EFFECTS OF SUPPLEMENTATION WITH ZINC AND CHROMIUM ON PLASMA GLUCOSE AND SERUM INSULIN IN ADULTS AGE 50 AND OLDER

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CHAPTER I

INTRODUCTION

World wide the United States has the third largest elderly population (people over age 65) and the largest old-old population (people age 80 and above). In 1987, one in eight Americans, or approximately 30 million people, were at least 65 years of age. By the year 2030 the number of persons aged 65 and above is expected to double to an estimated 23 percent of the total population (1). Due to the projected increase in life span, increasing attention is being given to research relating to the aging process, medical complications associated with aging, and to the nutritional status of older adults (2-4).

Although not inevitable, health status often declines with advancing age. Older adults have a high incidence of chronic diseases, medication use, and sedentary lifestyles (5). Poor dietary intake among the elderly may contribute to declining health status and increased risk of diet-related diseases such as coronary heart disease, glucose intolerance, and maturity-onset diabetes (5-7).

The elderly, as a group, are at increased risk of poor nutritional status due to several factors including socioeconomic pressures, depression, reduced mobility, loneliness, aging body tissues, physical and mental disabilities, and food and drug interactions (3,8,9). Nutrition intervention to improve dietary adequacy could possibly divert or slow the progression of many disease conditions that occur with aging (5).

Diabetes is of particular concern among older adults. Diabetes is one of the six leading causes of death after age 65 (1). Approximately 20-30 percent of individuals aged 65 or older have impaired glucose tolerance, as indicated by elevated fasting glucose concentrations, while 10-35 percent have actual diabetes

(10). Increased postprandial plasma glucose concentrations have been reported with age (11,12). In addition, decreased glucose tolerance has been reported with increased age (12,13). This is of concern because impaired glucose tolerance is a leading indicator of non-insulin dependent diabetes (14). One hypothesis for the observed decrease in glucose tolerance among older adults is that insulin is less biologically active with age (15). Inadequate dietary zinc and chromium intake also may be associated with the observed increase in glucose intolerance among older adults (5,7,14,16).

The micronutrients zinc and chromium both have roles in insulin action and glucose homeostasis (14,17). Zinc is involved in many enzyme systems involved in both the activation and inhibition of glucose metabolism (18). Zinc also has a role in insulin biosynthesis, storage and release from pancreatic beta cells, and is involved in insulin configurational changes which enhance its binding to receptors (18,19). Chromium also has a major role in maintaining normal glucose metabolism and in potentiating insulin response (14,20). Chromium is an integral part of the glucose tolerance factor, or dinicotinic acid glutathione complex (21).

The role of chromium and zinc with insulin, glucose tolerance and diabetes is a particular concern among older adults due to reported poor dietary intakes of these trace minerals among the elderly. Although there is minimal evidence of zinc deficiency among adults in the United States, several studies have indicated that many elderly have zinc intakes below the Recommended Dietary Allowance (RDA) for zinc of 15 and 12 mg per day for men and women aged 51 and above, respectively (3,22-24). Sandstead et al. (23) reported zinc intakes among the elderly to be approximately 10.5 mg and 7.6 mg per day for men and women, respectively, using the Nationwide Food Consumption Survey data. In addition, several studies have also reported that average dietary chromium intakes among the elderly are

below the adults estimated safe and adequate daily dietary intake (ESADDI) of 50 to 200 micrograms per day (6,20,25). Bunker et al. (20) reported average chromium intakes among the elderly of 25 ug/day.

As a result of the increased incidence of diabetes among the elderly, the roles of zinc and chromium with glucose and insulin metabolism, and the reported low dietary intake of these micronutrients among older adults, the effect of zinc or chromium supplementation on plasma glucose and serum insulin in adults age 50 and above needs further investigation.

Objective

The objective of this research project was to evaluate the effects of zinc or chromium supplementation on plasma glucose and serum insulin concentrations in adults age 50 and older.

Null Hypotheses

Ho 1: There will be no significant relationship between initial dietary intake and plasma glucose or serum insulin concentrations in adults age 50 and older.

Ho 2: There will be no significant effect due to eight weeks zinc supplementation on mean plasma glucose concentration in adults age 50 and older.

Ho 3: There will be no significant effect due to eight weeks zinc supplementation on mean serum insulin concentration in adults age 50 and older.

Ho 4: There will be no significant effect due to eight weeks chromium supplementation on mean plasma glucose concentration in adults age 50 and older.

Ho 5: There will be no significant effect due to eight weeks chromium supplementation on mean serum insulin concentration in adults age 50 and older.

Assumptions

It was assumed that the subjects:

1. were healthy for their age group.
2. were not diabetic.
3. took the supplement as directed (one capsule each morning and evening with meals).
4. did not change their exercise or eating habits during the course of the study.
5. correctly recorded their three day food intakes before each data collection period.
6. fasted twelve hours before each data collection period.
7. maintained their weight throughout the course of the study.

Limitations

Limitations of the study included:

1. Subjects participating in the study were volunteers.
2. Subjects were on self-selected diets.
3. Subjects food records were self-reported.
4. The sample size was small, less than 10 subjects per treatment group.
5. Subjects received supplementation for only eight weeks.
6. The majority of the subjects had normal fasting plasma glucose and serum insulin concentrations.

Definitions of Terms

ELDERLY ADULTS: Those persons age 65 and older (1).

OLDER ADULTS: Those persons age 50 and older.

Thesis Format

The bibliographic citations in this thesis follow the American Journal of Clinical Nutrition format.

CHAPTER II

REVIEW OF LITERATURE

Due to the increased incidence of diabetes and glucose intolerance with age, and the role of zinc and chromium with glucose tolerance and insulin action. Understanding trace minerals and their importance in the elderly, this chapter contains a review of the literature on zinc and chromium, their roles with glucose and insulin, and how these may affect the elderly.

ZINC

Food Sources

The recommended dietary allowance for zinc is 15 mg and 12 mg per day for adult men and women respectively (26). Meats and dairy products are the major dietary sources of zinc in typical American diets. Estimates indicate that 43% of dietary zinc is provided by meat, poultry and fish, and 25% is provided from milk, ice cream, eggs and cheese (23). Whole grain breads, nuts, wheat germ and shellfish are rich sources of zinc whereas white fish and vegetables are poor sources (27).

Zinc bioavailability from foods ranges between 14% and 41% (28). Animal products such as eggs, meat, seafood, and liver provide highly available zinc, whereas zinc from plant products such as whole grains is less available (23,28,29). Compounds in food, such as ferrous iron, phytates and phosphorus, can adversely affect zinc absorption (23,30,31,32). Lonnerdal et al. (33) reported low zinc absorption from soy formulas with high phytate content. In addition to compounds in food affecting zinc absorption, an individuals' need also can affect zinc

absorption. Increased need for zinc has been observed to result in increase zinc absorption (34).

The zinc body pool in a normal 70 kg adult is about 1.5 to 2.0 grams. Plasma zinc is present primarily bound to albumin, but can also bind to other proteins, such as transferrin and ceruloplasmin (34). Zinc is present in all body tissues, fluids, and secretions, but is chiefly found in skeletal muscle and bone (34).

Zinc is an essential trace mineral which has many important roles in the body (35,36). Zinc functions to preserve enzyme integrity, and is involved with more than 80 metalloenzymes and proteins, including the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (35,36,37). Zinc is necessary for growth, cell division, reproduction, wound healing, taste acuity, and normal immune function (23). Zinc also is directly involved in insulin metabolism (38).

Common signs of zinc deficiency include cell mediated immunological abnormalities, loss of appetite, decreased taste acuity, retarded growth and skin changes (23). Decreased insulin response and impaired glucose tolerance have also been observed with zinc deficiency (28). Zinc deficiency can have a significant effect on serum albumin, transferrin and prealbumin concentrations. Seven patients with various underlying diseases became zinc deficient on TPN solutions that contained only 0.5 mg/L of zinc. Compared to healthy controls, all patients had decreased mean serum concentrations, of 22 ug/dl for zinc, 3.2 g/dl for albumin, 211 g/dl for transferrin, and 14.5 mg/dl for prealbumin. All concentrations increased after supplementation with 220 mg zinc sulfate (50 mg of elemental zinc) up to three times daily or 3 to 12 mg zinc sulfate intravenously. All other nutritional support remained the same. The authors did not report duration or amount of zinc supplementation for each patient, but stated that serum zinc and prealbumin increased rapidly after zinc supplementation. After zinc

supplementation, improvements in skin lesions were observed among these subjects. Diarrhea and impaired immune function, problems associated with poor zinc status, also improved upon supplementation (39).

Zinc is considered to have a low toxicity threshold (40). Moderately elevated zinc intakes are not easily detected; however, zinc toxicity has been observed after ingestion of 2 or more grams of zinc as zinc sulfate. Symptoms of zinc toxicity include nausea, vomiting, abdominal cramps, and gastrointestinal irritation (41). Increased zinc intakes also have been shown to adversely affect copper status (42).

Zinc Effect on Glucose Tolerance and Insulin

Insulin is stored in the beta cells of the pancreas as zinc crystals (8,43). Zinc is involved in synthesis, storage, and release of insulin from pancreatic beta cells (19,44). Incorporation of zinc into insulin induces conformational changes which aids insulin in binding to receptors (43). In addition, zinc enhances cell uptake of insulin (45,46).

It is postulated that decreased zinc status may correlate with decreased glucose metabolism (18,47). Huber et al. (48) observed significant decreases in insulin concentrations among zinc deficient rats compared with pair fed controls. Zinc deficient rats demonstrated impaired glucose tolerance when a glucose challenge was administered at higher infusion rates versus lower rates, indicating peripheral insulin resistance (18). Hendricks and Mahoney (49) also observed impaired glucose tolerance among zinc deficient rats when glucose was administered intraperitoneally, although glucose intolerance was not evident when glucose was administered orally. This impaired glucose tolerance developed slowly with low zinc diets, and was corrected within one week with zinc supplementation. The

authors speculated that zinc deficiency impaired the release of insulin from the pancreas, which results in elevated glucose concentrations. It also has been proposed that decreased pancreatic insulin release observed with zinc deficiency may lead to increased insulin resistance (9, 40). However, in a study by Quarterman and Florence (50), no effects on glucose tolerance and plasma insulin were observed among zinc deficient rats.

One hypothesis is that zinc may stimulate glucose metabolism by some mechanism not yet identified that mimics the biological effects of insulin. Shisheva et al. (45) observed that oral zinc supplementation lowered plasma glucose concentration among diabetes-induced rats. Zinc stimulated glucose use in muscle tissue, which in turn lowered glucose concentrations in the entire animal. The researchers postulated that zinc is involved in mechanisms which stimulate hexose transport, and also that zinc may enhance the oxidation of glucose to carbon dioxide by glycolysis and by the pentose phoshate pathway. These researchers concluded that oral zinc supplementation results in the ability of tissues to metabolize glucose, although normal concentrations cannot be reached by a single treatment. Circulating zinc is often lower among diabetics, therefore, this information may also be useful in evaluating zinc homeostasis among humans and its' relationship to IDDM or NIDDM.

Diabetes itself may have an effect on zinc absorption (18). Car et al. (47) observed significantly decreased serum zinc concentrations among 30 IDDM and NIDDM subjects, mean age 46, compared to 15 controls of similar age. However, Kinlaw et al. (38) studied zinc metabolism in 20 controlled NIDDM patients, 25% of whom had hyperzincuria. Using fasting blood samples before insulin administration, it was determined that serum zinc concentrations did not differ between diabetic subjects and non-diabetic controls. However, zinc loss via urine

was greater when proteinuria was also present, which is explained by the fact that zinc is protein bound. In this study, urinary zinc excretion was correlated positively with serum glucose concentrations. Also, serum zinc did not correlate with glycosylated hemoglobin concentrations among these subjects. In addition, Honnorat et al. (45) studied 53 diabetic subjects including 18 with IDDM and 35 with NIDDM. Of the NIDDM subjects, 22 were treated with oral antidiabetic agents and 13 were treated with insulin. All of the subjects demonstrated normal mean plasma zinc concentrations of 15.3 umol/L. However, urinary zinc concentrations were increased in IDDM and NIDDM subjects being treated with insulin, with concentrations of approximately 16.2 umol/24 hours, whereas NIDDM subjects not being treated with insulin had normal urinary zinc values of 11.3 umol/24 hours. These data indicate that urinary losses of zinc were not related to decreases in plasma zinc or altered zinc status. Explanations for normal plasma zinc in the presence of hyperglycemia may be that the quantity of zinc is insufficient in long-acting insulin or that absorption of zinc may be enhanced in diabetic subjects. There also may be decreased intestinal excretion of zinc and increased tissue catabolism, although no relationship was observed between plasma or urinary zinc and Body Mass Index (BMI) or age among any subjects. The authors reported that hyperzincuria was not related to glycosylated hemoglobin concentrations. Hyperinsulinemia is not usual among IDDM subjects which may explain the increased urinary zinc excretion despite insulin treatment in this study.

CHROMIUM

Food Sources

The estimated safe and adequate daily dietary intake for chromium (ESADDI) in adults is 50 to 200 ug per day (26). Foods rich in chromium include turkey-ham, liver, whole grain products, brewer's yeast, raisins, black pepper, prunes, coffee, tea, beer, and wine (51,52). Dairy products are generally poor sources of chromium. Some types of food processing can increase the chromium content of foods through use of stainless steel equipment (51). Anderson et al. (52) found higher contents of chromium in packaged or prepared foods such as English muffins, waffles and bagels. However, milling has been observed to decrease chromium content of grain products. To date, accurate and complete data on chromium content of foods are limited (51).

Intestinal absorption of inorganic chromium is relatively low in humans. However, chromium absorption from the biologically active organic complex (GTF) is high (51). It has been reported that chromium from food sources and chromium chloride ($CrCl_3$) supplements have similar absorption rates (53). Dietary chromium intake level can also affect chromium absorption. Anderson and Kozlovsky (6) reported that chromium absorption was inversely related to dietary intake in a study including 10 males and 22 females, aged 25 to 65. Approximately 2% of dietary chromium is absorbed at intakes of 10 ug/day and 0.5% at chromium intakes of 40 ug/day.

The chromium body pool is approximately 1.7 mg (54). However, reliable methods for evaluating clinical chromium status are not available to date (16,55). Blood plasma, serum, urine and hair do not adequately reflect chromium status.

Currently, relative chromium status is commonly evaluated using effects of chromium supplementation on glucose tolerance (25,56).

Chromium is an essential trace element which functions to maintain normal glucose metabolism and facilitate insulin function (7,14,16,20). Trivalent chromium is an integral part of the glucose tolerance factor (GTF), or dinicotinic acid glutathione complex. The exact structure of GTF, first identified in brewer's yeast, has not been determined (21,57). Glucose tolerance factor is not considered a trace element or vitamin, instead the biologically active chromium complex is believed to resemble a hormone which is released in the blood in response to a stimulus, such as insulin. Chromium potentiates the movement of glucose into the tissues, increases insulin activity and therefore, can reduce the amount of insulin necessary to control blood sugar (14,16). Chromium is also believed to have a role in nucleic acid and lipid metabolism (6,21).

Impaired glucose tolerance has been observed with chromium deficiency (14,16,20,58,59). In patients receiving total parenteral nutrition (TPN) solutions containing no chromium, chromium supplementation resulted in greatly improved insulin status and glucose tolerance (58,60,61). In one case, chromium deficiency resulted after 5 months on TPN. The chromium content, if any, in the TPN solution was not provided by the author, however, daily supplementation with 150 ug of chromium resulted in improved glucose tolerance, decreased insulin requirement, weight gain and cessation of encephalopathy (60).

Due to low absorption of trivalent chromium, toxicity from dietary intake is rare (62). In one animal study, no toxicity was observed in rats consuming 5 mg/liter in water or 100 mg/kg in the diet throughout their lifetimes (63). Trivalent chromium does not appear to be carcinogenic. However, increased bronchial cancer incidence has been associated with occupational exposure among industrial workers

to hexavalent forms of chromium, which may be inhaled or absorbed through the skin. To prevent workers from the risks of excess contact and exposure to chromium compounds, the United States observes an atmospheric limit or a threshold limit value-ceiling (TLV-C), which should never be exceeded (64).

Chromium Effect on Glucose Tolerance and Insulin

Lowered dietary chromium intake may be a factor in the development of age related decreases in glucose tolerance and insulin response (7,65). However, current studies are inconclusive as to the effects of chromium supplementation on glucose tolerance, blood glucose concentrations, and insulin sensitivity among the elderly.

Lui et al. (59) observed a 50% improvement in glucose tolerance among older women, aged 40 to 75 years after three months daily supplementation with 4 ug of chromium in 5 g of brewer's yeast. In addition, decreased insulin release, indicating beneficial glucose control, was noted among both older and younger subjects following chromium supplementation.

Anderson et al. (7) also reported a beneficial effect of chromium supplementation among hyperglycemic subjects consuming controlled diets which contained inadequate dietary chromium, less than 20 ug per day. In these subjects, 0 to 90 minute glucose and insulin concentrations and glucagon were elevated on the controlled inadequate chromium diets. After supplementation with 200 ug $CrCl_3$ for five weeks glucose, insulin and glucagon concentrations decreased in these subjects. The authors suggested that these results could indicate that chromium may have an effect on glucose and insulin concentrations by increasing glucagon sensitivity.

In addition, a beneficial effect was observed by Offenbaucher and Pi-Sunyer (16), in a study including both nondiabetic and NIDDM subjects, mean age 78, supplemented daily for eight weeks with 9 grams of chromium-rich brewer's yeast

(experimental) or chromium-poor torula yeast (control). Improved glucose tolerance and insulin sensitivity were observed in the chromium supplemented groups compared to the control. Improvements in glucose tolerance and insulin sensitivity also were observed even when diabetic subjects were omitted from the analysis; these subjects were omitted in secondary analysis because they displayed better initial glucose tolerance than non-diabetic subjects.

However, Riales et al. (65) observed inconclusive results among 23 men, aged 31 to 60, supplemented with 200 ug/day of chromium chloride 5 days/week for 12 weeks. In this study, an improvement in glucose tolerance was observed at six weeks, but not at 12 weeks, among 8 subjects in the supplemented group (n=12) and among 6 subjects in the placebo group (n=11). The author stated that the observed decrease in glucose tolerance among both groups at 6, but not 12 weeks supplementation may be due to noncompliance at 12 weeks or an unconscious change in lifestyle. However, the authors did observe a trend towards decreased insulin concentrations in the chromium supplemented group after 12 weeks supplementation, possibly indicating increased insulin sensitivity.

No significant effect was observed by Uusitupa et al. (66) in fasting glucose tolerance or serum insulin concentrations after 6 weeks supplementation with 200 ug chromium chloride compared to a placebo among 6 male (mean age 58 years, range 37 to 65) and 4 female (mean age 63 years, range 59 to 68) NIDDM subjects.

In addition, no effect due to chromium supplementation was observed in fasting oral glucose or insulin concentrations in a study by Offenbacher et al. (67) including 23 healthy elderly adults, 19 females and 4 males with an age range from 63 to 86 years, supplemented with 5 ug chromium as brewer's yeast, 200 ug chromium chloride or a lactose placebo for 10 weeks. The researchers reported no significant effects in fasting oral glucose or insulin concentrations.

ELDERLY

Increased Incidence of Diabetes with Age

Although not inevitable, health status often declines with advancing age. Older adults have a high incidence of chronic disease, medication use and sedentary lifestyle (5). Poor dietary intake among the elderly may contribute to declining health status and increased risk of diet-related diseases such as obesity, coronary heart disease, glucose intolerance, and maturity-onset diabetes (5-7,68).

Diabetes is of particular concern among older adults. Diabetes is one of the six leading causes of death after age 65 (1). Approximately 20 to 30 percent of individuals aged 65 or older have impaired glucose tolerance, as indicated by elevated fasting glucose concentrations, while 10 to 35 percent have actual diabetes (10). Increased postprandial plasma glucose concentrations have been reported with age (11,12). Morley et al. (11) has reported increased postprandial plasma glucose of approximately 5 to 10 mg/dl per decade after age 50, while fasting glucose concentrations increased only 1 to 2 mg/dl per decade after age 50 (13). In addition, decreased glucose tolerance has been reported with increased age (12,13). This is of concern because impaired glucose tolerance is a leading indicator of non-insulin dependent diabetes (14). Hypotheses for the observed decrease in glucose tolerance among older adults include: decreased biologically active insulin, less responsive pancreatic function, or decreased insulin sensitivity resulting from altered pancreatic beta cell function (15,69). Increased glucose intolerance among older adults also may be associated with inadequate dietary zinc and chromium intake (5,7,14,16).

Dietary Intake Among the Elderly

Specific nutrient needs for the elderly have not been evaluated extensively. The RDA's for adults age 51 years and over are extrapolated from guidelines based on younger, healthy individuals (26). Therefore, it is difficult to determine whether reported intakes of older adults are adequate to maintain health (70). There may be an increased or decreased need for certain vitamins and minerals with advanced age (71).

The nutritional intake of older adults may be compromised by many factors including: socioeconomic pressures, depression, reduced mobility, loneliness, aging body tissues, physical and mental disabilities, and food and drug interactions (3,8,9).

Consumption of poor diets is common among individuals over age 65, and poor diets are increasingly prevalent among those 85 years of age and older (72). A decline in total energy intake implies micronutrients obtained from these energy sources will also decrease (70,72,73). The National Health and Nutrition Examination Survey (NHANES II) reported that mean caloric intakes decreased below recommended intakes among elderly men and women beginning at approximately age 50 and continuing through age 74 (74). For example, decreased zinc intake was reported among older adults who consumed inadequate calories (23,72). There is concern that decreased nutritional status or dietary intake among the elderly, especially related to zinc and chromium intake, may lead to increased insulin resistance and glucose intolerance (17,18,20,58).

Zinc Intake Among The Elderly

Although there is minimal evidence of zinc deficiency among adults in the United States, some studies have indicated that the elderly have zinc intakes below

the RDA (75). Using the Food and Drug Administration's Total Diet Study for 1974-1982, 1982-1984, and 1982-86, Pennington et al. (76) reported that mean intakes of women aged 60 to 65 years were 8.7 mg of zinc daily whereas mean intakes for men of the same age were 12.9 mg/day. In addition, using the Nationwide Food Consumption Survey data, Sandstead et al. (23) reported zinc intakes of elderly were approximately 10.5 mg and 7.6 mg daily for men and women, respectively.

It has been suggested that zinc deficiency may be underdiagnosed among the elderly (23). Decreased tissue zinc concentrations have been reported among elderly populations even though plasma zinc levels were similar to controls. Using 24-hour recalls, taste acuity tests, and hair zinc concentrations, Hutton et al. (77) determined that the elderly were at increased risk for zinc deficiency. Estimates of dietary zinc intake appear to be valid, however, some studies indicate that taste acuity tests and hair zinc levels are not indicative of zinc status (3,23).

Chromium Intake Among the Elderly

Several studies have reported that the elderly have higher chromium intakes than young adults, and while chromium intakes were independent of calorie consumption, they were still below the ESADDI (25). Anderson et al. (6) estimated that 90% of normal diets in the United States do not provide the minimum intake of 50 ug chromium per day as suggested by the U.S. National Academy of Sciences. In addition, Anderson reported that chromium absorption was significantly higher in women than men, which may be a result of lower chromium intake and lower caloric intakes among women (6).

Anderson et al. (78), chemically analyzed daily chromium intakes and reported mean daily intakes of approximately 38.8 ug/day for 8 male subjects and

23.1 ug/day for 11 female subjects, 22 to 65 years of age. These values are below the ESADDI of 50-200 ug/day (26). In another study, Anderson and Kozlovsky (6) analyzed the chromium content of self-selected diets by collecting duplicate daily composites of all foods and beverages consumed by subjects. Chromium content of dry ashed samples was determined by atomic absorption spectrophotometry. The reported mean daily chromium intakes were approximately 33 ug/day for 10 male subjects and 25 ug/day for 22 female subjects, 25 to 65 years of age (6). Among subjects aged 70 to 85, Bunker et al. (20) analyzed duplicate samples of self-selected diets of subjects by dry ashing and performing atomic absorption spectrophotometry, and reported chromium intakes of 29.8 ug/day for 11 male subjects and 20.1 ug/day for 12 female subjects.

Supplement Use Among the Elderly

Varying doses of single and multi-nutrient supplements are widely available in local grocery and drug stores (Appendix A). The use of one or more non-prescription supplements is common among the elderly (79,80).

Using NHANES I data, Kim reported 22.5% of subjects qualified as regular daily supplement users and 10% as irregular users (79). In another study, evaluation of 20,080 persons aged 18 to 99 years from the 1987 National Health Interview Survey (NHIS), a cross-sectional, continuing, nationwide survey, reported that approximately 51% of respondents consumed a vitamin and/or mineral supplement within the last year, while 23% of those consumed supplements daily (80). In this report, older adults, whites, and women were found to be more likely to use supplements regularly.

Concerning the use of single-mineral supplements, 13% and less than 0.5% of the respondents in the 1986 NHIS survey consumed a zinc or chromium

supplementation product, respectively (48). Zinc supplements were most commonly consumed as zinc sulfate, gluconate, oxide or chloride while chromium was most commonly consumed as chromium chloride.

EVALUATION METHODS

Evaluating Dietary Assessment Methods

Dietary assessments are important techniques used to evaluate dietary intakes of individuals; however, currently there is no ideal method for evaluating dietary intakes (81). To date, the most widely used methods for assessing dietary intake include 24-hour recalls, food frequency questionnaires, and food records (81,82).

A 24-hour recall is a retrospective method to evaluate dietary intake in which the subject relies on memory to recollect food intake from the past 24 hours (83). One concern with 24-hour recalls is that they may not represent a subjects usual intake (83); however, Howat et al. (81) reported 24-hour recalls were reliable for energy and macronutrient intake among 44 women age 18 to 49 years of age.

A food frequency questionnaire is an inexpensive food assessment method which consists of a record of "usual" or average dietary intake over a specific time period, such as one to twelve months (84). A food frequency questionnaire contains information concerning how often most common foods are consumed and standard portion sizes (85). It has been reported that older adults tend to significantly underestimate average food intakes using food frequency questionnaires (81,86,87), which may be due to decreasing short-term memory in advancing years (73,86,88). Younger adults aged 24 to 51 tend to overestimate food consumption using food frequency questionnaires (85).

A food record is a written record of food intake immediately at the time of consumption. This method requires participation from literate adults who have been trained to record food intake and estimate portion sizes (81). It is also important that food records are checked by trained interviewers. Food records can be recorded for several days. Seven-day records are considered to be the best representation of usual food intake, but may often be difficult to obtain from subjects (82). However, Gersovitz et al. (89) reported that food records for more than three days were not valid with population studies. Three-day food records are generally more accepted by subjects (82), and are considered an accurate assessment of dietary intake for moderately sized studies (87). One controversy concerning use of food records is whether or not to include food intake over weekends due to variation in food intake from weekend to weekday. St. Jeor et al. (90) included weekend days in their four-day food record to include any variation in nutrient intake from weekday to weekend. Decisions must be made as to which days of the week to record. In a recent study by Larkin et al. (82), estimation of energy and nutrient intakes from random three-day dietary records were similar to consecutive three-day dietary records.

Despite which method is used to evaluate dietary intake, it is important that the serving sizes reported be valid and reliable (91). Validity accurately assesses usual intake of subjects while reliability relates to reproducibility or consistency of reporting data across different measurement periods (81). Individual variation in estimating portion sizes are immense unless controlled by standard measures. Therefore it is advantageous to use food models to achieve consistent food portion from different subjects (92). Moore et al. (92) reported that graduated food models made from actual food covered in paraffin resulted in reduced frustration among

interviewers and respondents, as well as increased accuracy in reported serving size, volume or weight.

Evaluating Anthropometric Measures

Anthropometric measures are used to determine body weight, height, body fat and lean body mass. As age progresses, changes in anthropometric values are observed and need to be taken into account in measurement procedures.

Body weight is measured using a standard calibrated scale. A study by Schoeller (93) indicated total body water tends to decrease with age, indicating decreases in fat-free mass. Body height can be measured by standing in an upright position. However, because height tends to decrease with age, an alternate method to estimate height is knee height stature (94). The long bone below the knee is not affected by age-related shrinkage and is an accurate measure of overall height among the elderly. Accurate height estimates are important because height is used in other estimates of body composition such as determining lean body mass via bioelectrical impedance analysis (BIA) or body fat via body mass index.

Bioelectrical impedance analysis (BIA) measures electrical conductivity of fat-free tissue using whole body reactance and resistance to estimate lean body mass and total body fat. Bioelectrical impedance analysis is an ideal measurement of lean body mass because it is safe, reproducible, quick and painless, and does not require any physical demands or invasive procedures (95,96,97). Bioelectrical impedance does depend on subjects' body water. An individual's hydration status can be affected by alcohol, diuretics or caffeine, recent exercise or food intake, and abnormal body temperature (93). Some researchers report that BIA is valid for older adults even though body water tends to decrease with age, because there is little change in the relationship between total body water and fat-free mass in aging

(98). However, Deurenburg et al. (95) reported that BIA prediction formulas defined for younger populations overestimated fat free mass (FFM) by approximately 6 kg due to decreased total percent body water with age among elderly men and women. BIA age-specific equations are available (99), and the choice prediction formula for estimation of FFM among the elderly comes from a study by Deurenburg et al. (95) who measured body composition in 35 men and 37 women aged 60 to 83 years.

Body mass index provides a reasonable estimate of body fat if weight and height measures are valid (100). Body mass index is calculated as weight (kg)/height (meters)^2 (101,102). Deurenburg et al. (102) reported that BMI correlates with body fat among younger adults, but due to fat redistribution with age, as well as decreases in lean body mass and height, BMI may be misleading among older adults. However, Micozzi et al. (100) reported that BMI is reliable among the elderly. Body mass index values of greater than 27.8 for men and 27.3 for women indicate the person is 20% overweight whereas values between 22.7 to 27.8 for men and between 22.4 to 27.3 for women are considered to be within desirable body weight (101).

CHAPTER III

MATERIALS AND METHODS

EXPERIMENT DESIGN

This study was approved by the Institutional Review Board (IRB) for human subject research at Oklahoma State University (Appendix B). A randomized, block experimental design consisting of three treatment groups, and four measurement periods was used for this study. Male and female subjects were randomly assigned to one of three experiment groups 1) placebo, 2) chromium supplementation or 3) zinc supplementation. There were 8 subjects per experimental group. Data were collected at baseline, after four and eight weeks of supplementation and four weeks after supplementation ended.

The principal investigators and research assistants involved in data collection for the study received training on laboratory safety, biological hazard disposal and radioisotope handling.

Subjects

After approval by the Oklahoma State University IRB, subjects were recruited by announcement through local physicians' offices, college and university mailings, local newspaper and contacts with senior citizen organizations (Appendix C). Subjects reviewed and signed an informed consent prior to participating in the research study (Appendix D). Twenty five volunteers over the age of 50 who were free of chronic disease, not taking medication to lower blood cholesterol and who were not attempting to gain or lose weight were included in the study. Originally, it was intended that subjects' plasma cholesterol concentration be above 240 mg/dl to

be admitted into the study, but the majority of the subjects did not meet this guideline. Four women who were on hormone replacement therapy were included in the study, two were in the zinc supplemented group and two were in the chromium supplemented group. One subject was omitted from the study because he was nine years younger than other subjects. Another subject was omitted due to a high ferritin concentration which may be indicative of hemochromatosis, a condition which may lead to development of non-insulin dependent diabetes (103). However, Dinneen et al. (103) also reported that non-insulin dependent diabetes is not typically associated with iron overload, therefore, two subjects with ferritin concentrations indicative of iron overload were not omitted.

Twice each day during eight weeks of the study, subjects consumed a lactose placebo or a supplement containing either zinc (as zinc sulfate) or chromium (as chromium chloride). Subjects were instructed to take one capsule each morning and one each evening with meals. Subjects received a four week supply of capsules at the baseline measurement period. Subjects returned any capsules that were not consumed during the first four weeks of supplementation at the second measurement period. At the second measurement period, subjects received a second four week supply of capsules. Subjects returned any capsules that were not consumed during the second four weeks of supplementation at the third measurement period. During each measurement period, subjects kept a three-day written food record. At each measurement period, each subject also completed a health questionnaire and participated in anthropometric measurements and blood collection.

Supplements

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

(Apothecary Products, Inc., Burnsville, Minnesota) were filled using a gelatin capsule filling machine (Quanterron, Inc., Burnsville, Minnesota). Placebo capsules contained approximately 0.25 grams (g) lactose.

The chromium supplement consisted of .8611 g of $CrCl_3 \cdot 6H_20$ in 420 g lactose, so that 0.25 g of the chromium supplement mixture was calculated to contain 100 micrograms (ug) chromium as chromium chloride. The lactose and chromium chloride were mixed together for seven hours in a ball mill to ensure even distribution of the chromium chloride in lactose.

The zinc supplement was prepared in the same manner as the chromium supplement. The zinc supplement consisted of 110.7206 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) in 420 g lactose, so that .25 g of the zinc supplement mixture was calculated to contain approximately 15 milligrams (mg) zinc as zinc sulfate in lactose.

Due to hydration, the analyzed supplements' mineral concentrations were lower than calculated values. Therefore, the amounts of chromium chloride and zinc sulfate were adjusted and supplement mixtures were remixed to adjust the mineral concentrations of the supplements.

In order to evaluate the accuracy of the supplements, four capsules from each supplement group were chosen at random and analyzed for chromium and zinc content using an atomic absorption spectrophotometer, Model 5100PC (Perkin-Elmer Corp., Norwalk, CT). Supplement and capsules were wet and dry ashed using a modification of the Hill et al. (104) method. The average analyzed chromium content of the chromium supplements was 119.66 ug per capsule. The average analyzed zinc content of the zinc supplement was 15.18 mg per capsule.

Questionnaire

1. Health information questionnaire:

Prior to beginning the study, each subject was interviewed regarding information pertaining to past medical history, type of special diet, current medication, dietary supplements used in the past six months, recent serious illness or surgery, serum cholesterol concentrations, exercise, and family history of elevated cholesterol or heart disease (Appendix E).

2. Monthly data collection form:

Each subject completed a monthly data collection form at each of the four measurement periods. The monthly data collection form consisted of questions pertaining to presence of cold, flu or other illnesses in the last month, continuation of supplementation in the presence of illness if any, or a change in the exercise pattern (Appendix F).

DATA COLLECTION

Height, Weight, and Skinfold Measurements

Subject's standing height was measured at the first and third measurement periods. With the subject standing erect against the wall with barefoot heels together, a scaled rule attached to the wall was read to the nearest 1/4 inch. Subject's body weight was measured at all four measurement periods using a calibrated beam balance scale (Continental Co., Chicago). Accuracy was read to the nearest one/fourth pound increment.

Bioelectrical Impedance

Bioelectrical impedance is used to measure a subject's body fat. Body fat was measured at the first and third data collection periods by a research assistant trained in the procedure. The bioelectric impedance model used was Biodynamics Model 310 Body Composition Analyzer (Chattanooga Corporation, P.O. Box 4287, Chattanooga, TN 37405).

The subjects were briefed on the bioelectrical impedance procedure and then asked if any heart condition or pacemaker existed. If so, this procedure was eliminated from the data collection. The subjects were assumed to be in adequate hydration status and were measured after a 12-hour fast. Subjects were measured twice at each collection period using new electrodes for each measurement to ensure accuracy. The measurements were then averaged.

Deurenburg et al. (95) report a prediction formula, applicable to those persons aged 60 and above, for estimation of fat-free mass (FFM) from bioelectrical impedance and anthropometric variables as:

$FFM\ (kg) = (0.671 \times 10^4 \times H^2/R) + 3.1S + 3.9$

where H is height (m), R is resistance (ohms), and

S is gender (females, 0; males,1)

The procedure for bioelectrical impedance determination is found in Appendix G.

Knee Height

Often stature decreases with age or osteoporosis. Knee height measurement can be used to estimate stature of adults 60 to 90 years old. It also can be used with other anthropometric measures to predict weight. The knee height procedure is a

reliable estimate of stature when used to adjust body composition measurements included in bioelectrical impedance (94).

Subjects' knee height was measured during the second collection period. Measurements were performed in duplicate and later averaged. Measurements were read to the nearest 0.5 centimeter (cm).

The model used was the Ross Knee Height Caliper (1989), Ross Laboratories, Columbus, Ohio 43216. The procedure for knee height collection is found in Appendix G. A diagram of the knee height procedure is found in Appendix H. A nonogram or prediction equation can be used to determine height. Equations were provided by Ross Laboratories, Columbus, Ohio 43216, distribution # 50452, 1989. The gender and age specific equations are as follows:

Stature for men= [2.03 x knee height(cm)] - [0.04 x age(yr)] + 64.19

Stature for women=[1.83 x knee height(cm)]-[0.24 x age(yr)] + 84.8 (105).

Blood Collection

Subjects fasted for 12 hours prior to each blood collection. Due to variations in plasma mineral concentrations with the time of the day, all blood collections were performed between 7:30 and 9:30 AM. Blood collection was performed by a phlebotomist. The procedure for blood collection can be found in Appendix I.

Dietary Records

Before beginning the study, subjects were trained by a registered dietitian for recording three day dietary intakes. A modification of the Moore (92) method was used for food models. Food models constructed of dried beans wrapped in netting were used to illustrate 1 cup, 1/2 cup and 1/3 cup portion sizes. Wooden blocks were used to illustrate 3 oz meat/cheese portions. Registered dietitians or research

assistants reviewed the 3-day dietary records descriptions, corrections, and additions with each subject on the day of blood collection. The dietary data were recorded on three consecutive weekdays prior to each measurement period (Appendix J).

Dietary Record Analysis

The three-day dietary food records were analyzed using the Food Processor Plus program (version 5.03, ESHA Research, Salem OR). Diets were analyzed for each set of three day dietary records. Data used in this study from the diets analyzed using Food Processor Plus program included kilocalories, protein, carbohydrate, fiber, total fat, zinc, percent of calories from protein, percent of calories from carbohydrate, and percent of calories from fat.

BIOCHEMICAL ANALYSES

Glucose

Plasma glucose was measured by a quantitative, enzymatic (glucose oxidase) determination method (Sigma Diagnostics procedure No. 510, St. Louis, MO). At each measurement collection period, analysis was performed with three hours of blood collection. All samples were performed in duplicate and then averaged. If duplicate values were not within 5% of each other, the analysis was repeated. This method was modified to use smaller amounts of plasma that could still be detected by the spectrophoto-meter (Appendix K). All tubes were covered with foil to prevent exposure to light and incubated at room temperature for 45 minutes. Absorbances of the standard and test samples were read against a blank as referenced at 450 nm using a Gilford spectrophotometer (Response Series, Ciba Corning

Diagnostics, Corp., Oberlin, OH). All readings were completed within 30 minutes of initiating the procedure.

Albumin

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

RADIOIMMUNOASSAY

Insulin

Serum insulin was measured by double antibody radioimmunoassay (RIA). Insulin determination is based on the ability of an antibody to bind a fixed amount radioactive isotope. Frozen serum samples were thawed and 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. For each sample, duplicate values were averaged. If duplicate values were not within 5% of each other, samples were reanalyzed. The Equate (Equate RIA, South Portland, ME) option 1 procedure was followed. Insulin tracer and antiserum was added to all tubes using an Eppendorf repeating pipette. All tubes were vortexed, covered with parafilm, and incubated at room temperature for ten minutes. All tubes were centrifuged at 4 degrees Celsius at 1500 x g for 10 minutes. The supernatant of each tube was decanted using a blotting technique, so that only the pellet remained. The tubes were counted for one minute using a gamma counter (Packard Cobra II, Auto Gamma, Packard Instrument Co., Meriden, CT).

Ferritin

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

DATA ANALYSIS

Data were analyzed using the Statistical Analysis System (SAS Inst. Inc., Cary NC, 1987) version 6.06. Treatment means at baseline, four and eight weeks supplementation and four weeks post supplementation were compared to the placebo using the SAS General Linear Model procedure, significant differences were determined by Least Squared Means. Correlation coefficients were determined at baseline between dietary parameters and plasma glucose and serum insulin concentrations. Significance level was set at 0.05.

CHAPTER IV

RESULTS AND DISCUSSION

The subjects in this study were independently living volunteers from the community of Stillwater, Oklahoma. Initially, twenty-nine subjects participated in the study; however, three dropped out before completion. Two subjects completed the study but were not included in the data analysis, because one subject was outside of the age range of the other subjects, and another had an extremely elevated initial ferritin concentration of 752 ng/ml. Therefore, twenty-four subjects were included in the data analysis. Of these twenty-four subjects completing the study, there were 8 subjects per supplement group. Two subjects in the zinc supplement group did not have bioelectrical impedance measurements administered due to cardiac irregularities. One subject in each group, placebo, chromium, and zinc, missed the second data collection period.

Description of Subjects by Gender

For the entire sample population, there were 11 males and 13 females with a mean age of 65 years, and an age range of 51 to 82 years of age. There were 5, 2, and 4 males and 3, 6, and 4 females in the placebo, chromium and zinc supplement groups, respectively (Table 1). Male subjects had a mean age of 62 years, mean height of 70 inches, mean weight of 179 pounds, and a mean body mass index (BMI) of 25.8 (wt/ht^2). Female subjects had a mean age of 68 years, mean height of 63 inches, mean weight of 142 pounds, and a mean BMI of 25.0 (wt/ht^2).

TABLE 1

*Subjects' initial age, body weight, height, and body mass index (BMI)**

	Placebo	Chromium	Zinc
Sample size (n=)			
Total	8	8	8
Male	5	2	4
Female	3	6	4
Age (yrs)			
Total	63 ± 4^a	68 ± 4^b	64 ± 2^a
Male	58 ± 3^a	70 ± 12^b	63 ± 4^b
Female	72 ± 7	68 ± 4	65 ± 3
Body Weight (lb)			
Total	164 ± 13	159 ± 14	155 ± 12
Male	169 ± 14^a	194 ± 13^b	183 ± 9^b
Female	155 ± 29	147 ± 16	126 ± 10
Height (in)			
Total	68.0 ± 1.5^a	64.0 ± 1.2^b	66.1 ± 1.3^a
Male	70.4 ± 1.2^a	68.5 ± 0.5^b	69.3 ± 0.5^a
Female	64.0 ± 1.7	62.5 ± 0.8	63.0 ± 1.1
BMI (kg/m^2)			
Total	24.7 ± 1.5^a	26.9 ± 1.8^b	24.5 ± 1.3^a
Male	23.9 ± 1.3^a	29.1 ± 1.5^b	26.7 ± 1.1^c
Female	26.3 ± 3.6^a	26.2 ± 2.3^a	22.3 ± 1.8^b

*means ± standard error

Differences at Baseline Between Supplement Groups by Gender

Subjects' mean age, height, weight and BMI by gender and supplement groups are reported in Table 1. Body weight for all groups was considered to be within desirable ranges. Mean body mass index values for males in the chromium supplement group were considered overweight while placebo, zinc supplement groups, and all females were within desirable weight.

Significant differences were observed among males and females between placebo, chromium and zinc supplement groups for initial age, body weight, height, and BMI values. Males in the chromium supplement group were significantly older than males in placebo or zinc supplement groups (Table 1), while no significant differences were observed for females for age. Males in the chromium and zinc supplement groups had significantly higher body weights compared to placebo (Table 1); however, no significant differences were observed for height in females.

Males in the chromium supplement group were significantly shorter compared to placebo and zinc supplement groups (Table 1); however, no significance was observed for females.

Mean BMI values were significantly different for males in each supplement group (Table 1). Females in the placebo and chromium supplement groups had significantly higher mean BMI values compared to the zinc supplement group (Table 1).

Subjects' mean initial plasma glucose, serum insulin, ferritin and albumin concentrations by gender and supplement groups are reported in Table 2. All initial mean values for plasma glucose, serum insulin, ferritin and albumin concentrations were within normal parameters for males and females; however, significant differences were observed among males and females between placebo, chromium

TABLE 2

*Subjects' initial plasma glucose, serum insulin, ferritin and albumin concentrations**

	Placebo	Chromium	Zinc
Glucose (mg/dl)			
Total	93 ± 2^a	106 ± 7^b	92 ± 3^a
Male	90 ± 3^a	127 ± 6^b	94 ± 4^a
Female	96 ± 4	98 ± 6	90 ± 5
Insulin (μμ/ml)			
Total	22 ± 3	24 ± 2	20 ± 1
Male	19 ± 2^a	28 ± 1^b	20 ± 1^a
Female	27 ± 5^a	23 ± 2^b	21 ± 2^b
Ferritin (ng/ml)			
Total	133 ± 37	122 ± 49	106 ± 28
Male	155 ± 58^a	316 ± 122^b	158 ± 42^a
Female	97 ± 16^a	57 ± 11^b	55 ± 6^b
Albumin (g/dl)			
Total	4.8 ± 0.1	4.7 ± 0.2	4.7 ± 0.2
Male	4.9 ± 0.1	5.2 ± 0.1	4.8 ± 0.4
Female	4.7 ± 0.2	4.5 ± 0.2	4.5 ± 0.1

*means ± standard error

Different superscript letters within a row are significnatly different from one another ($p<0.05$)

and zinc supplement groups for initial plasma glucose, serum insulin and ferritin concentrations.

Mean initial plasma glucose concentrations were significantly higher among males in the chromium supplement group compared to males in the placebo and zinc supplement groups. However, no significant differences were observed for mean initial plasma glucose concentrations among females between supplement groups. (Table 2).

Mean initial serum insulin concentrations were significantly higher in males in the chromium supplement group compared to males in the placebo and zinc supplement groups. Females in the placebo group had significantly higher mean serum insulin concentration compared to females in the chromium and zinc supplement groups (Table 2).

Mean initial ferritin concentration was significantly higher among males in the chromium supplement compared to males in the placebo and zinc supplement groups. This difference is due to the two male subjects in the chromium supplement group who had elevated ferritin concentrations, but who were not omitted from the study. Females in the placebo group had significantly higher initial mean ferritin concentration compared to females in the chromium and zinc supplement groups (Table 2). No significant differences were observed for serum albumin concentrations for either males and females between supplement groups (Table 2).

No significant interaction was observed over time between supplement groups and gender or age with plasma glucose or serum insulin concentrations using repeated measures analysis of variance. Therefore, all further data analyses were analyzed only by supplement groups, and not by gender or age.

Differences at Baseline between Supplement Groups

At baseline the chromium supplement group had a significantly higher mean age than placebo or zinc supplement groups (Table 1). There were no initial significant differences for initial mean body weight between the placebo, chromium or zinc supplement groups (Table 1). The chromium supplement group also had significantly lower height and a significantly higher BMI compared to placebo and zinc supplement groups (Table 1).

At baseline there were no significant differences between the placebo, chromium and zinc supplement groups for initial mean serum insulin, ferritin or albumin concentrations (Table 2). Initial mean plasma glucose concentration was significantly higher in the chromium supplement group compared to placebo and zinc supplement groups (Table 2).

Initial mean fasting plasma glucose concentration for all groups in this study were similar to mean fasting plasma glucose concentrations of 97 mg/dl reported by Offenbacher et al. (16) among 24 combined diabetic and nondiabetic subjects, mean age 78 years. Initial mean plasma glucose concentrations among the placebo and zinc supplement groups were similar to plasma glucose concentrations of 5.11 mmol/L (92 mg/dl) observed by Hermann et al. (106) among 12 subjects aged 60 and older with elevated cholesterol concentrations of greater than 6.21 mmol/L. Lui et al. (59) reported initial mean fasting glucose concentrations of 101 mg/dl among 27 women aged 40 to 75 years of age, of whom 15 had normal glycemic control and 12 were hyperglycemic.

In the same study by Offenbacher (16), the initial mean serum insulin concentration of 11 uU/ml was lower than the initial mean serum insulin of 22 uU/ml reported for all subjects in this study. However, initial mean fasting insulin

concentration reported by Lui et al. (59) was 27 uU/ml, which was higher than the reported initial mean insulin concentration of 22 uU/ml observed in this study.

Effects of Eight Weeks Supplementation on
Plasma Glucose and Serum Insulin

An increase of approximately 7% was observed in assay controls during the second measurement period compared to the first, third and fourth measurement periods. As a result, effects observed after four weeks supplementation (second measurement period) may not be due to supplementation, but rather to malfunction of the instrument used for analysis. Therefore, data after four weeks supplementation are not presented.

Chromium. In this study, a significant decrease ($p=.0189$) was observed in mean plasma glucose concentrations in the chromium supplement group after eight weeks supplementation compared to the placebo. However, mean plasma glucose concentration in the chromium supplement group was similar to the baseline concentration at four weeks post supplementation (Table 3). These data indicate that chromium supplementation resulted in a beneficial decrease in plasma glucose compared to the placebo, and that values returned to initial concentrations at four weeks post supplementation suggesting better to no carry over effect of the supplemental inorganic chromium.

Lui et al. (59) also observed a decrease, although not significant, in mean fasting glucose concentration after 3 months supplementation with 4 ug of chromium as brewer's yeast. However, Uusitupa et al. (66) observed that mean fasting blood glucose concentration remained unchanged among subjects supplemented for six weeks with 200 ug chromium as chromium chloride solution compared to the

TABLE 3

Chromium supplementation effects on plasma glucose and serum insulin by measurement period

	Glucose* (mg/dl)	Change from baseline	Insulin* (μμ/ml)	Change from baseline
Placebo				
Baseline (n=8)	93 ± 2		22 ± 3	
8 weeks (n=8)	93 ± 4	0	26 ± 6	+4
12 weeks (n=8)	94 ± 2	+1	22 ± 3	0
Chromium				
Baseline (n=8)	106 ± 6		24 ± 2	
8 weeks (n=8)	100 ± 7	-6**	24 ± 3	0
12 weeks (n=8)	107 ± 6	+1	25 ± 3	+1

*means ± standard error

** significant change from baseline, supplemented group versus placebo, $p<0.05$

control. However, 1-hour blood glucose concentration was significantly decreased in the chromium supplement group compared to the control 1-hour blood glucose concentration.

Although not significant, an increase in mean serum insulin concentration was observed in our study in the placebo group compared to the chromium supplement group. Mean serum insulin concentrations were similar to baseline at four weeks post supplementation for both the placebo and chromium supplement groups.

In the same study as mentioned previously, Uusitupa et al. (66) also observed an increase, although not significant, in fasting mean serum insulin concentration in the placebo supplement group after 6 weeks supplementation with 200 ug chromium. However, in a study by Lui et al. (59), a significant decrease from 27 uU/ml to 13 uU/ml was observed in mean fasting insulin concentration among 27 women after 3 months supplementation with only 4 ug chromium from brewer's yeast. The difference observed by Uusitupa could be due to the form of chromium used. While not apparent in our study, Riales et al. (65) observed a decrease, although not significant, in mean insulin concentration among 7 healthy men aged 31 to 60 after 12 weeks of 200 ug chromium supplementation as chromium chloride compared to placebo. This observed difference could be due to the longer supplementation period by Riales' study.

Zinc. In this study, mean plasma glucose concentration among subjects in the zinc supplement group did not significantly change after eight weeks supplementation compared to the placebo. However, there was a significant increase ($p = .0004$) in mean plasma glucose concentration four weeks post

supplementation in the zinc supplement group compared to the placebo (Table 4). This increase may be due to the removal of the zinc supplement.

A significant decrease ($p=.0254$) in mean serum insulin concentration was observed among subjects in the zinc supplement group after eight weeks supplementation compared to the placebo. Mean serum insulin concentrations were similar to baseline at four weeks post supplementation for both the placebo and zinc supplement groups (Table 4), indicating concentrations returned to baseline within four weeks post supplementation. It is possible that a longer zinc supplementation period could result in a greater decrease in serum insulin concentration compared to the placebo. Longer supplementation may also result in adaptation or return to baseline values while on the supplement.

There are few studies concerning effects of zinc supplementation in humans on serum insulin concentrations. However, there are studies involving zinc deficient animals. Glucose intolerance during a glucose challenge, as well as insulin resistance has been observed in zinc deficient animals (18). However, no significant changes were observed by Quarterman et al. (48) in glucose tolerance curves and mean plasma insulin concentration among zinc deficient animals compared to control animals. A significant decrease in mean serum insulin concentration was also observed by Huber et al. (48) among zinc deficient rats compared with pair fed controls.

Dietary Intakes at Baseline

Mean initial caloric intakes by gender within supplement groups are presented in Table 5. The percentages indicate the majority of our subjects consumed less than 100% of the recommended energy intake (REI) for their age.

TABLE 4

Zinc supplementation effects on plasma glucose and serum insulin by measurement period

	Glucose* (mg/dl)	Change from baseline	Insulin* (μμ/ml)	Change from baseline
Placebo				
Baseline (n=8)	93 ± 2		22 ± 3	
8 weeks (n=8)	93 ± 4	0	26 ± 6	+4**
12 weeks (n=8)	94 ± 2	+1	22 ± 3	0
Zinc				
Baseline (n=8)	92 ± 3		20 ± 1	
8 weeks (n=8)	92 ± 6	0	19 ± 1	-1
12 weeks (n=8)	101 ± 5	+9**	21 ± 2	+1

*means ± standard error

** significant change from baseline, supplemental group versus placebo, $p<0.05$

TABLE 5

*Mean initial caloric intake**

Gender	Placebo	Chromium	Zinc
Male			
n=	5	2	4
daily calories	2187 ± 176	1988 ± 20	1648 ± 181
% of REI	95	86	72
Female			
n=	3	6	4
daily calories	1906 ± 262	1528 ± 291	1389 ± 98
% of REI	100	80	73

* means ± standard error
% of REI = Percent of Recommended Energy Intake for adults over age 51 (3)

However, only the male and female subjects in the zinc supplement group consumed less than 75% of the REI.

Mean initial total daily calories, percent of daily calories from carbohydrate, protein and fat, total fiber and zinc intakes by placebo, chromium and zinc supplement groups are reported in Table 6. A significant difference in mean initial total calorie intake was observed between supplement groups. Subjects in the placebo group consumed significantly higher mean initial calories than subjects in the chromium or zinc supplement groups (Table 6). Subjects in the placebo groups also consumed significantly lower mean initial percentage of daily calories from carbohydrate than subjects in the chromium or zinc supplement groups (Table 6). However, the mean initial percent daily calories from carbohydrate consumed by the placebo group was similar to the Food and Nutrition Board's current recommendations that over half of all daily calories be supplied by carbohydrate (26) while the mean initial percent of calories from carbohydrate consumed by the chromium and zinc supplement groups were similar to the Senate Select Committee on Nutrition and Human Needs recommendations that 58 percent of all calories come from carbohydrate (107).

No significant differences were observed between supplement groups in mean initial percent of daily calories from protein or total fat (Table 6). The mean initial percent daily calories from protein consumed by all groups was higher than the 12% suggested in the Dietary Goals for Americans (107). In addition, the mean initial total fat consumed was similar to the 30% of calories from fat suggested by the US Dietary Goals for Americans (107).

The placebo group did have a significantly higher mean initial fiber intake compared to the chromium and zinc supplement groups (Table 6). Mean initial fiber intake in the placebo group was similar to recommendations that Americans

TABLE 6

*Initial Dietary intakes for placebo, chromium, and zinc supplemented groups**

Nutrient	Placebo	Chromium	Zinc
	n=8	n=8	n=8
Total Calories	2082 ± 145[a]	1643 ± 226[b]	1518 ± 107[b]
% CHO	51 ± 2[a]	55 ± 3[b]	55 ± 3[b]
% PRO	17 ± 1	17 ± 3	18 ± 1
% FAT	31 ± 1	30 ± 3	29 ± 2
Fiber (g)	24 ± 2[a]	19 ± 2[b]	16 ± 1[b]
Zinc (mg)	12 ± 1[a]	12 ± 2[a]	8 ± 1[b]

* means ± standard error
% CHO = percent daily calories from carbohydrate
% PRO = percent daily calories from protein
% FAT = percent daily calories from total fat.
Different superscript letters within a row are significantly different from one another ($p<0.05$)

consume 20 to 35 grams of dietary fiber daily (108); however, the chromium and zinc supplement groups consumed less than this recommendation (Table 6). Mean initial dietary zinc intake in the zinc supplement group was significantly lower than the placebo and chromium supplement groups (Table 6).

Dietary Intakes Over Time Among Supplement Groups

Mean total daily calories, percent of daily calories from carbohydrate, protein and fat, fiber and zinc intakes were analyzed over time within the placebo, chromium and zinc supplement groups. No significant differences were observed over time among the placebo, chromium or zinc supplement groups for any dietary parameters, with the exception of total daily calories, which significantly increased in the zinc supplement group ($p=.0397$) after eight weeks supplementation. Mean total caloric intakes in the zinc supplement group were 1518, 1739 and 1432 calories for baseline, 8 weeks supplementation and 4 weeks post supplementation, respectively.

Correlations between Dietary Intakes at Baseline and Plasma Glucose and Serum Insulin in Supplement Groups

Correlations between baseline dietary intakes and baseline plasma glucose and serum insulin concentrations were determined for the total sample (Table 7). Correlation coefficients were considered significant if probability values were less than 0.05 (Table 7).

A significant positive correlation was observed between initial serum insulin and initial plasma glucose concentration ($r=.49306$, $p=.0001$). These data indicate that as serum insulin concentrations increased, plasma glucose concentrations increased and, conversely, as plasma glucose concentrations increased, serum insulin

TABLE 7

Correlation coefficients between dietary intakes at baseline and plasma glucose and serum insulin concentrations

	Calories	% CHO	% PRO	% FAT	Fiber	Zinc	Ferritin	Albumin	Weight
Glucose									
r	0.046	⁻0.178	0.085	0.211	⁻0.005	0.281	0.376	0.066	0.396
p	NS	NS	NS	0.0390	NS	0.0056	0.0002	NS	0.0001
Insulin									
r	0.268	⁻0.118	⁻0.174	0.222	0.099	0.130	0.110	-0.233	0.606
p	0.0082	NS	NS	0.0296	NS	NS	NS	0.0222	0.0001

r = correlation coefficient
p = statisical probability
NS = no significant difference
% CHO = percent daily calories from carbohydrate
% PRO = percent daily calories from protein
% FAT = percent daily calories from total fat
Weight = body weight

concentrations also increased. An increase in glucose concentrations will lead to an increase in insulin concentrations, which may be a consequence of insulin resistance (109).

No significant correlations were observed between initial total daily calories from carbohydrate or protein and initial plasma glucose or serum insulin concentration (Table 7). However, initial percent daily calories from fat was positively correlated with initial glucose and serum insulin concentrations (Table 7). This indicates that as total fat in the diet increased, plasma glucose and serum insulin concentrations also increased. In a study by Mayer et al. (110), dietary fat was also positively related to fasting insulin concentrations among 272 sets of female twins aged 30 to 84 years of age, mean age 51. However, in this study by Mayer, an estimated 1511 calories per day was reported. The mean percentage of daily calories from fat was higher than in our study (38%), while percent daily calories from carbohydrate was lower (43%) than in our study. Percent daily calories from protein was similar in both studies. Chen et al. (69) also observed that insulin sensitivity improved when non-diabetic elderly subjects of desirable weight consumed diets higher in carbohydrate than their typical intakes.

No significant correlations were observed between initial fiber intake and initial plasma glucose or serum insulin concentrations (Table 7). A significant positive correlation was observed between initial zinc intake and initial ferritin concentration with plasma glucose concentration; however, no significant correlations were observed between these parameters and serum insulin concentration (Table 7).

A significant negative correlation was observed between initial albumin concentration and serum insulin, indicating that as albumin concentrations decreased, serum insulin concentrations increased. However, no significant

correlation was observed between initial albumin concentration and initial plasma glucose concentration (Table 7).

Significant positive correlations were observed between initial body weight with initial plasma glucose and serum insulin concentrations, indicating that plasma glucose and serum insulin concentrations are increased among persons with higher body weights (Table 7). Insulin resistance is common among subjects with body weights that are 20% above their desirable weights. Improvements in insulin sensitivity have been observed following weight loss to within a desirable range (110,111).

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the effects of eight weeks chromium or zinc supplementation on plasma glucose and serum insulin concentrations in adults age 50 and above. This study was conducted in the spring and early summer of 1993, using twenty-four adult volunteers from the community of Stillwater, Oklahoma.

Subjects were randomly assigned to one of three supplement groups. The placebo group (n=8) consumed 0.25 mg/day lactose in a supplemental capsule, the chromium supplement group (n=8) consumed supplements containing 241.32 ug chromium per day as chromium chloride, and the zinc supplement group (n=8) consumed supplements containing 30.36 mg zinc per day as zinc sulfate. Supplementation lasted for eight weeks. Subjects consumed independently chosen diets throughout the study.

Blood samples were obtained at baseline, after four and eight weeks supplementation and four weeks post supplementation. Anthropometric data, including height, weight, bioelectrical impedance, and knee height were obtained at specific measurement periods.

Changes in plasma glucose and serum insulin concentrations after eight weeks supplementation and four weeks post supplementation were compared to changes in the placebo using SAS General Linear Models procedure. Significant differences were determined by Least Squares Means.

The initial dietary intakes from three day averages were used for dietary analysis. Dietary parameters included in diet analysis for placebo, chromium and

zinc supplement groups across measurement periods included total daily calories, percent daily calories from total carbohydrate, protein, and fat, fiber and zinc intakes. Correlation coefficients were also determined between initial dietary intakes and initial plasma glucose and serum insulin concentrations using SAS.

Missing data in the nutritional analysis program placed limitations on interpretations that can be made. Many foods that were added from label information had missing mineral or complex or simple carbohydrate information. Also, chromium was not included in the nutritional database.

In this study, we observed a beneficial decrease in plasma glucose after eight weeks chromium supplementation compared to the placebo and a significant decrease in serum insulin concentrations after eight weeks zinc supplementation compared to the placebo. We also observed positive correlations among initial dietary parameters with initial plasma glucose and serum insulin concentrations.

RECOMMENDATIONS

The author recommends the following:

1. Evaluation of a larger sample at low and high extremes of mineral status.
2. Evaluation of zinc and chromium supplementation using a diabetic population.
3. Supplementation for a longer time period, which may allow mineral status to better adapt to intake.
4. Supplementation with both zinc and chromium to determine effects of interaction.
5. Use of a food analysis program that contains chromium values, more accurate complex carbohydrate, simple sugars and other missing data.

6. Obtaining a detailed evaluation of activity levels to better determine individual calorie needs.

7. Training subjects as a group to record diet records.

8. Use of a pocket-sized food diary, to be carried with subjects at all times, to ensure compliance in recording all foods consumed, at or away from home.

CONCLUSIONS

Supplementation with chromium or zinc may have a beneficial effect on plasma glucose and serum insulin concentrations among adults aged 50 and older.

Hypothesis one stated that there will be no significant relationship between initial dietary intake and initial plasma glucose or serum insulin concentrations in adults age 50 and above. As seen in Table 7, significant correlations were observed between initial dietary intake with plasma glucose and serum insulin. Therefore, the first null hypothesis was rejected.

Hypothesis two stated that there will be no significant effect due to eight weeks zinc supplementation on plasma glucose concentration in adults age 50 and above. There was no significant effect due to eight weeks zinc supplementation on plasma glucose concentration. Therefore, the second null hypothesis was accepted.

Hypothesis three stated that there will be no significant effect due to eight weeks zinc supplementation on serum insulin concentration in adults age 50 and above. As seen in Table 4, a significant decrease was observed after eight weeks zinc supplementation in serum insulin concentration compared to the placebo group. Therefore, the third null hypothesis was rejected.

Hypothesis four stated that there will be no significant effect due to eight weeks chromium supplementation on plasma glucose concentration in adults age 50 and above. As seen in Table 3, a significant decrease was observed after eight

weeks chromium supplementation in plasma glucose concentration compared to the placebo. Therefore, the fourth null hypothesis was rejected.

Hypothesis five stated that there will be no significant effect due to eight weeks chromium supplementation on serum insulin concentration in adults age 50 and above. There was no significant effect due to eight weeks chromium supplementation on serum insulin concentration. Therefore, the fifth null hypothesis was accepted.

Due to the results observed in this study, it appears that a diet adequate in chromium and zinc may beneficial in achieving glucose control and increasing insulin sensitivity among aging adults. Additionally, diets that provide a good balance of carbohydrate, protein and fat, and adequate nutrients may have a positive influence on plasma glucose and serum insulin concentrations among older adults.

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APPENDIXES

APPENDIX A

AVAILABILITY OF MINERALS IN OVER-THE-COUNTER SUPPLEMENTS FROM IRB PROPOSAL # HES-93-011

Availability of Mineral in Over-the-Counter Supplements*

Supplements containing copper, chromium and zinc are readily available over the counter. Single nutrient supplements of chromium and zinc are available in nearly every grocery and drug store as well as in discount stores in Stillwater. The doses of minerals are 200 ug for chromium and range from 35 to 100 mg for zinc. In addition higher amounts of over the counter zinc can be ordered by request. Copper was found as a single nutrient in only one location in Stillwater; the amount of copper in this supplement was 2 mg with the recommended dose of one tablet per day.

Multi-nutrient supplements containing all three minerals with varying amounts of each are widely available in all the above locations. "Stress" and "high potency" supplements generally contain 3 mg of copper, and varying amounts of zinc. Not all of these supplements contain chromium. The range of chromium found in the multi-nutrient supplements was 15 to 1000 ug. For copper, the range was 2 to 3 mg, and for zinc the range was from 15 to 50 mg.

Some of the supplements suggested taking 2 to 3 packets or tablets per day which would increase the copper to 4-6 mg, zinc to 80-120 mg, and chromium to 400 ug per day.

Representative specific supplements are included on the next page. This listing does not include all of the supplements available but does include those with both the low and the high levels.

*Over the Counter Supplements Available in Stillwater
September 28, 1992.

Over the Counter Supplements Available in Stillwater
September 28, 1992

Single Nutrient Supplements

Nutrient	Brand	Amount of Mineral	Form
Chromium			
	Spring Valley	200 ug	Chromium picolinate
	BioFormed	200 ug	trivalent in Brewer's yeast
Copper			
	Twinlab Natural Chelated Copper Caps	2 mg	Copper gluconate
Zinc			
	Spring Valley	50 mg & 100 mg	Zinc gluconate
	Kal Zinc 100 + 5	100 mg	Zinc amino acid chelate
	Thompson Organic Thompson Zn Picolinate	50 mg 35 mg	Zinc gluconate Zinc picolinate
	Rugby Labs	200 mg	Zinc sulfate
	Upshire Smith	220 mg	Zinc sulfate
	United Reser	220 mg	Zinc sulfate

Multivitamin-mineral Supplements

Brand	Mineral	Amount of Mineral
Spring Valley		
	Copper sulfate	2 mg
Spring Valley Athletes		
	Copper gluconate	25 ug
	Zinc gluconate	25 mg
Spring VAlley Stress with Zn		
	Cupric oxide	3 mg
	Zinc sulfate	23.9 mg
Spring Valley Thera Plus		
	Chromium chloride	15 ug
	Copper sulfate	2 mg
	Zinc oxide	15 mg

APPENDIX B

APPROVAL FORM FOR INSTITUTIONAL REVIEW BOARD FOR HUMAN SUBJECTS RESEARCH

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
FOR HUMAN 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 [x]

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 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

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;

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

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

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

(14) this research is beneficial to the public in that many individuals take nutrient supplements without knowledge of the interactions among the nutrients; and

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

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

Date _____ Time _____(am/pm)

Signed _____

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

Signed _____
(project director or her authorized representative)

APPENDIX E

HEALTH INFORMATION 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 F

MONTHLY DATA COLLECTION FORM

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?_____

APPENDIX G

PROCEDURES FOR BIOELECTRICAL IMPEDANCE AND KNEE HEIGHT COLLECTIONS

Bioelectrical impedance procedure:

1) Height and weight were measured immediately before this procedure. This information along with age and gender were programmed into the bioelectrical impedance analyzer.

2) Fasted subjects lay in a supine position on a large heavy wooden table with the right arm and the portion of the right leg below the knee exposed. All jewelry was removed. Hands were placed six inches from the sides and feet were placed six inches apart.

3) Four sensor electrodes were placed on the right foot, ankle, wrist and hand. The right foot was relaxed and the first electrode was placed on the top center of the foot near the base of the toes. The same foot was dorsiflexed (flexed toward the head) and the second electrode was placed on the top of the relaxed ankle against the edge where the crease had formed. The right wrist was dorsiflexed and the third electrode was placed on the top of the relaxed wrist against the edge where the crease had formed. The hand was relaxed and the fourth electrode was placed on the center top of the hand near the edge of the first and second knuckles towards the thumb.

4) Red (positive) sensor cables were attached to electrodes on the wrist and ankle and black (negative) sensor cables were attached to electrodes on the hand and foot. The cables were connected to the bioelectrical impedance unit.

5) The subject was reminded to relax and remain motionless for the ten second test. Duplicate test results were printed out on a body composition test summary sheet. Information obtained included percent body fat, body fat weight, lean body weight, basal metabolic rate, total body water and bioresistance.

Knee height procedure:

1) Subjects lay in a supine position on a heavy wooden table with the left knee and left ankle bent at a 90 degree angle. Angles of the knee and foot were positioned with a triangle provided by Ross Labs. The knee caliper was placed with the fixed blade under the heel in line with the tibia (large bone in the lower leg) and over the lateral malleolus (ankle bone). The sliding blade was brought down against the thigh about two inches behind the patella (kneecap).

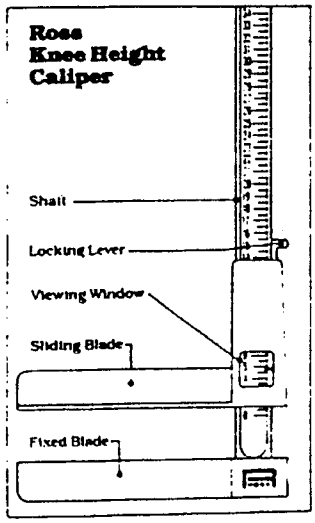
2) The measurement was held with a locking mechanism on the caliper and read to the nearest 0.1 cm through a viewing window.

APPENDIX H

KNEE HEIGHT MEASUREMENT DIAGRAM

DIRECTIONS FOR THE ROSS KNEE HEIGHT CALIPER

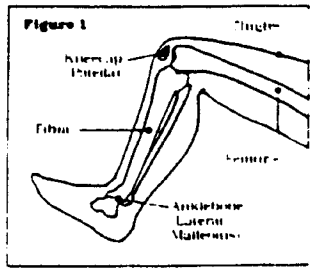
Stature for adults 60 to 90 years old can be estimated from knee height when standing height cannot be measured. Estimated stature can be used in nutritional assessment parameters, including reference weights for height, energy expenditure equations, body surface area equations, and the creatinine height and body mass indices. Knee height may also be used with other anthropometric measures to predict weight in elderly individuals who cannot be weighed by conventional methods.

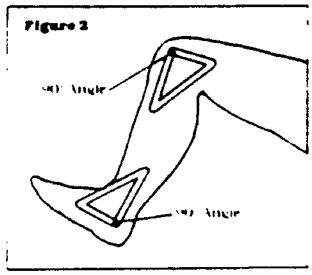


KNEE HEIGHT MEASUREMENT

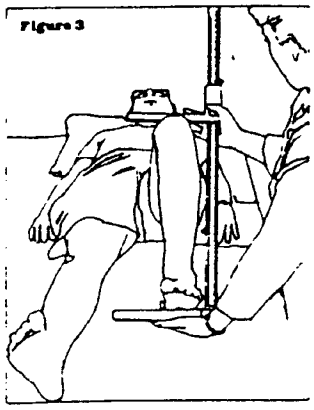
Directions:

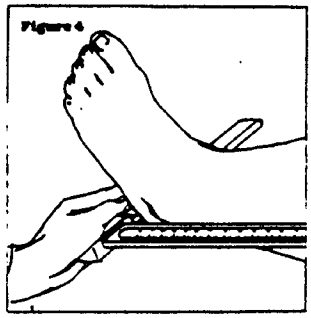
1. With the subject lying on his or her back, bend both the left knee and left ankle to a 90° angle. (Figure 1) Check the angles by using the triangle. (Figure 2)



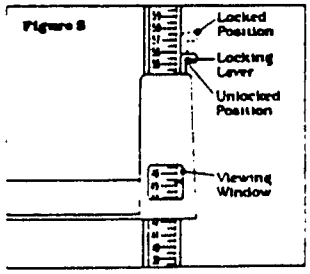


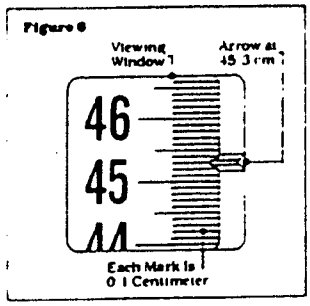
2. Open the caliper and place the fixed blade under the heel. Press the sliding blade down against the thigh about 2 inches behind the kneecap (patella). (Figure 3) The shaft of the caliper should be in line with the large bone in the lower leg (tibia) and be over the anklebone (lateral malleolus). (Figure 4)





3. To hold the measurement, push the locking lever away from the blades. (Figure 5) Read the measurement through the viewing window to the nearest 0.1 centimeter (cm). (Figure 6)





4. Release the locking lever by pushing it toward the caliper blades. Repeat the process to take a second measurement. Both measurements should be within 0.5 cm of each other.

Source: #50452, Ross Labs, Columbus, Ohio, 1989.

APPENDIX I

PROCEDURE FOR BLOOD COLLECTION

Blood collection procedure:

1. Site selection: A prominent vein was selected at the antecubital area of the non dominant arm. If there was no prominent vein in that area, a vein at the dorsum of the hand was used.

2. Site preparation: A tourniquet was placed approximately two inches above the antecubital area. An alcohol swab was used to clean the antecubital area.

3. Skin puncture: A 21 gauge butterfly needle was attached to the first plasma tube and used to puncture the antecubital vein. A piece of micropore tape was placed over the butterfly needle to stabilize it.

4. Specimen collection: After releasing the tourniquet, blood was allowed to flow into the tube by slowly pulling on the plunger. Ten milliliters of blood was collected in the first plasma tube. One hundred microliters of sodium citrate (trisodium citrate 300 g/L) was used as an anticoagulant. The end of the butterfly needle tube near the plasma tube was kinked to remove the first plasma tube and attach the second plasma tube. After 10 ml of blood was collected in the second plasma tube, the butterfly needle tube was kinked while the second plasma tube was removed and the serum tube was attached to collect ten milliliters of blood. The first anticoagulant tube was centrifuged at 2500 rpm for 30 minutes immediately after blood collection and the serum tubes were allowed to clot on ice for two hours.

5. Puncture treatment: After the blood samples were collected, a sterile cotton ball was applied to the puncture site with a slight pressure until the bleeding stopped. A bandage was then placed over the puncture wound.

6. Biohazard disposal: All butterfly needles were discarded into a biohazard container for sharps and all used cotton balls were discarded into a biohazard bag.

7. Immediately following the blood draw, subjects received orange juice and muffins to relieve their fasting state.

APPENDIX J

DIETARY RECORD FORM

DIET RECORD

Subject Number _____ Date _____

Time	Amount	Coding	Description/Brand Name

APPENDIX K

MODIFICATIONS TO PLASMA GLUCOSE ANALYSIS PROCEDURE

Modifications to glucose determination:

Plasma glucose was determined using Sigma Diagnostics procedure No. 510, St Loius, MO. Modifications to the procedure were made by decreasing sample amounts by three-fifths to minimize waste. To blank, standard, and test tubes, 200 ul of water was added. Ten ul of water, standard and sample was added to blank, standards and test tubes, respectively. Two ml of enzymatic color was added to each tube.

VITA

Michelle L. Burns

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF ZINC AND CHROMIUM SUPPLEMENTATION ON PLASMA GLUCOSE AND SERUM INSULIN IN ADULTS AGE 50 AND OLDER

Major Field: Nutritional Science

Biographical:

Personal Data: Born in Yuba City, California, On April 29, 1969, the daughter of Roger and Joanne Burns.

Education: Graduated from Yerington High School, Yerington, Nevada in June 1987; received Bachelor of Science degree in Foods and Nutrition from San Diego State University, San Diego, California in May 1992. Completed the requirements for the Master of Science degree with a major in Nutritional Science at Oklahoma State University in July 1994.

Professional Experience: Dietary Clerk, Caregiver Nursing Home, San Diego, California, January 1991-May 1992; Provisional Licensed Dietitian and Applied Pre-Professional Practice Program Intern, Oklahoma State University, at Jane Phillips Medical Center, Bartlesville, Oklahoma, August 1992-May 1993; Registered Dietitian, 1993; Graduate Research and Teaching Assistant, Department of Nutrition, Oklahoma State University, 1993-1994.

Professional Organizations: American Dietetic Association, Kappa Omnicron Nu