diabetes mellitus (Stremmel et al., 1988). However, a recent report (Dinneen et al., 1994) suggested that non-insulin-dependent diabetes mellitus is not associated with a substantial level of iron overload.

Baseline Dietary Intakes

Initial caloric intakes and recommended energy intake (REI) by gender within supplement groups are presented in Table IV. Significant differences were observed in daily calorie intake between supplement groups. Mean daily calorie intake for the placebo group was significantly higher than the chromium and copper groups. In addition, males in the copper group had significantly lower mean energy intake than males in the placebo and chromium groups; and females in the placebo group had significantly higher mean energy intake than females in the chromium group (TableIV). Except for females in the placebo group all other gender supplement groups consumed less than 100% of the recommended energy intake (REI) for their age (Table IV). Males

TABLE IV

Subjects' Caloric Intakes at Baseline

	Placebo*	Chromium*	Copper*
Total			
Kcal Intake	2081 ± 145[a] (n=8)	1643 ± 226[b] (n=8)	1527 ± 66[b] (n=8)
Male			
Kcal Intake	2187 ± 176[a] (n=5)	1987 ± 19[a] (n=2)	1514 ± 43[b] (n=4)
% of REI	95	86	66
Female			
Kcal Intake	1906 ± 262[a] (n=3)	1528 ± 291[b] (n=6)	1540 ± 137[b] (n=4)
% of REI	100	80	81

*means ± standard error.
Values with different superscript letters in a row are significantly different at ($p < 0.05$).
REI = recommended energy intake.

in copper group consumed only 66% of their REI.

No significant interaction was observed over time between supplement groups by gender for plasma lipids, apolipoproteins, plasma glucose or serum insulin concentrations. Therefore, all further analyses were performed only by supplement groups, and not supplement group by gender.

Baseline mean percentage of calories from protein, carbohydrate, fat, and saturated fat; and mean fiber, copper and zinc intakes by supplement groups are presented in Table V. Mean percent of calories from carbohydrate was significantly higher in the copper group than the placebo group (Table V). Nevertheless, all groups in this study consumed a higher percent of calories from carbohydrate than the 45% of calories from carbohydrate reported by the American Heart Association Nutrition Committee (1988). However, no groups met the 58% of calories from carbohydrate recommended by the Dietary Guidelines for Americans (U.S. Dept. Agriculture and U.S. Dept. of Health, 1980).

There was no significant difference in mean percent of calories from protein between supplement groups (Table V). Mean percent of calories from protein was similar to the 16% reported by the American Heart Association Nutrition Committee (1988). However, all groups had mean percent of calories from protein higher than the 12% recommended by the U.S. Dietary Guidelines for Americans (U.S. Dept. Agriculture and U.S. Dept. of Health, 1980).

The placebo group had significantly higher mean percent of calories from fat than the copper group (Table V). However, mean percent of calories from fat for all groups was similar to the 30% of calories from fat recommended in the U.S. Dietary Guidelines for Americans (U.S. Dept. of Agriculture and U.S. Dept. of Health, 1980) and by the American Heart Association (The Nutrition Committee, 1988). In addition, all groups' mean percent of calories from fat was lower than the reported 37% for Americans (The Nutrition Committee, 1988). Because the percent of calories from dietary fat and

TABLE V

Subject's Dietary Intakes at Baseline

	Placebo Group*	Chromium Group*	Copper Group*
Energy (Kcal/day)	2081 ± 145^{a}	1643 ± 226^{b}	1527 ± 66^{b}
% CHO	51.4 ± 2.3^{a}	54.8 ± 3.4^{ab}	56.6 ± 3.0^{b}
% Protein	17.3 ± 1.4	17.1 ± 1.0	15.9 ± 0.9
% Fat	31.4 ± 1.2^{a}	29.6 ± 3.2^{ab}	28.0 ± 2.3^{b}
%S-Fat	9.3 ± 0.6	9.2 ± 1.0	8.9 ± 0.9
Fiber (g)	24 ± 1.8^{a}	19 ± 1.9^{b}	16 ± 1.6^{b}
Copper (mg)	1.6 ± 0.1^{a}	1.6 ± 0.3^{a}	1.2 ± 0.2^{b}
Zinc (mg)	12.2 ± 3.5	11.7 ± 5.7	10.5 ± 3.7

*means ± standard error.
% CHO = percent daily calories from carbohydrates.
% protien = percent daily calories from protein.
% Fat = percent daily calories from total fat.
% S-Fat = percent daily fat calories from saturated fat.
Values with different superscript letters in a row are significantly different ($p < 0.05$).

carbohydrate are related reciprocally, the recommendation to lower total fat intake coincides with recommendation to increase dietary carbohydrate.

There was no significant difference in mean percent of calories from saturated fat between supplement groups (Table V). Mean percent of calories from saturated fat for all groups were similar to the U.S. Dietary Guidelines for Americans, of less than 10% of calories from saturated fat. Mean percent of calories from saturated fat for most groups were well below the reported mean intake for Americans of 16% (The Nutrition Committee, 1988). Emphasis often is placed on saturated fat intake because fat quality is more closely correlated with certain plasma lipid responses than fat quantity.

There was a significant difference in mean fiber intake between supplement groups (Table V). The placebo group had a significantly higher mean fiber intake than the chromium and copper groups (Table V). Reported American mean fiber intakes are between 10 to 20 grams dietary fiber per day (The Nutrition Committee, 1988). Mean fiber intakes for the chromium and copper groups were close to the upper reported American intake, whereas mean fiber intake for the placebo group was above the upper reported fiber intake for Americans.

The copper group consumed significantly less copper than the placebo and chromium groups. Mean copper intakes for the placebo and chromium groups were close to the lower limit of the estimated safe and adequate intake for copper of 1.5 to 3 mg/day, and mean copper intake for the copper group was below the lower limit (Food and Nutrition Board, 1989). Mean copper intakes for all groups were consistent with reports that the average American diet provides less than 2 mg copper per day (Pennington, et al., 1986).

There was no significant difference in mean zinc intake between supplement groups (Table V). Mean zinc intakes for males and females were 12.6 mg and 10.5 mg, respectively. Mean zinc intakes for males in the placebo, chromium and copper groups were 13.8 mg, 17.5 mg, 8.8 mg, respectively. Mean zinc intakes for females in the

placebo, chromium and copper groups were 9.7 mg, 9.8 mg, 12.2 mg, respectively. Only males in the chromium group and females in the copper group had zinc intakes above the zinc RDA for adults. Reported mean zinc intakes for American males and females are 67 to 47% of the RDA for adults (Solomons, 1982).

Thus, even though the subjects' distribution of calories from carbohydrate, protein and fat in this study was similar to recommended intakes, dietary copper and zinc intakes were below recommended levels. Animal products, which are a major source of dietary fat source, also contribute significant amounts of minerals in the diet. Thus, recommendations to limit animal products in an effort to lower fat intake may also lower mineral intakes.

Dietary Intakes Over Data Collection Periods

Dietary intakes across all four data collections within each supplement group were analyzed to determine if mean dietary intakes remained constant over time. Within each supplement group there were no significant differences in dietary intake between the four data collections. Thus, we presumed that significant changes in plasma lipids, apolipoproteins, plasma glucose and serum insulin concentrations after eight weeks' supplementation, or four weeks after supplementation ended were not due to changes in dietary intake during the study, but rather due to supplementation.

Correlations Between Dietary Intakes and Blood Parameters at Baseline

Correlations between dietary intakes and blood parameters at baseline revealed some interesting relationships (Table VI). Correlations were considered significant when probabilities were less than 0.05.

TABLE VI

Correlation Coefficients (r) Between Blood Parameters and Dietary Intakes at Baseline (n=24)

	% CHO	% Pro	% Fat	% S-Fat	Fiber	Copper	Zinc
Total-C (p=)	-0.2412 0.0179	0.1660 NS	0.2883 0.0044	0.1511 NS	-0.0420 NS	-0.1190 NS	-0.0671 NS
HDL-C (p =)	0.1511 NS	-0.2363 0.0204	-0.2002 0.0505	0.0356 NS	0.0619 NS	0.3773 0.0002	0.3115 0.0020
LDL-C (p =)	-0.1597 NS	0.2304 0.0239	0.1724 0.0931	0.1013 NS	-0.0350 NS	-0.3396 0.0007	-0.2622 0.0099
TG (p =)	-0.2403 0.0183	0.0021 NS	0.3347 0.0009	0.0748 NS	-0.0477 NS	0.0697 NS	0.2113 0.0388
Apo A-I (p =)	0.3361 0.0008	-0.3723 0.0002	-0.3206 0.0014	-0.1073 NS	-0.0044 NS	0.2524 0.0131	0.1426 0.1659

TABLE VI Continued

	% CHO	% Pro	% Fat	% S-Fat	Fiber	Copper	Zinc
Apo B	0.0928	-0.0104	-0.0745	-0.3080	-0.0569	0.0113	-0.2083
(p =)	NS	NS	0.4691	0.0023	NS	NS	0.0417
Glucose	-0.1944	0.0281	0.2424	0.1330	-0.0612	0.1131	0.1362
(p =)	NS	NS	0.0174	NS	NS	NS	NS
Insulin	-0.1060	0.0249	0.2500	-0.0227	0.0772	0.0393	0.0986
(p -=)	NS	NS	0.0140	NS	NS	NS	NS

P = statistical probability.
NS = no significance.
% CHO = percent daily calories from carbohydrate.
% Pro = percent daily calories from protein.
% Fat = percent daily calories from fat.
% S-Fat = percent daily fat calories from saturated fat.
Total-C = total cholesterol.
HDL-C = HDL cholesterol.
LDL-C = LDL cholesterol.
TG = triglyceride.

Percent of calories from carbohydrate had a significant negative correlation with plasma total cholesterol and triglyceride concentrations, and a significant positive correlation with Apo A-I concentration. These correlations indicate that subjects consuming a higher percent of calories from carbohydrate had lower plasma total cholesterol and triglyceride concentrations, and higher Apo A-I concentration. The higher Apo A-I concentration could be related to higher HDL cholesterol. However, though the correlation coefficient between percent of calories from carbohydrate and HDL cholesterol was positive, it was not significant. Consumption of a higher percent of calories from carbohydrate has been associated with favorable effects on plasma lipid concentrations (Hallfrisch et al., 1985). Since dietary fat and carbohydrate may be reciprocally related, recommendations to lower dietary fat may be synonymous with recommendations to increase dietary carbohydrate.

Percent of calories from protein had a significant positive correlation with HDL cholesterol and Apo A-I, and a significant negative correlation with LDL cholesterol concentration. These data indicate that subjects consuming a higher percent of calories from protein had lower plasma HDL cholesterol and Apo A-I concentrations and higher LDL cholesterol concentrations. The lower Apo A-I concentration was consistent with the lower HDL cholesterol concentration. Animal studies have reported that animal proteins such as meat, milk, and egg may be hypercholesterolemic and vegetable proteins hypocholesterolemic (Van Raaij et al., 1981). However, further research is needed to investigate protein source on plasma lipids.

Percent of calories from fat had a significant positive correlation with plasma total cholesterol, triglyceride, glucose and insulin concentrations, and a significant negative correlation with Apo A-I concentration. These data indicate that subjects consuming a higher percent of calories from fat had higher total cholesterol, triglyceride, glucose and insulin concentrations, and lower Apo A-I concentration. Again, the lower Apo A-I concentration could be related to lower HDL cholesterol. However, though the

correlation coefficient between percent of calories from carbohydrate and HDL cholesterol was negative, it was not significant. Kay and his associates (1980) also reported a positive correlation between total fat intake and plasma total cholesterol. Substituting unsaturated fats for saturated fats in the diet without changing the quantity of fat in the diet has been reported to modify plasma lipid concentrations (Keys et al., 1957). There was a significant negative correlation between percent of calories from saturated fat and Apo B concentration in this study. These data indicate that those subjects consuming a higher percent of calories from saturated fat had lower Apo B concentration. The lower Apo B concentration could be related to a lower LDL cholesterol concentration, and in fact the correlation coefficient between percent of calories from saturated fat and LDL cholesterol concentration, though no significant was negative.

Dietary fiber intake was not significantly correlated with any blood parameters in this study. In general, soluble fibers such as oat and pectin often favorably affect plasma total cholesterol and lipoprotein cholesterol concentrations (Hillman et al., 1985).

Mineral intakes also were correlated with plasma lipids. Copper intake had a significant positive correlation with plasma HDL cholesterol and Apo A-I, and a significant negative correlation with LDL cholesterol. These data indicate that subjects who consumed a higher copper intake had higher HDL cholesterol and Apo A-1 concentrations, and lower LDL cholesterol concentration. The higher Apo A-I concentration would be consistent with the higher HDL cholesterol concentration. Zinc intake had a significant positive correlation with HDL cholesterol and triglyceride concentrations and a significant negative correlation with LDL cholesterol and Apo B concentrations. These data indicate that subjects with higher zinc intakes had higher HDL cholesterol concentration, but also higher triglyceride concentrations. In addition, subjects with higher zinc intakes had lower LDL cholesterol and Apo B concentrations. The lower Apo B concentration would be consistent with the lower LDL concentration.

Hermann et al. (1993) reported that subjects with lower zinc intakes had elevated plasma total and LDL cholesterol concentrations. It has also been reported that rats red zinc deficient diets became hypercholesterolemic (Koo & Williams, 1981, Koo & Lee, 1988)

Effects of Supplementation on Blood Parameters

Changes in blood concentrations after eight weeks of chromium or copper supplementation (zero to eight weeks) and four weeks after supplementation ended (eight to twelve weeks) were compared to the placebo group. A 7% increase in assay controls occurred at the second data collection, after four of weeks supplementation, therefore effects after four weeks supplementation may not be due to supplementation, but rather to instrument malfunction. As a result, data after four weeks of supplementation are not presented.

Effects of Chromium Supplementation on Plasma Lipids, Apolipoproteins and Glucose, and Serum Insulin

In this study, eight weeks chromium supplementation resulted in a significant decrease in mean plasma total cholesterol concentration compared to the placebo (Table VII). There was no significant change in plasma total cholesterol concentration four weeks after chromium supplementation ended compared to the placebo (Table VII). These results are similar to those reported by Offenbacher and Pi-Sunyer (1980). They reported a decrease in mean serum cholesterol concentration after eight weeks chromium supplementation with nine grams brewer's yeast in nondiabetic older adults. Press and associates (1990) also reported a decrease in mean serum cholesterol concentration after supplementing 28 healthy adults for 42 days with 200 ug chromium per day as chromium picolinate.

TABLE VII

Effects of Chromium Supplementation on Plasma Lipids Over Experimental Period

	Total-C[a] (mmol/L)	HDL-C[a] (mmol/L)	LDL-C[a] (mmol/L)	Triglyceride[a] (mmol/L)
Placebo				
Baseline (n=8)	5.35 ± 0.33	0.94 ± 0.08	3.60 ± 0.30	1.76 ± 0.32
8 weeks (n=8)	5.42 ± 0.27	0.96 ± 0.10	3.48 ± 0.27	2.14 ± 0.44
12 weeks (n=8)	5.57 ± 0.30	0.98 ± 0.11	3.75 ± 0.30	1.82 ± 0.39
C 8	0.07 ± 0.18	0.01 ± 0.02	-0.11 ± 0.19	0.37 ± 0.20*
C 12	0.14 ± 0.20	0.03 ± 0.03	0.26 ± 0.18	-0.32 ± 0.26
Chromium				
Baseline (n=8)	5.63 ± 0.39	1.04 ± 0.11	3.41 ± 0.38	2.56 ± 0.46
8 weeks (n=8)	5.32 ± 0.28	1.02 ± 0.12	3.13 ± 0.28	2.54 ± 1.15
12 weeks (n=8)	5.51 ± 0.15	1.01 ± 0.10	3.42 ± 0.43	2.35 ± 0.46
C 8	-0.31 ± 0.16*	-0.02 ± 0.04	-0.28 ± 0.14	-0.02 ± 0.24
C 12	0.20 ± 0.20	-0.01 ± 0.02	0.29 ± 0.20	-0.19 ± 0.14

[a] means ± standard error.

*significant change from baseline in a column, supplement group versus placebo, $p < 0.05$.

C 8; change from baseline at 8 weeks.

C 12; change from 8 weeks at 12 weeks.

Total-C = total cholesterol; HDL-C = HDL cholesterol; LDL-C = LDL cholesterol.

There were no significant changes in mean HDL or LDL cholesterol concentrations after eight weeks' chromium supplementation compared to the placebo, or four weeks after chromium supplementation ended compared to the placebo (Table VII). In a study by Riales and Albink (1981), supplementation with 200 ug trivalent chromium daily for 12 weeks increased HDL cholesterol concentration, but had no effect on LDL cholesterol concentration among healthy adults aged 31 to 60 years. However, Press and associates (1990) reported supplementation with 200 ug chromium as chromium picolinate daily for 42 days increased HDL cholesterol concentration and decreased LDL cholesterol concentration among adults aged 25 to 80 years. Differences in our results and these studies could be due to differences in subjects' initial chromium status

Eight weeks' chromium supplementation did not significantly affect the chromium group's plasma triglyceride concentration; however, there was a significant decrease in plasma triglyceride concentration in the placebo group after eight weeks supplementation (Table VII). There was no effect on plasma triglyceride concentration four weeks after chromium supplementation ended compared to the placebo. These results are similar to those reported by Riales and Albrink (1981) who observed no significant change in triglyceride concentration after 12 weeks chromium supplementation among healthy adults aged 31 to 63 years.

After eight weeks' chromium supplementation, mean plasma Apo A-I concentration was significantly decreased in the placebo group compared to the chromium supplemented group (Table VIII). However, there was no significant change in mean plasma Apo A-I concentration four weeks after chromium supplementation ended compared to the placebo.

Chromium supplementation for eight weeks resulted in a significant increase in mean plasma Apo B concentration compared to the placebo. However, there was no

TABLE VIII

Effects of Chromium Supplementation on Plasma Apolipoproteins Over Experimental Period

	Apolipoprotein A-I[a] (g/L)	Apolipoprotein B[a] (g/L)
Placebo		
Baseline (n=8)	1.11 ± 0.04	0.89 ± 0.11
8 weeks (n=8)	1.24 ± 0.06	0.87 ± 0.09
12 weeks (n=8)	1.11 ± 0.03	0.85 ± 0.15
C 8	0.13 ± 0.02*	-0.02 ± 0.05
C 12	-0.13 ± 0.04	-0.19 ± 0.07
Chromium		
Baseline (n=8)	1.39 ± 0.12	1.00 ± 0.08
8 weeks (n=8)	1.32 ± 0.13	1.12 ± 0.15
12 weeks (n=8)	1.17 ± 0.12	1.09 ± 0.12
C 8	-0.07 ± 0.08	0.13 ± 0.14*
C 12	-0.14 ± 0.12	-0.03 ± 0.05

[a] means ± standard error.
*significant change from baseline in a column, supplement group versus placebo, $p < 0.05$.
C 8 = change from baseline at 8 weeks.
C 12 = change from 8 weeks at 12 weeks.

significant change in mean plasma Apo B concentration four weeks after supplementation ended compared to the placebo (Table VIII). Plasma Apo B is the major apolipoprotein in LDL cholesterol. However, the increase in plasma Apo B concentration after eight weeks' chromium supplementation did not coincide with an increase in plasma LDL cholesterol concentration after eight weeks' chromium supplementation. In fact, though not significant, plasma LDL cholesterol concentration decreased after eight weeks chromium supplementation. However, Press and associates (1990) reported an increase in Apo A-I and HDL cholesterol concentrations, and a decrease in Apo B and LDL cholesterol concentrations after 42 days supplementation with 200 ug chromium as chromium picolinate daily in adults aged 25 to 80. However, in elderly subjects with impaired glucose tolerance, serum Apo A-I and Apo B concentrations were not affected by six months of chromium supplementation (Uusitupa et al., 1992). Currently there is no clear explanation for the effects of chromium on plasma Apo A-I or Apo B concentrations. Further research is needed in investigating the effects of chromium supplementation on blood apolipoproteins.

Mean plasma glucose concentration was significantly decreased after eight weeks' chromium supplementation compared to the placebo group (Table IX). In addition, four weeks after chromium supplementation ended mean plasma glucose concentration significantly increased compared to the placebo (Table IX). Four weeks after chromium supplementation ended, mean plasma glucose concentration was similar to baseline concentration (Table IX). These data indicate that eight weeks of chromium supplementation resulted in a beneficial decrease in plasma glucose compared to the placebo, and that glucose concentrations returned to initial concentrations four weeks after chromium supplementation ended. However, in other studies (Anderson et al., 1983b; Offenbacher et al., 1985), no significant effect on mean plasma glucose concentration was observed among chromium-supplemented subjects with normal glucose tolerance.

TABLE IX

Effects of Chromium Supplementation on Plasma Glucose and Serum Insulin Over Experimental Period

	Glucose[a] (mmol/L)	Insulin[a] (pmol/L)
Placebo		
Baseline (n=8)	5.1 ± 0.1	158 ± 19
8 weeks (n=8)	5.2 ± 0.2	189 ± 42
12 weeks (n=8)	5.2 ± 0.1	158 ± 19
C 8	0.0 ± 0.3	31 ± 36*
C 12	0.0 ± 0.2	31 ± 35**
Chromium		
Baseline (n=8)	5.8 ± 0.4	174 ± 14
8 weeks (n=8)	5.5 ± 0.4	173 ± 19
12 weeks (n=8)	5.9 ± 0.3	179 ± 19
C 8	-0.3 ± 0.2*	-1 ± 10
C 12	0.4 ± 0.2**	6 ± 10

[a] means ± standard error.

*significant change from baseline in a column, supplement group versus placebo, $p < 0.05$.

**significant change from 8 weeks in a column, supplement group versus placebo, $p < 0.05$

C 8 = change from baseline at 8 weeks.

C 12 = change from 8 weeks at 12 weeks.

After eight weeks' supplementation there was a significant increase in serum insulin concentration in the placebo group compared to the chromium supplemented group (Table IX). In addition, mean serum insulin concentration in the placebo group significantly decreased back to baseline concentrations four weeks after supplementation ended (Table IX). However, Uusitupa and associates (1983) observed an increase, although not significant, in fasting mean serum insulin concentration after 6 weeks' supplementation with 200 ug chromium. However, Riales and Albrink (1981) reported chromium supplementation for 12 weeks tended to decrease serum insulin concentrations among healthy men. Lui and Morris (1978) also reported a significant decrease in fasting insulin concentration among 27 women after 3 month supplementation with only 4 ug chromium from brewer's yeast.

Effects of Copper Supplementation on Plasma Lipids, Apolipoprotein and Glucose and Serum Insulin

Eight weeks' copper supplementation resulted in a significant decrease in plasma total cholesterol compared to the placebo (Table X). In addition, there was a significant increase in mean plasma total cholesterol four weeks after copper supplementation ended compared to the placebo (Table X). These results indicate that eight weeks' copper supplementation resulted in a beneficial decrease in mean plasma total cholesterol, and that plasma total cholesterol concentration significantly increased four weeks after copper supplementation ended.

Mean plasma HDL cholesterol concentration was also significantly decreased after eight weeks copper supplementation compared to the placebo (Table X). In addition, mean plasma HDL cholesterol concentration continued to decrease, though not significantly, four weeks after copper supplementation ended compared to the placebo (Table X).

TABLE X

Effects of Copper Supplementation on Plasma Lipids Over Experimental Period

	Total-C[a] (mmol/L)	HDL-C[a] (mmol/L)	LDL-C[a] (mmol/L)	Triglyceride[a] (mmol/L)
Placebo				
Baseline (n=8)	5.35 ± 0.33	0.94 ± 0.08	3.60 ± 0.30	1.76 ± 0.38
8 weeks (n=8)	5.42 ± 0.27	0.96 ± 0.10	3.48 ± 0.27	2.14 ± 0.44
12 weeks (n=8)	5.57 ± 0.30	0.98 ± 0.11	3.75 ± 0.30	1.82 ± 0.39
C 8	0.07 ± 0.18	0.01 ± 0.02	-0.11 ± 0.19	0.37 ± 0.20*
C 12	0.14 ± 0.20	0.03 ± 0.03	0.26 ± 0.18	-0.32 ± 0.26
Copper				
Baseline (n=8)	5.76 ± 0.34	0.99 ± 0.11	4.12 ± 0.34	1.42 ± 0.15
8 weeks (n=8)	5.31 ± 0.36	0.95 ± 0.09	3.74 ± 0.38	1.36 ± 0.13
12 weeks (n=6)	5.54 ± 0.22	0.90 ± 0.10	3.81 ± 0.22	1.79 ± 0.27
C 8	-0.44 ± 0.18*	-0.04 ± 0.04*	-0.37 ± 0.20*	-0.06 ± 0.07
C 12	0.50 ± 0.23**	-0.01 ± 0.04	0.35 ± 0.22	0.34 ± 0.14**

[a] means ± standard error.

*significant change from baseline in a column, supplement group versus placebo, $p < 0.05$.

**significant change from 8 weeks in a column, supplement group versus placebo, $p < 0.05$.

C 8 = change from baseline at 8 weeks.

C 12 = change from 8 weeks of 12 weeks.

Total-C = total cholesterol; HDL-C = HDL cholesterol; LDL-C = LDL cholesterol.

Mean plasma LDL cholesterol concentration significantly decreased after eight weeks of copper supplementation compared to the placebo. However, there was no significant change in mean plasma LDL cholesterol concentration four weeks after copper supplementation ended compared to the placebo (Table X).

In the placebo group mean plasma triglyceride concentration significantly decreased after eight weeks supplementation compared to the copper supplemented group (Table X). However, a significant increase in mean plasma triglyceride concentration was observed four weeks after copper supplementation ended compared to the placebo (Table X).

Most studies investigating the effects of copper on blood lipids and lipoprotein distribution are copper deficiency studies using rats as the experimental model. However, Klevay and associates (1984) reported a increase in both total and LDL cholesterol concentrations in a 29-year-old male consuming a diet supplying only 0.89 mg of copper per day. Reiser and associates (1987) also reported that human subjects fed an experimental diet inadequate in copper (0.36 mg per 1000 Kcal) for 11 weeks had increased HDL cholesterol concentration compared to those fed a control diet (0.57 mg copper per 1000 Kcal).

Important differences exist in lipoprotein metabolism between man and rat. In rats, about two-thirds of total blood cholesterol is carried in the high density lipoprotein (Allen & Klevay, 1980; Lei, 1983). In humans, the majority of blood cholesterol is carried by the low density lipoprotein. Copper deficiency in rats has been reported to decrease plasma lipoprotein lipase activity and increase HMG CoA reductase activity (Harvey & Allen, 1981); Lau & Klevay, 1982; Yount et al., 1991). If copper deficiency produced similar effects in humans, one would expect decreased plasma total cholesterol and LDL cholesterol concentrations among humans supplemented with copper. Further research is needed in humans as to the copper's mechanism of action with plasma cholesterol.

After eight weeks' copper supplementation there was a significant increase in plasma Apo A-I concentration in the placebo group compared to the copper supplement group (Table XI). In addition, a significant decrease in mean plasma Apo A-I concentration was observed in the placebo group four weeks after supplementation ended compared to the placebo.

Although not significant, after eight weeks copper supplementation, mean plasma Apo B concentration decreased compared to the placebo (Table XI). However, four weeks after copper supplementation ended there was a significant increase in mean plasma Apo B concentration compared to the placebo (Table XI). This decrease in mean plasma Apo B concentration after eight weeks copper supplementation coincides with the decrease in LDL cholesterol after eight weeks copper supplementation.

No information is available concerning copper effects on apolipoproteins in humans. Although not significant, Lei (1983) reported elevated Apo A-I concentration in the HDL fraction in rats after seven weeks on a copper deficient diet. Hing and Lei (1991) also reported increased Apo B concentration increased in rabbits fed copper deficient diets four weeks. Nassir and associates (1993) observed that Apo B-100 liver synthesis increased in copper deficient rats. Thus further research is needed on the copper's mechanism of action on apolipoproteins.

After eight weeks' copper supplementation there was a significant decrease in mean plasma glucose concentration compared to the placebo (Table XII). In addition there was a significant increase in mean plasma glucose concentration four weeks after copper supplementation ended compared to the placebo (Table XII). Little information is available on copper supplementation on plasma glucose. Klevay and associates (1983) reported impaired glucose tolerance in copper deficient humans compared to a copper adequate group. They also reported that glucose concentration increased during copper depletion and decreased during copper repletion.

TABLE XI

Effects of Copper Supplementation on Plasma Apolipoproteins Over Experimental Period

	Apolipoprotein A-I[a] (g/L)	Apolipoprotein B[a] (g/L)
Placebo		
Baseline (n=8)	1.11 ± 0.04	0.89 ± 0.11
8 weeks (n=8)	1.24 ± 0.06	0.87 ± 0.09
12 weeks (n=8)	1.11 ± 0.03	0.85 ± 0.15
C 8	0.13 ± 0.02*	-0.02 ± 0.05
C 12	-0.13 ± 0.04**	-0.19 ± 0.07
Copper		
Baseline (n=8)	1.21 ± 0.11	0.98 ± 0.07
8 weeks (n=8)	1.12 ± 0.06	0.90 ± 0.07
12 weeks (n=68)	1.13 ± 0.06	1.04 ± 0.04
C 8	-0.09 ± 0.05	-0.08 ± 0.03
C 12	0.06 ± 0.05	0.15 ± 0.08**

[a] means ± standard error.
*signficant change from baseline in a column, supplement group versus placebo, $p < 0.05$.
**signficant change from 8 weeks in a column, supplement group versus placebo, $p < 0.05$.
C 8 = change from baseline at 8 weeks.
C 12 = change from 8 weeks at 12 weeks.

TABLE XII

Effects of Copper Supplementation on Plasma Glucose and Serum Insulin Over Experimental Period

	Glucose[a] (mmol/L)	Insulin[a] (pmol/L)
Placebo		
Baseline (n=8)	5.1 ± 0.1	158 ± 19
8 weeks (n=8)	5.2 ± 0.2	189 ± 42
12 weeks (n=8)	5.2 ± 0.1	158 ± 19
C 8	0.0 ± 0.3	31 ± 36*
C 12	0.0 ± 0.2	-31 ± 35**
Copper		
Baseline (n=8)	5.4 ± 0.3	149 ± 11
8 weeks (n=8)	5.1 ± 0.4	142 ± 8
12 weeks (n=8)	5.8 ± 0.4	160 ± 13
C 8	-0.3 ± 0.2*	-7 ± 9
C 12	0.3 ± 0.3 **	14 ± 7

[a] means ± standard error.
*signficant change from baseline in a column, supplement group versus placebo, $p < 0.05$.
**signficant cha.nge from 8 weeks in a column, supplement group versus placebo, $p < 0.05$.
C 8 = change from baseline at 8 weeks.
C 12 = change from 8 weeks at 12 weeks.

After eight weeks copper supplementation there was a significant increase in mean serum insulin concentration in the placebo group compared to the copper supplemented group (Table XII). Additionally, there was a significant decrease in mean serum insulin concentration in the placebo group four weeks after supplementation ended compared to the copper supplemented group (Table XII). Mean serum insulin concentration in the placebo group was similar to baseline concentration four weeks after supplementation ended. In an animal study (Fields et al., 1983), copper supplementation increased insulin binding to fat cells and increased the number of insulin receptor sites. If copper stimulates insulin binding in humans, copper supplementation could improve plasma glucose homeostasis.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study investigated the effects of eight weeks of chromium or copper supplementation on plasma lipids, apolipoproteins, glucose and serum insulin concentrations in adults over age 50. The subjects in this study were free-living volunteers from the community of Stillwater, Oklahoma. Subjects were randomly assigned to one of three treatments; placebo, chromium or copper supplementation. The placebo group (n=8) consumed 0.25 mg lactose daily, the chromium group (n=8) consumed supplements containing 241.3 ug chromium daily as chromium chloride, and the copper group (n=8) consumed supplements containing 3.2 mg copper daily as copper carbonate. Subjects consumed self selected diets throughout the study. Subjects participated in four data collections; baseline, four and eight weeks after supplementation and four weeks after supplementation ended. Plasma lipids (total cholesterol, HDL cholesterol, and triglycerides), apolipoproteins (Apo A-I and Apo B), plasma glucose and serum insulin concentrations were measured at each data collection period. LDL cholesterol was calculated. Changes in blood concentrations after eight weeks of supplementation and four weeks after supplementation ended were compared to changes in the placebo using the SAS General Linear Models procedure and Least Squared Means.

Chromium supplementation for eight weeks significantly decreased plasma total cholesterol and glucose concentrations and significantly increased Apo B concentrations in adults over age 50. However, the increase in Apo B concentration did not coincide

with a significant increase in LDL cholesterol concentration. Four weeks after chromium supplementation ended plasma glucose concentration significantly decreased.

Copper supplementation for eight weeks significantly decreased plasma total, HDL, and LDL cholesterol, and glucose concentrations in adults over age 50. However, the decrease in HDL and LDL cholesterol concentrations did not coincide with a significant a decrease in Apo A-I and Apo B concentrations, respectively. Four weeks after copper supplementation ended plasma total cholesterol, triglycerides, Apo B, and glucose significantly increased.

The results of this study indicate that diets adequate in chromium and copper may have beneficial effects on plasma lipids and glucose in adults over age 50.

Test of Null Hypotheses

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

Ho 1. There will be no statistically significant effects of eight weeks of chromium or copper supplementation on plasma total, LDL, HDL cholesterol, or triglyceride concentrations in adults over age 50.

Eight weeks chromium supplementation significantly decreased plasma total cholesterol concentration compared to the placebo in adults over age 50. Eight weeks copper supplementation significantly decreased plasma total, HDL, and LDL cholesterol concentrations compared to the placebo in adults over age 50. Therefore, null hypothesis one is rejected.

Ho 2. There will be no statistically significant effect of eight weeks of chromium or copper supplementation on plasma apolipoprotein A-I and apolipoprotein B concentrations in adults over age 50.

Eight weeks' chromium supplementation significantly increased plasma Apo B concentration compared to the placebo in adults over age 50. Therefore, null hypothesis two is rejected.

Ho 3. There will be no statistically significant effects from eight weeks of chromium or copper supplementation on plasma glucose and serum insulin concentrations in adults over age 50.

Eight weeks' chromium supplementation significantly decreased plasma glucose concentrations compared to the placebo in adults over age 50. In addition, eight weeks' copper supplementation significantly decreased plasma glucose concentrations compared to the placebo in adults over age 50. Therefore, null hypothesis three is rejected.

Implications

Our findings support the hypothesis that eight weeks of chromium or copper supplementation decrease plasma lipids and glucose concentrations and thereby may have a role in reducing risk of atherosclerosis or glucose intolerance. Adequate dietary chromium and copper intakes are important, particularly for those persons who are at risk for coronary heart disease, abnormal glucose tolerance, or have low dietary mineral intakes.

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APPENDIXES

APPENDIX A

APPROVAL FORM FOR INSTITUTIONAL REVIEW BOARD FOR HUMAN SUBJECT RESEARCH

[redacted] STATE UNIVERSITY
[redacted] REVIEW BOARD
FOR [redacted] SUBJECTS RESEARCH

Proposal Title: EFFECTS OF SUPPLEMENTATION WITH CHROMIUM, COPPER AND ZINC ON PLASMA LIPIDS, BONE METABOLISM AND INDICATORS OF TRACE MINERAL STATUS IN ADULTS

Principal Investigator: ANDREA B. ARQUITT/JANICE R. HERMANN

Date: 10-22-92 IRB # HES-93-011

This application has been reviewed by the IRB and

Processed as: Exempt [ ] Expedite [ ] Full Board Review [xx]

Renewal or Continuation [ ]

Approval Status Recommended by Reviewer(s):

Approved [xx] Deferred for Revision [ ]

Approved with Provision [ ] Disapproved [ ]

Approval status subject to review by full Institutional Review Board at next meeting, 2nd and 4th Thursday of each month.

Comments, Modifications/Conditions for Approval or Reason for Deferral or Disapproval:

PROVISIONS RECEIVED

Signature: Marcia K Tilley Date: 11-13-92

Chair of Institutional Review Board

APPENDIX B

INDIVIDUAL'S CONSENT TO PARTICIPATE IN RESEARCH

Individual's Consent to Participate in Research

Effect of Supplementation with Chromium, Copper and Zinc on Plasma Lipids, Bone Metabolism and Indicators of Trace Mineral Status in Adults

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

I understand that:

(1) the purpose of the study is to measure the effect of mineral supplementation on plasma lipids, bone metabolism and status of other trace minerals in adults;

(2) I will receive supplements containing either a placebo or ONE of the following minerals or mineral combinations which are equal to or less than those available in over-the-counter mineral supplements:
   (a) 15 mg zinc
   (b) 1.5 mg copper
   (c) 100 ug chromium
   (d) 15 mg zinc plus 1.5 mg copper
   (e) 1.5 mg copper plus 100 ug chromium;

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

(4) I understand that these supplements may cause slight nausea if taken on an empty stomach and that is the reason for the above statement; however, if I have any adverse reactions I will contact one of the principal investigators;

(5) I will be requested to record three days of food intake four times during this study;

(6) during this study period I should attempt to avoid consumption of oysters, and the following breakfast cereals: General Mill's Total (all varieties) and Kellogg's Nutri-grain Raisin Bran, Just Right, and Just Right Fruit & Nut;

(7) I will not take any nutrient supplements other than those that are a part of this study;

(8) a phlebotomist will draw fasting blood samples of 30 ml (about 6 teaspoons) by venipuncture prior to the study, at midpoint and end of the supplementation, and four weeks following supplementation and that slight bruising or discomfort may result from the venipuncture;

(9) I understand that this blood will only be used for analyses which involve lipid status, mineral status and bone mineral metabolism and that after these analyses are performed the remaining blood will be incinerated and that no perpetual cell lines will be maintained;

(10) as a reward for participation and as an incentive to complete the study, I will receive one coupon for a complimentary luncheon at the Taylor Dining Room at the end of the study;

APPENDIX C

RECRUITMENT ANNOUNCEMENT

DO YOU HAVE HIGH BLOOD CHOLESTEROL?

Have you ever wondered what it would be like to be a participant in a research study?

Would you like to know the effects of nutrient supplements on blood cholesterol, bone metabolism and nutritional status?

We have an ***opportunity*** for you if you meet the following conditions:

Over the age of 55

Not using estrogen replacement therapy or drugs to control blood cholesterol

Do not have a chronic disease

Blood cholesterol levels greater than 240 mg/dl

This study is designed to determine the effect of minerals on blood lipids, bone metabolism and measures of trace mineral status. The study involves participation for 12 weeks during which time you will participate in body composition measurements (height, weight, skin-fold measurements, and bioelectric impedance), record food intakes and blood collections.

The first collection period will be at the beginning of the study to provide baseline data. For eight weeks you will take a supplement twice a day. The supplement will contain either lactose, 15 mg zinc, 1.5 mg copper, 100 ug chromium or combinations of these concentrations of copper/zinc or copper/chromium. Data will be collected after four and eight weeks of supplementation , and then four weeks after the end of supplementation.

All that is required of you is to record food intakes for three days prior to each data collection, take the supplement twice daily for eight weeks, and come to the Department of Nutritional Sciences at OSU at four week intervals for blood collection (30 ml or about 6 teaspoons). Weight and body composition measurements will be recorded at each data collection period. We ask that you do not attempt to lose weight or change your usual eating and exercising habits during this period.

Volunteers completing the study will receive a complementary lunch at Taylors Dinning Room.

This study has been approved by the Oklahoma State University Internal Review Board for the protection of human subjects.

Sound like fun?!! If you're interested or for further information please contact or tear off and send this response to:

Andrea B. Arquitt, PhD, RD/LD
Janice R. Hermann, PhD, RD/LD
Department of Nutritional Sciences
425 HES
College of Human Environmental Sciences
Oklahoma State University
Stillwater, OK 74078
tel. no. 744-5040

I am interested in more information on the blood lipid, bone mineral metabolism and trace mineral status study.

Name _____

Telephone number _____

APPENDIX D

MEDICAL HISTORY QUESTIONNAIRE

HEALTH INFORMATION QUESTIONNAIRE
Mineral Supplementation Study
Nutritional Sciences Department
Oklahoma State University

Subject Number _____ Date of Birth _____ Race _____ Gender _____

Do you have or have you had any of the following diseases?

	No	Yes	When	Specify
Allergies	_____	_____	_____	_____
Inherited disorder	_____	_____	_____	_____
Uremia	_____	_____	_____	_____
Sickle cell anemia	_____	_____	_____	_____
Cancer	_____	_____	_____	_____
Diabetes	_____	_____	_____	_____
Heart disease	_____	_____	_____	_____
Liver disease	_____	_____	_____	_____
G I disorder	_____	_____	_____	_____
Blood Clotting Disorder	_____	_____	_____	_____

Are you on any type of special diet: Specify _____

Allergy	_____	_____	
Weight loss	_____	_____	
Weight gain	_____	_____	
Low fat, low cholesterol	_____	_____	
Diabetic diet	_____	_____	(kcal level_____)
Low sodium	_____	_____	
Other	_____	_____	Specify _____

Do you currently take any medications on a regular basis? _____

Specify all drugs taken _____

_____

_____

_____

In the last 6 months have you taken any nutrient (dietary) supplements? YES _____ NO

IF YES, Specify how recently _____

How regularly _____

IF YES, Specify brand and frequency of consumption:

_____

_____

_____

Have you recently had a serious illness or surgery? Yes _____ NO _____

Specify _____

Do you know your cholesterol "number" (concentration)? YES _____ NO _____

IF YES, what is it? _____ total _____ HDL

When did you learn you had high blood cholesterol ? _____(year)

_____(don't recall)

Do you regularly exercise? Yes _____ No _____

What type of exercise do you do? _____

How many times a week do you exercise? _____

What length of time do you exercise? _____

Do you have a family history of elevated blood cholesterol or heart disease?

Yes _____No _____

IF YES, Which family member?

	Elevated blood cholestrol	Heart disease
Father	_____	_____
Mother	_____	_____
Brothers	_____	_____
Sisters	_____	_____
Grandfather	_____	_____
Grandmother	_____	_____

APPENDIX E

DIETARY RECORD FORM

DIET RECORD

Subject Number_____ Date _____

Time	Amount	Coding	Description, Brand Name

APPENDIX F

HEALTH INFORMATION QUESTIONNAIRE

MONTHLY DATA COLLECTION FORM

Mineral Supplementation Study
Nutritional Sciences Department
Oklahoma State University

Subject Number _____

Date _____

1. Have you had a cold in the last month? Yes _____ No _____

   IF YES, when? _____

   how long did it last? _____

   did you have a fever? _____

2. Have you had the flu in the last month? Yes _____ No _____

   IF YES, when _____

   how long did it last? _____

   did you have a fever? _____

3. Have you had any other illness last month? Yes _____ No _____

   IF YES, what type of illness? _____

   how long did it last? _____

   did you have a fever? _____

4. IF YES TO ANY OF QUESTIONS 1 - 3, did you continue to take your supplement during the illness ? Yes _____ No _____

5. Did your exercise pattern change last month? Yes _____ No _____

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

   Type of exercise? _____

   IF YES, how often did you exercise?_____

   IF YES, how long do you presently exercise?_____

VITA

Hyunhee Chung

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF CHROMIUM OR COPPER SUPPLEMENTATION ON PLASMA LIPIDS, PLASMA GLUCOSE AND SERUM INSULIN IN ADULTS OVER AGE 50

Major Field: Human Environmental Sciences

Biographical:

Personal Data: Born in Yaesan, Korea, On October 4, 1962, the daughter of Gapyoung and Heejoe Chung.

Education: Graduated from Chunan Girls' High School, Chunan City, Korea, in March 1980; received Bachelor of Science degree in Food and Nutrition from Kyunghee University, Seoul, Korea in March 1985; received the Master of Science degree with a major in Food and Nutrition at Kyunghee University in August 1987. Completed the requirements for the Doctor of Philosophy degree with a major in Nutritional Science at Oklahoma State University in December 1994.

Experience: Licensed Dietitian and Licensed Teacher in Home Economics, Korea, 1985; Graduate Research and Teaching Assistant, Department of Food and Nutrition, Kyunghee University, Seoul, Korea, 1985-1986; Graduate Research Assistant, Department of Nutritional Science, Oklahoma State University, 1990-1994.

Professional Membership: Korean Scientists & Engineers Association in America.