EFFECTS OF CHROMIUM SUPPLEMENTATION ON INDICATORS OF TYPE 2 DIABETES IN HYPERGLYCEMIC MEN

By

JOSHUA ANDREW PHELPS

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University of Michigan

Ann Arbor, Michigan

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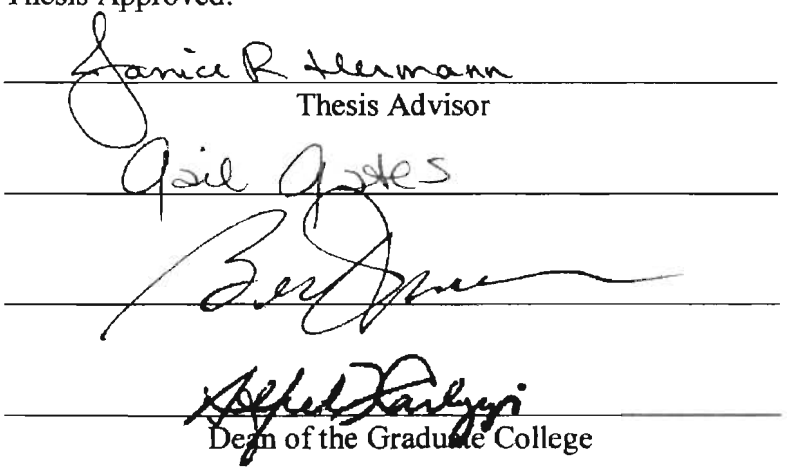

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Dean of the Graduate College

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CHAPTER I

RESEARCH PROBLEM

Introduction

According to the Centers for Disease Control and Prevention, 5.9% of the U.S. population, or 15.7 million people have diabetes (Centers for Disease Control, 1998). There are approximately 800,000 new cases of diabetes diagnosed each year (Centers for Disease Control, 1998). Of the 15.7 million individuals with diabetes, 90%-95% have type 2 diabetes (Centers for Disease Control, 1998). Major risk factors for developing type 2 diabetes, formerly known as non-insulin dependent diabetes mellitus (NIDDM), include older age, obesity, family history of the disease, and impaired glucose tolerance (Centers for Disease Control, 1998).

Complications that stem from diabetes include heart disease, stroke, high blood pressure, kidney disease, blindness, nervous system disease, and amputation (Centers for Disease Control, 1998). The most recent report from the American Diabetes Association indicated that the total cost of the disease is estimated at $98 billion (Centers for Disease Control, 1998). Direct medical costs accounted for $44 billion, while indirect costs, such as loss of work, disability, and early death accounted for $54 billion of the total cost of illness (Centers for Disease Control, 1998). In an effort to reduce costs and complications associated with type 2 diabetes, one goal of current research is to identify

new treatments and methods for the prevention of the disease. Lifestyle modifications such as increased physical activity and improved dietary practices are proposed to reduce the incidence of type 2 diabetes by as much as 58% in individuals who are at high risk for diabetes (American Diabetes Association, 2000).

Inadequate chromium status may be associated with exacerbating risk factors for developing type 2 diabetes. Studies have shown that an inadequate chromium intake leads to decreased insulin sensitivity and increased blood glucose concentration in humans (Anderson, 1992). Impaired glucose tolerance and insulin sensitivity, which lead to elevated insulin concentration, are risk factors for developing type 2 diabetes (Anderson, 1992). Several controlled research studies have indicated that adequate chromium intake improves glucose tolerance and reduces insulin concentrations required to handle the uptake of glucose into cells (Glinsmann & Mertz, 1966; Martinez et al., 1985; Anderson et al., 1983; Anderson et al., 1991; Anderson, 1997a; Guan et al., 2000). Other studies have shown no improvement in glucose tolerance or insulin sensitivity with increased chromium intake (Abraham et al., 1992; Trow et al., 2000; Amato et al., 2000). Researchers continue to debate if chromium is effective as a preventive measure or a treatment for those who already have diabetes, and the exact level of chromium required to show a beneficial effect on glucose tolerance (Hellerstein, 1998).

Objectives

Developing treatments to prevent diseases such as type 2 diabetes will aid in reducing both direct and indirect health care costs as well as improve the quality of life

for individuals at risk for developing diabetes. The purpose of this study is to examine the effects of twelve weeks of chromium supplementation, at 200 mcg/day as chromium chloride, on indicators of glucose metabolism in hyperglycemic men. The following objectives were developed for this study:

1. Determine the effects of twelve weeks of chromium supplementation on fasting serum glucose concentrations in hyperglycemic men.
2. Determine the effects of twelve weeks of chromium supplementation on fasting serum insulin concentration in hyperglycemic men.
3. Determine the effects of twelve weeks of chromium supplementation on three-hour glucose tolerance curves in hyperglycemic men.
4. Determine the effects of twelve weeks of chromium supplementation on glycosylated hemoglobin concentration in hyperglycemic men.
5. Determine the effects of twelve weeks of chromium supplementation on percent specific insulin binding in hyperglycemic men.

Null Hypotheses

The following null hypotheses were developed for this study:

1. There will be no statistically significant effect of twelve weeks chromium supplementation on fasting serum glucose concentrations in hyperglycemic men.
2. There will be no statistically significant effect of twelve weeks

chromium supplementation on fasting serum insulin concentrations in hyperglycemic men.

3. There will be no statistically significant effect of twelve weeks chromium supplementation on three-hour glucose tolerance curves of hyperglycemic men.
4. There will be no statistically significant effect of twelve weeks chromium supplementation on percent glycosylated hemoglobin of hyperglycemic men.
5. There will be no statistically significant effect of twelve weeks chromium supplementation on percent specific insulin binding of hyperglycemic men.

Assumptions

The following assumptions were made for this research:

1. It was assumed that the participants accurately filled out their one-week food frequency questionnaire.
2. It was assumed that the participants took their supplements as directed.
3. It was assumed that the participants fasted for twelve hours before blood was drawn.
4. It was assumed that the participants did not change their diet or exercise patterns over the course of the study.

5. It was assumed that the participants did not take any other supplements containing chromium over the course of the study.
6. It was assumed that the participants did not undergo events causing lifestyle changes, such as medication consumption and surgical events.

Limitations

1. The one week food frequencies were limited by the participants' knowledge of portion size and food composition.
2. An inadequate data base for chromium limited analysis of chromium content of participants' diets.
3. The results of the study are relevant to this sample group limiting extrapolation of results to the general population.
4. The results of this study are limited by outside factors affecting participants, such as changes in medication and surgical events.
5. The results of the study are limited by the tendency for health conscious individuals to volunteer to participate in this type of study.

CHAPTER II

LITERATURE REVIEW

Type 2 Diabetes

According to the Centers for Disease Control and Prevention, 5.9% of the U.S. population, or 15.7 million people have diabetes (Centers for Disease Control, 1998). There are approximately 800,000 new cases of diabetes diagnosed each year (Centers for Disease Control, 1998). Of the 15.7 million individuals with diabetes, 90%-95% have type 2 diabetes (Centers for Disease Control, 1998). Risk factors for developing type 2 diabetes include being over 40 years of age, overweight, physically inactive, having a family history of diabetes or a prior history of gestational diabetes, or impaired glucose tolerance (Centers for Disease Control, 1998; American Diabetes Association, 2000). Type 2 diabetes is a disease characterized by insulin resistance (American Diabetes Association, 2000). Insulin binding to cell surface insulin receptors enables a cell to take glucose from the bloodstream. Insulin resistance means that insulin is not binding to its receptors. Defects in insulin action result in high blood glucose concentrations due to the cells' inability to remove glucose from the bloodstream (Centers for Disease Control, 1998).

The most common procedure for diagnosing type 2 diabetes is a fasting plasma glucose test (Centers for Disease Control, 1998). A diagnosis of diabetes requires a twelve-hour fasting plasma glucose concentration to be greater than or equal to 126 mg/dl

(Centers for Disease Control, 1998). A previous history of excessive thirst and urination, unexplainable fatigue, constant hunger or weight loss, or a non-fasting plasma glucose concentration greater than or equal to 200 mg/dl would be indicative of type 2 diabetes (Centers for Disease Control, 1998). A further means of diagnosing type 2 diabetes is an oral glucose tolerance test. This procedure involves the consumption of a glucose tolerance beverage containing 75 g of dextrose dissolved in water (Centers for Disease Control, 1998). Two hours after consumption of the beverage, two-hour plasma glucose concentration is measured. A plasma glucose concentration greater than or equal to 200 mg/dl two hours after consuming a glucose tolerance beverage would be indicative of type 2 diabetes (Centers for Disease Control, 1998).

Early diagnosis is essential to avoiding further complications of diabetes. Complications that stem from diabetes include heart disease, stroke, high blood pressure, kidney disease, blindness, nervous system disease, and amputation (Centers for Disease Control, 1998). Heart disease death rates for adults with diabetes are two to four times higher than heart disease death rates for adults without diabetes (Centers for Disease Control, 1998). Stroke risk is two to four times higher for individuals who have diabetes (Centers for Disease Control, 1998). Among those who have diabetes, an estimated 60%-65% have high blood pressure (Centers for Disease Control, 1998). Approximately 40% of new end-stage renal disease cases are individuals with diabetes (Centers for Disease Control, 1998). Blindness associated with diabetic retinopathy is responsible for 12,000-24,000 new cases of blindness each year (Centers for Disease Control, 1998). Nervous system diseases related to diabetes account for the majority of lower extremity amputations (Centers for Disease Control, 1998).

Complications that stem from diabetes account for much of the disease cost. The most recent report from the American Diabetes Association indicated that the total annual cost of the disease is estimated at $98 billion (Centers for Disease Control, 1998). Direct medical costs accounted for $44 billion, while indirect costs, such as loss of work, disability, and early death accounted for $54 billion of the total cost of illness (Centers for Disease Control, 1998).

According to the World Health Organization, if current trends continue, the number of individuals worldwide with diabetes will increase from 140 million to 300 million by the year 2025 (World Health Organization, 2000). The development of effective treatment and prevention programs is essential for reducing the costs and complications associated with type 2 diabetes. Increased physical activity and weight control are two important factors that influence the onset of type 2 diabetes (Pi-Sunyer, 1996). Studies have shown that weight loss improves insulin sensitivity, and thus improves glucose control (Pi-Sunyer, 1996; American Diabetes Association, 2000). Improvement of dietary habits is a cost-effective way to reduce risk, and minimize the complications associated with type 2 diabetes (Franz et al., 1995).

Chromium

Chromium (Cr) is a "shiny, hard, white metal, belonging to the first series of transition elements" (Ducros, 1992). Chromium can occur in various oxidation states, ranging from –2 to +6 (Ducros, 1992). The most common oxidation states are 0, +2, +3, and +6 (Ducros, 1992; Jeejeebhoy, 1999). Divalent (+2) chromium is oxidized to trivalent (+3) chromium by "simple exposure to air" and, therefore, "does not exist in

biological systems" (Ducros, 1992). Hexavalent (+6) chromium can "readily penetrate cells" at a rate perhaps 1000 times faster than trivalent chromium (Jeejeebhoy, 1999). After penetrating the cell, hexavalent chromium is reduced to trivalent chromium (Jeejeebhoy, 1999). The process of reduction forms reactive oxygen intermediates that react with DNA (Jeejeebhoy, 1999). The reaction between DNA and reactive oxygen intermediates "is believed to mediate the genotoxic effects of hexavalent chromium" (Jeejeebhoy, 1999). Hexavalent chromium also "penetrates the mitochondria and depresses oxygen consumption" (Jeejeebhoy, 1999). Hexavalent chromium is an industrial pollutant that is "very toxic to tissues because of its oxidizing properties" (Jeejeebhoy, 1999). Trivalent chromium "diffuses slowly across the cell membrane" and is the most stable form of chromium (Jeejeebhoy, 1999). Trivalent chromium exists in various forms, such as chromic oxide and chromic chloride (Jeejeebhoy, 1999). Trivalent chromium is "the form of Cr found in foods and nutrient supplements" (Anderson et al., 1997). This study utilized trivalent chromium in the form of chromium chloride hexahydrate ($CrCl_3.6H_2O$).

For this particular study, chromium's function of interest is enhancing insulin sensitivity and maintenance of glucose tolerance. Convincing evidence of chromium's role in maintaining glucose metabolism comes from patients receiving total parenteral nutrition (Vincent, 2000a; Brown et al., 1986). In one such case, a patient receiving total parenteral nutrition (TPN) developed glucose intolerance (Brown et al., 1986). After a series of tests and analysis of medical records, chromium deficiency was determined as the cause (Brown et al., 1986). Subsequent supplementation of chromium into the TPN solution corrected the glucose intolerance (Brown et al., 1986).

Chromium is believed to maintain glucose metabolism through the activation of insulin receptors (Vincent, 2000a; Anderson, 2000). The proposed biochemical mechanism tying chromium to insulin receptor activity involves the oligopeptide chromodulin, also known as low-molecular weight chromium-binding substance (Vincent, 2000a). Binding of insulin to insulin receptors triggers the movement of chromium, "presumably in the form of Cr-transferrin", into insulin-dependent cells (Vincent, 2000a). Once inside the cell, chromium binds to apochromodulin (Vincent, 2000a). Newly formed chromodulin binds to the insulin receptor, "further activating the receptor kinase activity" (Vincent, 2000a). Increased kinase activity is believed to be due to "stabilization of the active conformation of insulin receptors" by binding of chromodulin (Vincent, 2000b).

Before chromium can enter insulin-dependent cells, it must first enter the blood stream. Although the exact mechanism of chromium absorption remains uncertain, it is known that chromium is absorbed through the intestinal mucosa (Ducros, 1992; Vincent, 2000a). Factors affecting chromium absorption include "oxalate intake, iron and zinc deficiency, and diabetes," which increase absorption (Jeejeebhoy, 1999). Factors that decrease chromium absorption include phytate intake and aging (Jeejeebhoy, 1999).

Once absorbed, transferrin and albumin transport chromium (Ducros, 1992; Vincent, 2000a). Transport of chromium via albumin occurs only after transferrin has become saturated (Ducros, 1992). Varying amounts of chromium are transported to and accumulate in the liver, soft tissue, spleen, and bone (Ducros, 1992). Chromium is excreted mainly via urine with smaller quantities lost in hair, sweat, and bile (Ducros, 1992).

As trivalent chromium is poorly absorbed, it is "difficult to determine whether trivalent chromium itself is toxic once inside the cell" (Jeejeebhoy, 1999). Concern has been expressed toward the "validity of the cell culture studies reporting clastogenic effects of Cr picolinate and extrapolating the results" to mammalian and "ultimately human studies" (Anderson et al., 1997). In such a cell culture study, "the minimum value where an effect was observed was several thousand-fold higher than any levels observed in the blood of humans following Cr supplementation" (Anderson et al., 1997; Sterns et al., 1995). Several studies involving both animals and humans have shown no toxic effects from usage of chromium supplementation. One particular study fed pregnant mice "up to 15 mg/kg per day trivalent chromium" (Jeejeebhoy, 1999; Danielsson et al., 1982). Accumulation of trivalent chromium in fetal tissues "did not cause any cytotoxicity in fetal mice" (Jeejeebhoy, 1999; Danielsson et al., 1982). As for human clinical studies, in "19 randomized controlled trials in which individuals received between 175 and 1000 mcg/day chromium for durations of between 6 and 64 weeks, there was no evidence of any toxic effects" (Jeejeebhoy, 1999). Perhaps discrepancies between cell culture studies and animal and human studies regarding trivalent chromium toxicity exist because in cell culture studies "protective mechanisms," such as "absorption, transport, conversion to a useful form and/or mechanisms for the conversion to a form that is nontoxic" have been removed or "minimized" (Anderson et al., 1997).

Chromium's "essentiality in humans was documented in 1977" as the result of a patient receiving inadequate long-term TPN (Anderson, 1997b; Jeejeebhoy et al., 1977). Symptoms, such as glucose intolerance, were corrected upon addition of chromium to the TPN solution (Jeejeebhoy et al., 1977). Signs and symptoms of chromium deficiency

that "are routinely observed in the general population" include impaired glucose tolerance, fasting hyperglycemia, glycosuria, hypoglycemia, elevated circulating insulin, decreased insulin receptor number, decreased insulin binding, decreased lean body mass, elevated percent body fat, and increased ocular pressure (Anderson, 1997b). As research interest grows in identifying subclinical deficiency states as causing many of today's health problems, more attention will be focused on the importance of maintaining optimal health through the consumption of a well-rounded diet, and supplementation when necessary.

Foods containing whole grains are considered a good source of dietary chromium (Anderson et al., 1992). Organ meats, as well as "cheese, mushrooms, various condiments and spices" are considered foods that provide "relatively high amounts of chromium" (Groff and Gropper, 2000; Khan et al., 1990; Kumpulaineu, 1992).

When our study began in May of 2000, the estimated safe and adequate daily dietary intake for chromium was 50-200 mcg/day for adults (National Research Council, 1989). According to newly released Dietary Reference Intakes, an Adequate Intake of 35 mcg/day for men and 25 mcg/day for women has been set for chromium consumption (Institute of Medicine, 2001). The new values were released in January 2001, well after our study had been completed. Due to the lack of available data pertaining to "adverse effects resulting from chronically high intake of the chromium contained in supplements," no Upper Limit was set (Institute of Medicine, 2001).

Chromium and Glucose Tolerance

The relationship between chromium and glucose tolerance dates back to a 1959 study involving rats. The study demonstrated a "requirement for chromium to maintain normal glucose tolerance in rats" (Mertz, 1993; Schwarz and Mertz, 1959). Research has continued to try and discover the link between chromium and glucose tolerance. Studies have been conducted involving, among other species, horses, pigs, rats, and humans. Significant evidence has yet to be revealed that will whole-heartedly support beneficial effects of chromium on maintenance of glucose tolerance. Supporters point to cases where individuals receiving total parenteral nutrition (TPN) have developed symptoms, such as hyperglycemia, glycosuria, peripheral neuropathy, and unexpected weight loss, that were corrected with the addition of chromium to TPN solution (Brown et al., 1986; Jeejeebhoy et al., 1977; Freund et al., 1979). Detractors point to studies involving non-obese healthy humans, and patients with Type 2 diabetes that have shown no beneficial effects from chromium supplementation (Amato et al., 2000; Trow et al., 2000).

Other researchers argue that people are missing the point. Researchers such as these argue that chromium is effective at preventing the onset of further glucose intolerance by regulating insulin sensitivity before the individual develops type 2 diabetes (Anderson, 1992). Chromium would not be as effective as a treatment for type 2 diabetes as it would a preventive measure (Anderson, 1992). Likewise, a healthy individual with adequate glucose tolerance and insulin sensitivity would not show benefits from consuming extra chromium in the form of supplementation, as it would appear that they are getting a sufficient amount from their diet (Anderson, 1992). Studies that support some of these claims have shown that chromium is beneficial for people who have

elevated plasma insulin with no signs of elevated plasma glucose (Riales and Albrink, 1981). A study such as this may support the claim that chromium supplementation is an effective preventive measure against the onset of type 2 diabetes. These articles and others will be discussed in this section of the literature review.

Researchers who believe in chromium's ability to maintain glucose tolerance point to TPN patients as providing evidence to support their belief. A 63 year old woman with an ileocolostomy was on TPN for 6 ½ months when she presented with "disturbed glucose metabolism, hyperglycemia, and glycosuria" (Brown et al., 1986). After ruling out other causes, "the assumption that chromium deficiency was responsible for glucose intolerance" led to an infusion of chromium chloride to her TPN solution (Brown et al., 1986). For a two week period, 200 mcg of chromium chloride was added to the TPN regimen daily (Brown et al., 1986). During the first five days of added chromium supplementation, extra insulin that was added to the TPN solution to combat the glucose intolerance "was gradually withdrawn" (Brown et al., 1986). After addition of the chromium chloride and withdrawal of insulin, the patient's serum glucose dropped to 169 mg/dl from well over 200 mg/dl and the glycosuria ended (Brown et al., 1986). The patient was eventually released from the hospital and remained on home TPN containing 32 mcg chromium daily (Brown et al., 1986).

Another patient who had a complete bowel resection and was on TPN for 5 months developed "severe glucose intolerance, weight loss, and a metabolic encephalopathy-like confusional state" (Freund et al., 1979). The patient's "serum chromium level was 0.5 mcg/dl (0.005 ppm), the lowest level of normal (normal, 0.5 to 9.0 mcg/dl or 0.005 to 0.09 ppm)" (Freund et al., 1979). The patient began to receive 150

mcg of chromium chloride daily in her TPN solution (Freund et al., 1979). A few days later, extra insulin (20-30 units/day) that had been added to her TPN prior to chromium supplementation was removed (Freund et al., 1979). When the patient was receiving the 20-30 units of insulin per day, her blood glucose level varied "between 72 to 400 mg/dl, with fluctuations as high as 581 mg/dl and erratic spilling of glucose in the urine" (Brown et al., 1986). After the addition of chromium chloride and removal of insulin from her TPN solution, the patient's blood glucose "was maintained at around 130 to 140 mg/dl (range, 73 to 200 mg/dl), with no spilling of glucose in the urine" (Freund et al., 1979). The patient's "encephalopathy cleared completely, and she started gaining weight" (Freund et al., 1979).

An earlier case of chromium deficiency diagnosed in a patient receiving TPN involved a woman who "underwent a complete enterectomy" (Jeejeebhoy et al., 1977). She was on TPN for 3 years when she developed "weight loss, neuropathy, and glucose intolerance" (Jeejeebhoy et al., 1977). After a clinical neurological examination, the "findings were considered to be consistent with a diabetic neuropathy" (Jeejeebhoy et al., 1977). Insulin was administered in order to control glucose intolerance. After having "45 units of insulin added to each daily infusion", the patient was still below her average weight and the neuropathy "was found to have persisted despite maintenance of near euglycemia" (Jeejeebhoy et al., 1977). Insulin was discontinued and plasma glucose levels "taken at midnight varied between 207 and 265 mg/dl" (Jeejeebhoy et al., 1977). Since "plasma insulin concentrations were normal and the insulin response in the intravenous glucose tolerance tests had been only very mildly reduced, other causes of glucose intolerance were sought" (Jeejeebhoy et al., 1977). Chromium levels "in blood

and hair were found to be low, and a balance study was strongly negative" (Jeejeebhoy et al., 1977). Chromium deficiency was considered, and "it was decided to check the effect of infusing 250 mcg of chromium daily for 2 weeks" (Jeejeebhoy et al., 1977). After two weeks of chromium supplementation via TPN solution, 2 hour postinfusion plasma glucose concentrations "varied between 111 and 118 mg/dl without added insulin" (Jeejeebhoy et al., 1977). Plasma glucose concentrations taken at midnight without insulin "varied between 177 and 196 mg/dl" which were "significantly lower ($P<0.05$) than levels observed before the administration of chromium (207 to 265 mg/dl)" (Jeejeebhoy et al., 1977). After continued chromium supplementation the neuropathy "was no longer detectable either clinically or by nerve conduction studies" (Jeejeebhoy et al., 1977). The patient maintained a sense of well being, normal plasma glucose, and no neuropathy "on a daily infusion of chromium amounting to 20 mcg/day" (Jeejeebhoy et al., 1977).

Case studies of patients receiving TPN demonstrate the deleterious effects of chromium deficiency. Glucose intolerance, decreased insulin sensitivity, and neurological problems are three symptoms associated with chromium deficiency. These symptoms were clearly corrected through the introduction of supplemental chromium to their TPN solutions.

Studies have been conducted revealing that TPN patients are not the only individuals at risk for potential chromium deficiency. Anderson et al. conducted a study on free-living individuals consuming controlled low-chromium diets (Anderson et al., 1991). This 14-week crossover study involved 17 subjects (11 females, 6 males) aged 22-65 years. Screening procedures separated the subjects into two groups,

hyperglycemic (90-min glucose values > 5.56 but < 11.1 mmol/L) and control (90-min glucose values < 5.56 mmol/L) (Anderson et al., 1991). People with diabetes or "clinically significant abnormal blood or urine profiles were excluded from the study" (Anderson et al., 1991). During the study all participants consumed a low chromium diet containing less than 20 mcg per day (Anderson et al., 1991). After a 4 week pretest period, "subjects received tablets containing either 200 mcg Cr as chromium chloride or placebo" (Anderson et al., 1991). At week 10 the subjects "received the opposite tablets" (Anderson et al., 1991).

The results of this study favored chromium supplementation for those individuals who were in the hyperglycemic group. There was no significant difference in the glucose tolerance or insulin values for the control group during the placebo period and their values "remained constant during the chromium-supplementation period" (Anderson et al., 1991). There was a significant difference in glucose tolerance and insulin values for the hyperglycemic group (Anderson et al., 1991). Their values deteriorated as time went on while consuming the low chromium diet (Anderson et al., 1991). However, the sum of the 0 to 90 and 0 to 240 minute glucose values "were significantly lower for the hyperglycemic subjects after chromium supplementation [26.5 and 39.4 mmol/L respectively] then for comparable placebo values [30.0 and 42.9 mmol/L respectively]" (Anderson et al., 1991). Insulin values also significantly improved for the hyperglycemic group while receiving chromium supplementation (1320 and 1703 mmol/L respectively) when compared to values obtained during the placebo period (1787 and 2285 mmol/L respectively) (Anderson et al., 1991). This study showed the beneficial effects of

chromium supplementation for hyperglycemic individuals consuming a low chromium diet.

Another study that demonstrated beneficial effects of chromium supplementation on glucose concentrations involved 76 free-living individuals (48 men and 28 women) aged 21-69 years (Anderson et al., 1983). This double-blind crossover study lasted for 6 months and involved taking of a placebo and a 200 mcg supplement of chromium chloride (Anderson et al., 1983). There were two experimental periods lasting 3 months each (Anderson et al., 1983). During the first three-month period, one group received 200 mcg of chromium chloride per day and the other group received a placebo (Anderson et al., 1983). During the second three-month period the group that had been receiving the chromium took the placebo and vice versa (Anderson et al., 1983). At the beginning of the study, 20 of the 76 subjects "had serum glucose concentrations greater than or equal to 100 mg/dl 90 minutes after a glucose challenge (1 g glucose per kilogram of body weight)" (Anderson et al., 1983). According to the results of this study, "chromium supplementation significantly decreased ($P<0.05$) the 90-minute glucose concentrations of these subjects from 135 ± 9 to 116 ± 11 mg/dl" (Anderson et al., 1983). The fasting serum glucose concentrations of these subjects also significantly ($P<0.05$) decreased "from 90 ± 3 mg/dl to 80 ± 2 and 84 ± 2 mg/dl after 2 and 3 months Cr supplementation, respectively" (Anderson et al., 1983). Serum insulin values did not significantly decrease for these subjects (Anderson et al., 1983). However, as the researchers involved with this study concluded, "the insulin in the serum appeared to be more effective . . . following Cr supplementation" and an "increase in insulin sensitivity would be a prerequisite to reduced circulating levels" (Anderson et al., 1983).

Other studies are not as quick to make a positive association between chromium supplementation, glucose tolerance, and insulin sensitivity. However, what seems to be the underlying characteristic of these studies is that they either involved people who were already diagnosed with diabetes, or were healthy and nonobese. For example, one such study looked at the effects of chromium supplementation on glucose tolerance and plasma insulin levels in patients with type 2 diabetes (Trow et al., 2000). This uncontrolled, pilot study used 12 free-living patients with type 2 diabetes (Trow et al., 2000). The subjects took a chromium rich yeast capsule containing 100 mcg of chromium per day for 8 weeks. According to their results, "fasting glucose concentrations and glucose area under the curve profiles did not alter significantly post supplementation with the chromium rich yeast (Trow et al., 2000). There were also no significant changes in insulin concentrations (Trow et al., 2000). The researchers pointed out that it was "possible that an effect could have been observed with a larger study group" (Trow et al., 2000).

Another study that obtained results casting doubt on the beneficial effects of chromium involved healthy, nonobese, older men and women (Amato et al., 2000). This was "a randomized, double-blind, placebo-controlled study with 19 subjects (9 men and 10 women), aged 63-77" (Amato et al., 2000). The subjects were given "either chromium picolinate, 1000 mcg/d, or a placebo for 8 weeks" (Amato et al., 2000). Amato et al. reported that "chromium picolinate supplementation alone does not appear to improve insulin sensitivity . . . in nonobese, healthy men and woman of advanced age" (Amato et al., 2000). The researchers from this study stated that their results "cast doubt on the role of chromium picolinate supplementation for the maintenance of glucose

homeostasis in healthy men and women of advanced age" (Amato et al., 2000). What has been said in the past is that chromium "improves glucose and insulin function of people with varying degrees of glucose intolerance that range from hypoglycemia to uncontrolled steroid diabetes with no detectable effects on glucose and insulin in people with good glucose tolerance" (Anderson, 2000). To state that this study by Amato et al. might cast doubt on the beneficial effects of chromium supplementation is a bit premature since the study dealt with healthy, nonobese individuals.

A study with similar results brought a different conclusion. A study by Offenbacher et al. that involved "twenty-three healthy, well nourished, free-living elderly volunteers" found no significant changes in glucose tolerance and insulin associated with 10 weeks of supplementation with "5 g brewer's yeast, 200 mcg Cr^{3+} as chromic chloride ($CrCl_3$), or placebo" (Offenbacher et al., 1985). However, rather than stating that their findings cast doubt on the beneficial effects of chromium supplementation, the researchers stated that this study "suggests that age per se is not a factor leading to chromium deficiency" (Offenbacher et al., 1985). This sentiment coincides with Anderson, who stated a healthy individual with adequate glucose tolerance and insulin sensitivity would not show benefits from consuming extra chromium in the form of supplementation, as it would appear that they are getting a sufficient amount from their diet (Anderson, 1991).

CHAPTER III

MATERIALS AND METHODS

Research Design

This was a double blind study that followed a pretest-posttest control group experimental design. Two groups were involved with this study, a control group receiving a lactose placebo, and a treatment group receiving a supplement containing 200 mcg chromium as chromium chloride in lactose. Two data collections included a baseline pretest collection at week zero and a posttest collection after twelve weeks of supplementation.

Participants

The Oklahoma State University Institutional Review Board approved this study for human subject research (Appendix A). Participants were solicited via flyer through campus wide mailing, newspaper ads, and local posting of flyers throughout the Stillwater area (Appendix B). Male participants were solicited for screening if they felt they had or met one or more of the criteria that increased risk for diabetes, such as having a family history of diabetes, or being overweight or over forty years of age. Participants were screened for fasting blood glucose concentrations less than 126 mg/dl, and two-hour

serum glucose concentrations between 130 mg/dl and 199 mg/dl following a 75 g oral glucose challenge (American Diabetes Association, 2000).

Supplement Preparation

Supplements for this study were prepared in the Department of Nutritional Sciences laboratory at Oklahoma State University. A gelatin capsule filler machine (Quanterron, Inc., Burnsville, MN) was used to fill number two gelatin capsules (Apothecary Products Inc., Minneapolis, MN).

On average, placebo capsules contained 0.24 g of U.S.P. grade lactose monohydrate (Spectrum Chemical Mfg. Corp., Gardena, CA). The chromium supplement mixture consisted of 2.0502 g of U.S.P. grade $CrCl_3.6H_2O$ (Professional Compounding Centers of America, Inc. Houston, TX) and 477.95 g lactose, so that 0.24 g of chromium/lactose supplement mixture contained 200 mcg of chromium as $CrCl_3.6H_2O$. The lactose and $CrCl_3.6H_2O$ were mixed for twenty-four hours in a ball mixer (U.S. Stoneware, East Palestine, OH) to ensure that the $CrCl_3.6H_2O$ was mixed evenly with the lactose. Eight 0.1 g samples were randomly taken from the chromium and placebo mixtures, four from the chromium supplement mixture and four from the placebo mixture. Samples were wet and dry ashed using a modification of the Hill et al method (Hill et al., 1986). Samples were then analyzed for chromium content using an atomic absorption spectrophotometer (Model 5100 PC, Perkin-Elmer Corp., Norwalk, CT). The average analyzed chromium content of the placebo was –0.484 mcg/g. The

average analyzed chromium content of the chromium supplement mixture was 658.3 mcg/g. The capsule free weight of the supplement mixture was adjusted to 0.3038 g so that each capsule would contain 200 mcg chromium, as chromium chloride hexahydrate.

Data Collection

Screening

In March 2000 participants came to the Student Health Center on the campus of Oklahoma State University after a twelve-hour fast. Participants were instructed to drink water during the fast to ensure an easier blood draw. At the screening, participants signed an informed consent for the screening (Appendix C). A HemoCue B-Glucose Photometer (HemoCue Inc., Mission Viejo, CA) analyzed a finger-prick fasting blood sample. If the fasting blood glucose concentration was greater than or equal to 126 mg/dl participants were excluded from the study and referred to a doctor from the Student Health Center. Participants with fasting blood glucose concentrations lower than 126 mg/dl had a fasting blood sample taken by a licensed phlebotomist in a 6 ml Vacutainer serum tube (Franklin Lakes, NJ). Participants were then given a Glucose Tolerance Beverage containing 75 g of dextrose (Appendix D). Following consumption of the beverage, blood samples were taken at 120 minutes in 6 ml Vacutainer serum tubes (Franklin Lakes, NJ).

While participants waited for blood draw, they completed a health questionnaire (Appendix E) and heights and weights were recorded. Participants' heights were

measured while standing erect with heels together and back against a wall. Heights were measured from a scaled ruler taped to the wall. Participants' weights were measured with a battery-powered scale. Body Mass Index (BMI) was calculated for each participant based on the equation: BMI = body weight (kg) / height (m^2) (Meisler and St. Jeor, 1996). After the final blood draw, participants were provided with nutritional support.

After laboratory analysis of fasting and two-hour serum glucose concentrations, participants were contacted by telephone and given results. Participants with two-hour serum glucose concentrations between 130 mg/dl and 199 mg/dl were invited to continue participation in the study. Participants with two-hour serum glucose concentrations greater than or equal to 200 mg/dl were informed they could not continue with the study and should consult a physician. Written copies of laboratory results were mailed to all participants.

Twenty individuals who qualified for the study and volunteered to participate were matched pair-wise based on their fasting serum glucose, two-hour serum glucose, age, and BMI and placed into two groups. Ten participants were placed in each group. The groups were randomly assigned as treatment and placebo. The assignation of groups as treatment or placebo was double blind.

Baseline Collection

In May 2000 participants came to the Student Health Center on the campus of Oklahoma State University after a twelve-hour fast. Participants were instructed to drink water during the fast to ensure an easier blood draw. At the baseline collection, participants signed an informed consent for the study (Appendix F). A licensed

phlebotomist drew a fasting blood sample in a 10 ml Vacutainer tube containing sodium heparin and a 10 ml Vacutainer serum tube (Franklin Lakes, NJ). Participants were then given a Glucose Tolerance Beverage containing 75 g of dextrose (Appendix D). Following consumption of the beverage, blood samples were taken at 30, 60, 120, and 180 minutes in 6 ml Vacutainer serum tubes for determinations requiring serum (Franklin Lakes, NJ).

Participants were instructed on how to fill out a seven-day food frequency questionnaire (Appendix G). While participants were waiting for blood draws, they completed the seven-day food frequency questionnaire, and height and body weight was recorded. Participants' heights were measured while standing erect with heels together and back against a wall. Heights were measured from a scaled ruler taped to the wall. Heights were measured to check for consistency with the screening heights. Participants' weights were measured with a battery-powered scale. The participants were also given the first four-week supply of the randomly assigned supplement and instructed to take one capsule daily with food. Six extra capsules were added to each four-week supply. Remaining capsules were counted at the end of each four-week period to monitor for compliance. Following the final blood draw, participants were provided nutritional support. The second and third four-week supplies of supplements were delivered to the participants on a monthly basis. Participants received $150.00 for their participation in the baseline data collection.

Post-data Collection

In August 2000, at the end of twelve weeks supplementation, subjects returned after a twelve-hour fast to the Student Health Center on the campus of Oklahoma State University for the posttest data collection. A licensed phlebotomist drew a fasting blood sample in a 10 ml Vacutainer tube containing sodium heparin and a 10 ml Vacutainer serum tube (Franklin Lakes, NJ). Participants were then given a Glucose Tolerance Beverage containing 75 g of dextrose (Appendix D). Following consumption of the beverage, blood samples were taken at 30, 60, 120, and 180 minutes in 6 ml Vacutainer serum tube for determinations requiring serum (Franklin Lakes, NJ). Participants were instructed on how to fill out a seven-day food frequency questionnaire (Appendix G). While participants were waiting for blood draws, they completed the seven-day food frequency questionnaire, and body weight was recorded. Following the final blood draw, participants were provided nutritional support. Participants received $150.00 for their participation in the posttest data collection.

Blood Handling

The same blood handling techniques were followed for both baseline and posttest data collections. Fasting heparin tubes were inverted and set on ice for thirty minutes. At the Nutritional Sciences laboratory, two 0.5 ml whole blood samples were transferred from the fasting heparin tubes into 0.5 ml plastic storage tubes. The tubes were covered with parafilm and stored at –20°C for future analysis of glycosylated hemoglobin concentration. Erythrocyte membrane ghosts were prepared from the remaining fasting

heparinized blood samples using a method developed by Dodge et al. (Dodge et al., 1963). Erythrocyte ghosts were stored at –70°C for future analysis of percent specific insulin binding and protein concentration.

Fasting, 30, 60, 120, 180 minute serum tubes were placed on ice until centrifugation at the Nutritional Sciences laboratory. The serum tubes were centrifuged at 4,000 rpm for 30 minutes in a Jouan Inc. CR3I centrifuge. Serum was transferred with transfer pipettes into individual 0.5 ml plastic storage tubes. The storage tubes were wrapped with parafilm and stored at –20°C for future analysis of serum glucose and serum insulin concentrations.

Biochemical Analysis

Glycosylated Hemoglobin

Whole blood glycosylated hemoglobin concentrations were determined using Roche HbA1c kit number 075563 (Roche Diagnostic System, Inc., Somerville, NJ) on a FARA clinical analyzer (COBAS FARA, Roche Diagnostic System, Inc., Montclair, NJ). Calibration factors and control materials available from Roche Diagnostic Systems Inc. were used for quality control to ensure validity and reliability of the testing procedure.

Serum Glucose

Serum glucose concentrations were determined using Roche kit number 47383 (Roche Diagnostic Systems Inc., Somerville, NJ) on a FARA clinical analyzer (COBAS

Protein Concentration of Erythrocyte Ghosts

Protein concentrations of erythrocyte ghost samples were determined using BIO-RAD protein assay kit number 500-001 (Los Angeles, CA) on a spectrophotometer (Beckman, DU 640 Spectrophotometer). A standard curve of control samples was used to ensure validity and reliability of the testing procedure.

Food Frequency Questionnaire Analysis

The seven-day food frequency questionnaires were used to monitor the participants' dietary intake (Eck et al., 1991). The questionnaire was a modified version of Willett's one-year food frequency questionnaire that was tested for reliability and validity (Eck et al., 1991). The questionnaires were analyzed using the Food Processor Plus Program (version 7.11, ESHA Research, Salem, OR). The same food codes were used for all participants' entries. Food frequency questionnaires were analyzed to monitor for changes in participants' diets.

Data Analysis

Data were analyzed using the Statistical Analysis System (SAS) for repeated measures and least squared means (SAS Inst. Inc., Cary, NC, 1989). The level of significance was set at $p \leq 0.05$. Effect of the independent variable, chromium supplementation, on the dependent variables, fasting serum glucose, fasting serum

insulin, three-hour glucose curves, glycosylated hemoglobin, and percent specific insulin binding, was determined.

CHAPTER IV

RESULTS AND DISCUSSION

Results

Four participants were excluded from the data analysis. Two participants received 100 g of dextrose rather than the 75 g dose they were supposed to have received at the posttest collection. A third participant underwent open-heart surgery. A fourth participant lost a substantial amount of weight after changing medications. These four participants experienced events outside the realm of focus for this study. There were two participants from each group therefore the groups remained even. Data from 16 participants, 8 from the chromium group and 8 from the placebo group, were analyzed for this study.

Age

There was no significant difference between the mean age of the participants in the two groups at baseline (Table 1). The mean age of the chromium group was 46 ± 4 years, while the mean age of the placebo group was 49 ± 4 years (Table 1).

Weight

Repeated measures analysis did not show a significant time by group interaction for total body weight (Table 2). The mean total body weights of the chromium group at baseline and posttest were 245 ± 15 lbs. and 246 ± 14 lbs., respectively (Table 1). The mean total body weights of the placebo group at baseline and posttest were 236 ± 16 lbs. and 237 ± 17 lbs., respectively (Table 1). Repeated measures analysis showed a significant group effect (Table 2). The mean total body weight of the placebo group was 9 lbs. heavier than the chromium group at baseline and posttest.

Body Mass Index

Repeated measures analysis did not show a significant time by group interaction for body mass index (BMI) (Table 2). The mean BMI of the chromium group was 35 ± 2 kg/m^2 at baseline and posttest (Table 1). The mean BMI of the placebo group was 32 ± 2 kg/m^2 at baseline and posttest (Table 1). Repeated measures analysis showed a significant group effect (Table 2). The mean BMI of the chromium group at baseline and posttest (35 ± 2 kg/m^2) was 3 kg/m^2 greater than the mean BMI of the placebo group at baseline and posttest (32 ± 2 kg/m^2). According to the 2000 Dietary Guidelines for Americans, a BMI greater than 30 kg/m^2 refers to obesity (United States Department of Agriculture, 2000). The study participants were not body builders or extremely thin giants, so the classification of obese fits both groups.

Dietary Intake

Repeated measures analysis did not show a significant time by group interaction for dietary intake (Table 3). Repeated measures analysis showed a significant time effect for total kilocalorie, carbohydrate, and fiber intakes (Table 3). The mean total kilocalorie intakes for both the chromium and placebo groups were higher at baseline than at posttest (Table 4). The mean total carbohydrate intakes for both the chromium and placebo groups were higher at baseline than at posttest (Table 4). The mean total fiber intakes for both the chromium and placebo groups were higher at baseline than at posttest (Table 4).

According to the 1977 Dietary Goals for the United States, which was issued by the U.S. Senate Select Committee on Nutrition and Human Needs, participants of this study did not consume the necessary nutrient quantities. In order to meet the goals set forth by the Committee, American diets should be proportioned in such a way that 58% of total kilocalories come from carbohydrate, 12% of total kilocalories come from protein, and less than 30% of total kilocalories come from fat (Select Committee on Nutrition and Human Needs, 1977). The percentages of kilocalories coming from carbohydrate for the chromium group at baseline and posttest were 51% and 49%, respectively. The percentages of kilocalories coming from carbohydrate for the placebo group at baseline and posttest were 48% and 47%, respectively. Therefore the chromium and placebo groups were below the recommended dietary intake for carbohydrate consumption. The percentages of kilocalories coming from protein for the chromium group at baseline and posttest were 15% and 17%, respectively. The percentages of kilocalories coming from protein for the placebo group at baseline and posttest were 15% and 17%, respectively. Therefore the chromium and placebo groups were above the

recommended dietary intake for protein consumption. The percentages of kilocalories coming from fat for the chromium group at baseline and posttest were 35% and 32%, respectively. Therefore the chromium group was above the recommended dietary intake for fat consumption. The percentages of kilocalories coming from fat for the placebo group at baseline and posttest were 29% and 30%, respectively. Therefore the placebo group consumed the recommended dietary intake for fat consumption.

Serum Glucose

Repeated measures analysis did not show a significant time by group interaction for serum glucose concentration in response to an oral glucose tolerance test (OGTT) (Table 5). Repeated measures analysis showed a significant time effect for serum glucose concentration in response to an oral glucose tolerance test (Table 5), which was expected since serum glucose concentrations naturally change over time during an OGTT.

At baseline, both groups had mean fasting serum glucose concentrations indicative of impaired glucose tolerance (fasting glucose concentrations $\geq$110 mg/dl but <126 mg/dl) (Wallach, 2000). The mean fasting serum glucose concentration for the chromium group at baseline was 116 $\pm$ 7 mg/dl (Table 6). The mean fasting serum glucose concentration for the placebo group at baseline was 128 $\pm$ 8 mg/dl (Table 6). At baseline, both groups had two-hour serum glucose concentrations indicative of impaired glucose tolerance (two-hour glucose concentration following an OGTT $\geq$140 mg/dl but <200 mg/dl) (Wallach, 2000). The mean two-hour serum glucose concentration for the

chromium group at baseline was 150 ± 16 mg/dl (Table 6). The mean two-hour serum glucose concentration for the placebo group at baseline was 167 ± 15 mg/dl (Table 6).

At posttest, the mean fasting serum glucose concentration for the chromium group was 106 ± 5 mg/dl (Table 6). While this concentration was below the 110 mg/dl concentration that is indicative of impaired glucose tolerance, it was not a significant difference. At posttest, the mean fasting serum glucose concentration for the placebo group was 120 ± 10 mg/dl (Table 6). At posttest, both groups still had two-hour serum glucose concentrations indicative of impaired glucose tolerance (two-hour glucose concentration following an OGTT ≥140 mg/dl but <200 mg/dl) (Wallach, 2000). The two-hour serum glucose concentration for the chromium group at posttest was 150 ± 16 mg/dl, which was still above the reference concentration of 140 mg/dl (Table 6). The two-hour serum glucose concentration for the placebo group at posttest was 140 ± 12 mg/dl (Table 6).

Serum Insulin

Repeated measures analysis did not show a significant time by group interaction for serum insulin concentration in response to an OGTT (Table 7). Repeated measures analysis showed a significant time effect for serum insulin concentration in response to an OGTT (Table 7), which was expected since serum insulin concentrations naturally change over time during an OGTT. Both groups experienced a reduction in their serum insulin concentrations from baseline to posttest OGTT (Table 8).

Glycosylated Hemoglobin

Repeated measures analysis did not show a significant time by group interaction for mean percent glycosylated hemoglobin (Table 9). Repeated measures analysis showed significant group and time effects for mean percent glycosylated hemoglobin (Table 9). Due to the lack of a significant time by group interaction, there was no adjustment for covariance. The mean percent glycosylated hemoglobin of the chromium group at baseline was 5.6 ± 0.1% (Table 10). The mean percent glycosylated hemoglobin of the placebo group at baseline was 6.0 ± 0.1% (Table 10). The mean percent glycosylated hemoglobin of the chromium group at posttest was 5.9 ± 0.1% (Table 10). The mean percent glycosylated hemoglobin of the placebo group at posttest was 6.4 ± 0.2% (Table 10). At baseline and pretest the mean percent glycosylated hemoglobin for both groups fell within the 4% to 8% range associated with "persons without diabetes" (Lee & Nieman, 1996).

Percent Specific Insulin Binding

Repeated measures analysis did not show a significant time by group interaction for mean percent specific insulin binding (Table 9). Repeated measures analysis showed a significant time effect for mean percent specific insulin binding. The mean percent specific insulin binding/100 mcg of protein for the chromium group at baseline was and 1.7 ± 0.2 (Table 10). The mean percent specific insulin binding/100 mcg protein for the placebo group at baseline was 2.0 ± 0.1 (Table 10). The mean percent specific insulin binding/100 mcg of protein for the chromium group at posttest was and 1.4 ± 0.2 (Table 10). The mean percent specific insulin binding/100 mcg protein for the placebo group at

posttest was 1.1 ± 0.1 (Table 10). Values for percent specific insulin binding/100 mcg protein from other studies range from 0.93% to 2.51% for healthy participants (Bhathena et al., 1989; Bhathena et al., 1995).

Table 1: Mean age, weight, and body mass index at baseline and after 12 weeks of supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Experimental (n=8)		Control (n=8)	
	Baseline	Posttest	Baseline	Posttest
Weight (lbs.)	245 ± 15	246 ± 14	236 ± 16	237 ± 17
Body Mass Index (kg/m^2)	35 ± 2	35 ± 2	32 ± 2	32 ± 2
Age (year)	46 ± 4		49 ± 4	

*Values are mean ± SEM

Table 2: Analysis of variance summary table for weight, and body mass index after 12 weeks supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Source of Variance	F value	Probability
Weight	supplement	33.81	< 0.0001*
	time	0.65	0.4325
	supp*time	0.01	0.9229
	code (supp)	186.60	< 0.0001*
Body Mass Index	supplement	127.09	< 0.0001*
	time	0.60	0.4510
	supp*time	0.01	0.9081
	code (supp)	201.38	< 0.0001*

*Significance at $p \leq 0.05$

Table 3: Analysis of variance summary table for kilocalorie, protein, carbohydrate, fiber, and fat intakes after 12 weeks supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Source of Variance	F value	Probability
Kilocalorie intake	supplement	1.43	0.2518
	time	6.56	0.0226*
	supp*time	0.33	0.5751
	code (supp)	2.14	0.0834
Protein intake	supplement	0.81	0.3828
	time	0.87	0.3665
	supp*time	0.56	0.4649
	code (supp)	1.48	0.2347
Carbohydrate intake	supplement	2.99	0.1056
	time	9.77	0.0074*
	supp*time	0.20	0.6602
	code (supp)	2.64	0.0398*
Fiber intake	supplement	0.06	0.8135
	time	10.92	0.0052*
	supp*time	0.01	0.9261
	code (supp)	2.40	0.0563
Fat intake	supplement	2.55	0.1323
	time	3.14	0.0981
	supp*time	0.02	0.9011
	code (supp)	1.40	0.2700

*Significance at $p \leq 0.05$

Table 4: Mean kilocalorie, protein, carbohydrate, fiber, and fat intakes at baseline and after 12 weeks of supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Experimental (n=8)		Control (n=8)	
	Baseline	Posttest	Baseline	Posttest
Kilocalorie intake	2534 ± 284	2109 ± 193	2400 ± 286	1730 ± 296
Protein intake (g)	93 ± 10	91 ± 12	91 ± 11	74 ± 12
Carbohydrate intake (g)	322 ± 40	256 ± 33	290± 29	202 ± 31
Fiber intake (g)	23 ± 3	16 ± 2	22 ± 4	16 ± 3
Fat intake (g)	97 ± 13	74 ± 7	76 ± 19	57 ± 10

* Values are mean ± SEM

Table 5: Analysis of variance summary table for serum glucose concentration following a 75 g OGTT after 12 weeks supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Source of Variance	F value	Probability
0 minutes	supplement	12.24	0.0035*
	time	5.42	0.0355*
	supp*time	0.03	0.8605
	code (supp)	7.40	0.0003*
30 minutes	supplement	5.53	0.0339*
	time	3.91	0.0681
	supp*time	3.70	0.0750
	code (supp)	23.37	< 0.0001*
60 minutes	supplement	6.03	0.0277*
	time	6.80	0.0206*
	supp*time	0.00	0.9512
	code (supp)	11.99	< 0.0001*
120 minutes	supplement	0.04	0.8433
	time	1.58	0.2294
	supp*time	3.09	0.1008
	code (supp)	4.77	0.0030*
180 minutes	supplement	0.40	0.5386
	time	5.27	0.0390*
	supp*time	0.02	0.9008
	code (supp)	6.66	0.0008*

*Significance at $p \leq 0.05$

Table 6: Mean serum glucose concentration following a 75 g OGTT at baseline and after 12 weeks of supplementation with $CrCl_3$ (experimental) or placebo (control)[1,2]

	Experimental (n=8)		Control (n=8)	
	Baseline	Posttest	Baseline	Posttest
0 minutes	116 ± 7	106 ± 5	128 ± 8	120 ± 10
30 minutes	203 ± 15	185 ± 12	205 ± 19	205 ± 18
60 minutes	229 ± 27	205 ± 16	250 ± 24	227 ± 24
120 minutes	150 ± 16	154 ± 18	167 ± 15	140 ± 12
180 minutes	98 ± 9	89 ± 10	101 ± 8	91 ± 7

[1]Values are given in mg/dl [2]Values are mean ± SEM

Table 7: Analysis of variance summary table for serum insulin concentration following a 75 g OGTT after 12 weeks supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Source of Variance	F value	Probability
0 minutes	supplement	0.33	0.5759
	time	2.27	0.1538
	supp*time	0.57	0.4639
	code (supp)	2.05	0.0964
30 minutes	supplement	1.31	0.2721
	time	3.23	0.0937
	supp*time	0.29	0.6016
	code (supp)	3.01	0.0239*
60 minutes	supplement	12.31	0.0038*
	time	3.40	0.0880
	supp*time	1.75	0.2090
	code (supp)	8.06	0.0003*
120 minutes	supplement	1.56	0.2334
	time	1.03	0.3293
	supp*time	1.94	0.1874
	code (supp)	8.72	0.0002*
180 minutes	supplement	15.13	0.0016*
	time	0.85	0.3715
	supp*time	0.45	0.5149
	code (supp)	16.46	< 0.0001*

*Significance at $p \leq 0.05$

Table 8: Mean serum insulin concentration following a 75 g OGTT at baseline and after 12 weeks of supplementation with $CrCl_3$ (experimental) or placebo (control)[1,2]

	Experimental (n=8)		Control (n=8)	
	Baseline	Posttest	Baseline	Posttest
0 minutes	27 ± 5	24 ± 6	27 ± 5	19 ± 2
30 minutes	96 ± 15	82 ± 16	89 ± 21	63 ± 10
60 minutes	132 ± 26	126 ± 33	107 ± 20	71 ± 11
120 minutes	94 ±24	79 ± 21	95 ± 19	71 ± 23
180 minutes	46 ± 14	45 ± 16	34 ± 6	27 ± 8

[1]Values are given in μIU/ml [2]Values are mean ± SEM

Table 9: Analysis of variance summary table for percent glycosylated hemoglobin and percent specific insulin binding after 12 weeks supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Source of Variance	F value	Probability
Glycosylated Hemoglobin	supplement	74.66	< 0.0001*
	time	54.31	< .00001*
	supp*time	4.06	0.0635
	code (supp)	17.02	< 0.0001*
Percent Specific Insulin Binding (per 100mcg protein)	supplement	0.00	0.9907
	time	10.45	0.0060*
	supp*time	1.97	0.1825
	code (supp)	0.36	0.9659

*Significance at $p \leq 0.05$

Table 10: Mean percent glycosylated hemoglobin and percent specific insulin binding at Baseline and after 12 weeks of supplementation with $CrCl_3$ (experimental) or placebo (control)*

	Experimental (n=8)		Control (n=8)	
	Baseline	Posttest	Baseline	Posttest
Glycosylated Hemoglobin (%)	5.6 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	6.4 ± 0.2
Specific Insulin Binding (%) (per 100mcg protein)	1.7 ± 0.2	1.4 ± 0.2	2.0 ± 0.1	1.1 ± 0.1

*Values are mean ± SEM

Discussion

Body Mass Index

There was no significant time by group interaction in BMI. This was not unexpected since the study was only 12 weeks in length and participants were instructed to maintain a consistent diet throughout the duration of the study.

Dietary Intake

Even though participants were instructed to not change their diets, decreases in total kilocalorie, carbohydrate, and fiber intakes were reported by the two groups. Repeated measures analysis showed a significant time effect for total kilocalorie, carbohydrate, and fiber intakes.

Serum Glucose

Chromium's "essentiality in humans was documented in 1977" as a result of a patient receiving inadequate long-term TPN (Anderson, 1997b; Jeejeebhoy et al., 1977). Symptoms, such as glucose intolerance, were corrected upon the addition of chromium to the TPN solution (Jeejeebhoy et al., 1977). Since then, signs and symptoms of chromium deficiency that are "routinely observed in the general population" include impaired glucose tolerance, and decreased insulin binding (Anderson, 1997b).

Repeated measures analysis did not show a significant time by group interaction for serum glucose concentrations. While a previous study involving 76 participants, of

which 20 were glucose intolerant, demonstrated a significant decrease in fasting serum glucose concentrations in glucose intolerant participants receiving 200 mcg of chromium chloride per day for 3 months (Anderson et al., 1983), our study revealed no such effect. The 1983 study was a double-blind crossover design involving 76 participants, of which 13 males and 7 females were glucose intolerant (Anderson et al., 1983). The participants received 3 months of chromium and then 3 months of placebo without a wash-out period (Anderson et al., 1983).

In our study, the mean glucose tolerance curve of the chromium group when compared to the mean glucose tolerance curve of the placebo group showed no significant improvement in glucose concentrations due to 12 weeks of chromium supplementation. These results conflict with the findings of the 1983 Anderson et al. study, which demonstrated a significant improvement in glucose concentrations during an OGTT for glucose intolerant participants who had received chromium supplementation (Anderson et al., 1983).

A study that had a similar sample size to ours but employed a crossover design demonstrated that during an OGTT, glucose concentrations were significantly lower for the hyperglycemic subjects after chromium supplementation than for comparable placebo values (Anderson et al., 1991). The 1991 study involved two groups, a control group of 6 healthy females and 3 healthy males aged 22-65 years and an experimental group of 5 hyperglycemic females and 3 hyperglycemic males aged 22-65 years (Anderson et al., 1991). The study looked at the effects of supplemental chromium on glucose and insulin in participants consuming controlled low-chromium diets (Anderson et al., 1991). The supplementation consisted of 200 mcg per day of chromium as chromium chloride or a

placebo. The supplementation periods lasted five weeks without a wash-out period (Anderson et al., 1991). Perhaps the larger sample size (Anderson et al., 1983) and crossover designs increased the potential for demonstrating significance in the 1983 and 1991 studies.

Serum Insulin

Repeated measures analysis did not show a significant time by group interaction for serum insulin concentrations. A previous study demonstrated a significant improvement in insulin concentrations "for the hyperglycemic group while receiving chromium supplementation when compared to values obtained during the placebo period," our study revealed no such effect on insulin concentrations (Anderson et al., 1991). The 1991 study observed during a four hour OGTT using 1 g glucose/kg body weight, that the "sums of insulin values from 0 to 90 and from 0 to 240 minutes" of the hyperglycemic participants significantly "decreased after chromium supplementation" (Anderson et al., 1991). This study had a similar sample size to ours but employed a crossover design. The 1991 study involved two groups, a control group of 6 healthy females and 3 healthy males aged 22-65 years and an experimental group of 5 hyperglycemic females and 3 hyperglycemic males aged 22-65 years (Anderson et al., 1991). The study looked at the effects of supplemental chromium on glucose and insulin in participants consuming controlled low-chromium diets (Anderson et al., 1991). The supplementation consisted of 200 mcg per day of chromium as chromium chloride or a placebo. The supplementation periods lasted five weeks without a wash-out period

(Anderson et al., 1991). Perhaps the observed significance in their study was a result of their crossover experimental design.

Another study by Anderson et al. that also used a crossover design and had a larger sample size than our study did not have significantly improved serum insulin concentrations from baseline (Anderson et al., 1983). The 1983 study was a double-blind crossover design involving 76 participants, of which 13 males and 7 females were glucose intolerant (Anderson et al., 1983). The participants received 3 months of chromium supplementation and then 3 months of placebo without a wash-out period (Anderson et al., 1983). The chromium supplementation period consisted of 200 mcg of chromium per day as chromium chloride (Anderson et al., 1983). However, as was mentioned earlier about this study, there were significant improvements in serum glucose concentrations. The improved serum glucose concentrations prompted Anderson and colleagues to conclude that "the insulin in the serum appeared to be more effective . . . following Cr supplementation" and an "increase in insulin sensitivity would be a prerequisite to reduced circulating levels" (Anderson et al., 1983). Since we did not observe any significant improvements in the serum glucose concentrations due to 12 weeks of chromium supplementation compared to the placebo, we cannot make the same assumption regarding improved insulin sensitivity.

Glycosylated Hemoglobin

Repeated measures analysis did not show a significant time by group interaction for the percentage of glycosylated hemoglobin. At baseline and pretest the mean percent

glycosylated hemoglobin for both groups fell within the 4% to 8% range associated with "persons without diabetes" (Lee & Nieman, 1996).

Glycosylated hemoglobin is a long-term indicator of glucose tolerance (Lee & Nieman, 1996). Improved glucose tolerance would lead to a reduced level of circulating glucose, which would then correspond with a lower percentage of glycosylated hemoglobin. Since we did not observe a significant improvement in glucose tolerance, it was not unexpected that the percent of glycosylated hemoglobin did not significantly change.

Percent Specific Insulin Binding

Repeated measures analysis did not show a significant time by group interaction for mean percent specific insulin binding. No other chromium supplementation studies have looked at percent specific insulin binding/100mcg protein. Values for percent specific insulin binding/100 mcg protein from studies with different objectives and sample populations range from 0.93% to 2.51% for healthy participants (Bhathena et al., 1989; Bhathena et al., 1995). At baseline and posttest the mean percent specific insulin binding for both the chromium and placebo groups fell within the 0.93% to 2.51% range.

CHAPTER V

SUMMARY, CONCLUSIONS, IMPLICATIONS, AND RECOMMENDATIONS

Summary

This study investigated the effect of twelve weeks chromium supplementation at 200 mcg per day on fasting serum glucose concentration, fasting serum insulin concentration, three-hour glucose tolerance curves, percent glycosylated hemoglobin, and percent specific insulin binding in hyperglycemic men. This study followed a pretest-posttest control group experimental design. Ten human participants in the control group received a lactose placebo each day for twelve weeks. Ten participants in the experimental group received a 200 mcg chromium supplement as chromium chloride each day over the same twelve week period. The two groups were randomly assigned as control and experiment. Due to extraneous factors outside the focus of the study, four participants were excluded from the data analysis leaving eight participants per group. Twelve weeks of chromium supplementation did not significantly affect fasting serum glucose concentrations, fasting serum insulin concentrations, three-hour glucose tolerance curves, percent glycosylated hemoglobin, or percent specific insulin binding in the hyperglycemic men who participated in this study.

Conclusions

Five null hypotheses were developed for this study.

H_o 1: There will be no statistically significant effect of twelve weeks chromium supplementation on fasting serum glucose concentrations in hyperglycemic men.

There was no significant time by group interaction on fasting serum glucose concentrations of hyperglycemic men receiving twelve weeks of chromium supplementation and hyperglycemic men receiving twelve weeks of placebo supplementation, therefore we failed to reject this null hypothesis.

H_o 2: There will be no statistically significant effect of twelve weeks chromium supplementation on fasting serum insulin concentrations in hyperglycemic men.

There was no significant time by group interaction on serum insulin concentrations of hyperglycemic men receiving twelve weeks of chromium supplementation and hyperglycemic men receiving twelve weeks of placebo supplementation, therefore we failed to reject this null hypothesis.

H_o 3: There will be no statistically significant effect of twelve weeks chromium supplementation on three-hour glucose tolerance curves of hyperglycemic men.

There was no significant time by group interaction on three-hour glucose tolerance curves of hyperglycemic men receiving twelve weeks of chromium supplementation and hyperglycemic men receiving twelve weeks of placebo supplementation, therefore we failed to reject this null hypothesis.

H_o 4: There will be no statistically significant effect of twelve weeks chromium supplementation on percent glycosylated hemoglobin of hyperglycemic men.

There was no significant time by group interaction on percent glycosylated hemoglobin concentrations of hyperglycemic men receiving twelve weeks of chromium supplementation and hyperglycemic men receiving twelve weeks of placebo supplementation, therefore we failed to reject this null hypothesis.

H_o 5: There will be no statistically significant effect of twelve weeks chromium supplementation on percent specific insulin binding of hyperglycemic men.

There was no significant time by group interaction on percent specific insulin binding of hyperglycemic men receiving twelve weeks of chromium supplementation and hyperglycemic men receiving twelve weeks of placebo supplementation, therefore we failed to reject this null hypothesis.

Assumptions

The following assumptions were made for this research:

1. It was assumed that the participants accurately filled out their one-week food frequency questionnaire.
2. It was assumed that the participants took their supplements as directed.
3. It was assumed that the participants fasted for twelve hours before blood was drawn.
4. It was assumed that the participants did not change their diet or exercise patterns over the course of the study.
5. It was assumed that the participants did not take any other supplements containing chromium over the course of the study.

6. It was assumed that the participants did not undergo events causing lifestyle changes, such as medication consumption and surgical events.

Limitations

1. The one week food frequencies were limited by the participants' knowledge of sample size and food composition.
2. An inadequate data base for chromium limited analysis of chromium content of participants' diets.
3. The results of the study are relevant to this sample group limiting extrapolation of results to the general population.
4. The results of this study are limited by outside factors affecting participants, such as changes in medication and surgical events.
5. The results of the study are limited by the tendency for health conscious individuals to volunteer to participate in this type of study.

Implications

This study involving hyperglycemic men and chromium supplementation as 200 mcg of chromium chloride per day for twelve weeks did not show a significant effect on indicators of glucose metabolism. Perhaps increasing sample size or pooling the two groups and creating a crossover design would have enhanced our prospects for finding significance. However, larger sample sizes do not always guarantee success.

Recommendations

In future studies I recommend doing away with the two-group design and pooling participants into one group. Participant recruitment in Stillwater is difficult. When participant numbers are low, a single study group with incorporation of a crossover period would create a more powerful experimental design. Although this design would not make assumptions and limitations obsolete, it would make them less confounding. I would increase contact with participants during the study to establish a closer tie with the participants. In order to monitor compliance more closely, I recommend taking urine samples. Urine samples would allow researchers to monitor compliance by comparing urine chromium levels at baseline to urine chromium levels at posttest.

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APPENDICES

APPENDIX A

APPROVAL FORMS FOR INSTITUTIONAL REVIEW BOARD FOR HUMAN PARTICIPANT RESEARCH

DEC 13 1999

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

DATE: 02-09-99 **IRB #: HE-99-058**

Proposal Title: EFFECTS AND INTERACTIONS OF CHROMIUM AND COPPER SUPPLEMENTATION ON INDICATORS OF DIABETES IN PREDIABETIC MEN WITH HYPERINSULINEMIA

Principal Investigator(s): Janice R. Hermann

Reviewed and Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

Signature: Carol Olson Date: February 10, 1999

Carol Olson, Director of University Research Compliance

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modification to the research project approved by the IRB must be submitted for approval. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board.

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

Date: December 9, 1999 IRB #: HE-99-058

Proposal Title: "EFFECTS AND INTERACTIONS OF CHROMIUM AND COPPER SUPPLEMENTATION ON INDICATORS OF DIABETES IN PREDIABETIC MEN WITH HYPERINSULINEMIA"

Principal Investigator(s): Janice Hermann

Reviewed and Processed as: Continuation

Approval Status Recommended by Reviewer(s) Approved

Signature:

Carol Olson

Carol Olson, Director of University Research Compliance

December 9, 1999
Date

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modification to the research project approved by the IRB must be submitted for approval with the advisor's signature. The IRB office MUST be notified in writing when a project is complete. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

Date: January 31, 2000 IRB #: HE-99-058

Proposal Title: "EFFECTS AND INTERACTIONS OF CHROMIUM AND COPPER SUPPLEMENTATION ON INDICATORS OF DIABETES IN PREDIABETIC MEN WITH HYPERINSULINEMIA"

Principal Investigator(s): Janice Hermann

Reviewed and Processed as: Modification

Approval Status Recommended by Reviewer(s) Approved

Signature:

Carol Olson, Director of University Research Compliance

January 31, 2000
Date

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modification to the research project approved by the IRB must be submitted for approval with the advisor's signature. The IRB office MUST be notified in writing when a project is complete. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board

APPENDIX B

RECRUITMENT ANOUNCEMENTS

ARE YOU AT RISK FOR DEVELOPING DIABETES ?

Male Participants Wanted Who Are At Risk For Diabetes

Did you know that low intake of certain minerals may increase your risk of developing diabetes?

Would you like to know if an adequate mineral intake lowers your risk of developing diabetes?

You may be at risk of developing diabetes if you have one or more of the following:

- Over 40 years of age
- Overweight
- Family history of diabetes

Volunteers who meet the study criteria will receive $300 for completing the study.

For further information about the men's diabetes study please contact:

Janice R Hermann, PhD, RD/LD
Department of Nutritional Sciences
Oklahoma State University
Stillwater, Oklahoma 74078
(405) XXX-XXX

Men at risk for diabetes wanted for OSU nutrition study.

Would you like to know if an adequate mineral intake lowers your risk of developing diabetes?

You may be at risk of developing diabetes if you have one or more of the following:

- Over 40 years of age
- Overweight
- Family history of diabetes

Men who meet the study criteria will receive $300 for completing the study. For more information call XXX-XXX.

Men at risk for diabetes wanted for OSU nutrition study. Factors increasing risk of diabetes include family history of diabetes, overweight, over 40 years of age. Men meeting the study criteria will receive $300 for completing the study. Call XXX-XXX.

APPENDIX C

INDIVIDUAL CONSENT TO PARTICIPATE IN SCREENING PROCESS

Screening For Men's Diabetes Study: Effect of Chromium and Copper Supplementation on Indicators of Diabetes in Men at Risk For Diabetes

"I,_____. hereby volunteer to participate in the screening for the above titled study and authorize or direct Janice R. Hermann, Ph.D., R.D./L.D., or associates of her choosing, to perform the following treatment or procedure."

I understand that:

(1) this is a screening for fasting and 2 hour glucose as criteria for participating in the above titled study which will measure the effects of chromium and copper supplementation on indicators of diabetes in men at risk for diabetes;

(2) A licensed phlebotomist will draw a fasting blood sample of 5 ml by venipuncture. After which I will consume a sugar drink containing 75 g glucose. I will have a 5 ml blood sample drawn by venipuncture by a licensed phlebotomist 120 minutes after consuming the glucose drink.

(3) I may have slight discomfort and/or bruising from the venipuncture.

(4) after the two-hour blood sample I will receive a light meal for nutritional support;

(5) my blood will only be used to measure blood glucose, and any remaining blood will be discarded and no further tests will be run;

(6) I will complete a Health Questionnaire concerning health conditions, medication use, vitamin and mineral supplement use and exercise practices.

(7) all my records are confidential and my name will not be associated with any reports;

(8) I will receive a form with my personal laboratory results with accepted ranges for each laboratory value. There will be a statement at the bottom of the form indicating that I should see a physician if my personal laboratory results are not in the accepted ranges.

(9) my participation in the screening is voluntary, that there is no penalty for refusal to participate;

(10) I will be notified by the project director if I meet the criteria to participate in the study;

(11) meeting the criteria for the study does not mean I am committed to participate in the study. my participation in the study is voluntary, that there is no penalty for refusal to participate, and that I am free to withdraw my consent and participation in this project at any time without penalty after notifying the project director;

(12) this research is beneficial to the public in that the risk of diabetes increases with age and low mineral intake.

(13) I may contact Dr Janice Hermann at (405) 269-1002 should I wish further information. I may also contact Sharon Bacher. IRB Executive Secretary. 203 Whitehurst. Oklahoma State University. Stillwater. OK 74078. telephone (405) 744-6244

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

Date_____ Time_____(a.m./p.m.)

Signed_____
Signature of Subject

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

Signed _____
(project director or her authorized representative)

APPENDIX D

GLUCOSE TOLERANCE BEVERAGE

APPROX. OUNCES DISPENSED

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CASCO·NERL DIAGNOSTICS

Trutol 100

Glucose Tolerance Beverage

Orange Flavored

Carbonated, Caffeine Free

For prescription use only

Catalog No. 401207 **100 g Dextrose, 10 fl. oz. (296 mL)**

Manufactured by:

CASCO-NERL Diagnostics Baltimore, MD 21224 1-800-631-9800 Rev 9/98

CAUTION: Federal law (U.S.A.) prohibits dispensing without a prescription.

Active Ingredient: 10.0 g glucose per fl. oz. (29.6 mL). Contains carbonated water, dextrose (d-glucose, source: corn), natural flavoring, citric acid, sodium benzoate, FD&C Yellow No. 6 (Sunset Yellow) as a color additive.

Indications and Usage: Trutol® 100 is indicated for the detection of glucose intolerance in the evaluation of gestational diabetes mellitus. For oral consumption only.

Precautions: Pregnant or nursing women and persons having diabetes take Trutol® 100 only under the direction of a physician.

Adverse Reactions: The most common adverse reactions to Trutol® 100 are nausea, vomiting, abdominal bloating and/or headache.

Dosage and Administration: The recommended usual dosage is 10.0 oz. (100g dextrose) for pregnant women. Instruct the patient to remain quiet, refrain from smoking and from drinking beverages containing caffeine just before and during the test. Start timing when the patient begins to drink Trutol® 100 (drink in about 5 min.). Obtain blood sample 1 hr later. To enhance palatability, chill before serving. Protect from light. Do not freeze. Discard unused contents.

Reference: For further details on the test and its interpretation see *Summary and Recommendations of the Second International Workshop—Conference on Gestational Diabetes Mellitus*, 34 (2) DIABETES 123, June 1985.

GLUCOSE TOLERANCE BEVERAGE • GLUCOSE TOLERANCE BEVERAGE • GLUCOSE TOLERANCE BEVERAGE • GLUCOSE TOLERANCE BEVERAGE •

APPENDIX E

HEALTH QUESTIONNAIRE

Monthly Health Questionnaire

Subject Number_____

Height_____

Weight_____

1. Did you have any illness in the last month? No___ Yes___

   IF YES TO QUESTION 1

   When where you ill? _____

   What type of illness?_____

   Did you take any medication when you were ill? No___ Yes___

   What type of medication did you take? _____

   Did you have a fever? No_____ Yes_____

   Did you continue to take your supplement during the illness? No___ Yes___

2. Did your exercise pattern change in the last month? No___ Yes___

   IF YES TO QUESTION 2

   In what way did your exercise pattern change?

APPENDIX F

INDIVIDUAL CONSENT TO PARTICIPATE IN RESEARCH

Men's Diabetes Study: Effect of Chromium Supplementation
on Indicators of Diabetes in Men at Risk for Diabetes

"I,_____, hereby volunteer to participate in the above titled study and authorize or direct Janice R. Hermann, Ph.D., R.D./L.D., or associates or assistants of her choosing, to perform the following treatment or procedure."

I understand that:

(1) the purpose of the study is the measure the effect of chromium supplementation on indicators of diabetes in prediabetic men with elevated insulin;

(2) I will receive one month's of supplements containing ONE of the following.

(a) a placebo
(b) 200 ug chromium

(3) I will take one supplement a day with a meal for 24 weeks;

(4) I will not take any other vitamin/mineral supplements or herbal/dietary supplements that contain chromium other than those that are part of this study;

(5) I will return each month to receive the next month's supplements.

(6) I will participate in a fasting blood collection and 3-hour glucose tolerance test at the beginning of the study, after 12 weeks supplementation and after 24 weeks supplementation. A licensed phlebotomist will draw a fasting blood sample of 20 ml by venipuncture. After which I will consume a glucose drink containing 75-g glucose. I will have a 6 ml blood sample drawn by venipuncture by a licensed phlebotomist 30, 60, 120, and 180 minutes after consuming the glucose drink.

(7) I may have slight discomfort and/or bruising from the venipuncture.

(8) after the 3-hour glucose tolerance test I will receive a light lunch for nutritional support.

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

(10) routine measurements of my body weight will be taken at the beginning of the study, after 12 weeks supplementation and after 24 weeks supplementation;

(11) I will complete a Health Questionnaire concerning health conditions, medication use, vitamin and mineral supplement use and exercise practices at the beginning of the study, and a Follow-up Health Questionnaire after 12 weeks supplementation and after 24 weeks supplementation;

(12) I will complete a 1-week food frequency questionnaire at the beginning of the study, after 12 weeks supplementation, and after 24 weeks supplementation;

(13) as a reward for participation and as an incentive to complete the study, I will receive $150 after completing each of the three data collections for a total of $450; $150 after the data collection at the beginning of the study, $150 after the data collection after 12 weeks supplementation, and $150 after the data collection after 24 weeks supplementation

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

(15) I will receive a form with my personal laboratory results with accepted ranges for each laboratory value. There will be a statement at the bottom of the form indicating that I should see a physician if my personal laboratory results are not in the accepted ranges.

(16) my participation is voluntary, that there is no penalty for refusal to participate, and that I am free to withdraw my consent and participation in this project at any time without penalty after notifying the project director;

(17) I will withdraw from the project if I need to begin taking medication for diabetes during this study;

(18) this research is beneficial to the public in that the risk diabetes increasing with age and low mineral intake.

(19) I may contact Dr Janice Hermann at (405) 269-1002 should I wish further information. I may also contact Sharon Bacher, IRB Executive Secretary, 203 Whitehurst, Oklahoma State University, Stillwater, OK 74078; telephone (405) 744-6244..

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

Date_____ Time_____(a.m./p.m.)

Signed_____
Signature of Subject

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 G

FOOD FREQUENCY QUESTIONNAIRE

Code Number_____

Men's Diabetes Study
Seven Day Food Frequency Questionnaire

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

EXAMPLES:

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

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

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
DAIRY FOODS						
Whole milk (1 cup, 8 oz)						
2% milk (1cup, 8 oz)						
1% milk (1 cup, 8 oz)						
Skim milk (1 cup, 8 oz)						
Rice milk (1 cup, 8 oz)						
Soy milk (1 cup, 8 oz)						
Milk Shake (16 oz)						
Pudding (1/2 cup)						
Cream, whipped (1 Tbsp)						
Sour cream (1 Tbsp)						
Coffee cream (1 Tbsp)						
Ice cream (½ cup)						
Low fat ice cream (½ cup)						
Frozen yogurt (½ cup)						
Yogurt (1 cup)						
Low fat yogurt (1 cup)						
Cottage cheese (½ cup)						
Cream cheese (1 oz)						
Low fat cream cheese (1 oz)						
Other cheese (1 slice or 1 oz)						
Low fat cheese (1 slice or 1 oz)						
Margarine (1 tsp)						
Butter (1 tsp)						
Reduced fat margarine (1 tsp)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
FRUITS, FRUIT JUICES						
Raisins (1 oz or 1 sm box)						
Grapes (20)						
Prunes (½ cup)						
Bananas						
Cantaloupe (¼ melon)						
Watermelon (1 slice)						
Apples, applesauce or pears (1 fresh, ½ cup)						
Apple juice (½ cup)						
Oranges						
Orange juice (½ cup)						
Grapefruit (½ cup)						
Grapefruit juice (½ cup)						
Cranberry juice (½ cup)						
Other fruit juices (½ cup)						
Strawberries—fresh, frozen, or canned (½ cup)						
Blueberries—fresh, frozen, or canned (½ cup)						
Peaches (1 fresh, ½ cup canned)						
Apricots (1 fresh, ½ cup canned)						
Plums (1 fresh, ½ cup canned)						
Honeydew melon (¼ melon)						
Kiwi (1 medium)						
Fruit Cocktail (½ cup)						
Mango (½ cup, or ½ small)						
Raspberries (½ cup)						
Blackberries (½ cup)						
Dried fruit (1/4 cup)						
Pears (1 medium)						
Pineapple chunks (½ cup)						
Lemon juice (1/4 cup)						
Lime juice (1/4 cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
VEGETABLES, VEGETABLE JUICE						
Tomatoes (1)						
Tomato juice (½ cup)						
Tomato sauce (½ cup)						
Spaghetti sauce (½ cup)						
Red chili sauce, taco sauce, or salsa (1 Tbsp)						
Tofu or soybeans (3-4 oz)						
String beans, green beans (½ cup)						
Broccoli (½ cup)						
Cabbage (½ cup)						
Cole slaw (½ cup)						
Cauliflower (½ cup)						
Brussels sprouts (½ cup)						
Carrots, raw (½ carrot or 2-4 sticks)						
Carrots, cooked (½ cup)						
Corn (1 ear or ½ cup frozen or canned)						
Peas (½ cup fresh, frozen or canned)						
Lima beans (½ cup frozen, or canned)						
Mixed vegetables (½ cup)						
Beans or lentils, baked or dried (½ cup)						
Summer or yellow squash (½cup)						
Winter squash (½ cup)						
Zucchini (½ cup)						
Yam or sweet potato (½ cup)						
Spinach, (cooked ½ cup, raw 1 cup)						
Iceberg lettuce, romaine or leaf (1 cup)						
Celery (4" stick)						
Beets (½ cup)						
Alfalfa sprouts (½ cup)						
Kale, mustard, or chard greens (½ cup)						
Vegetable, vegetable beef, minestrone or tomato soup (1 cup)						
Okra (½ cup)						
Cucumber slices (½ cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
VEGETABLES, VEGETABLE JUICE (Cont.)						
Mushrooms (½ cup)						
Collard greens (½ cup)						
Turnip greens (½ cup)						
Onion (½ cup)						
Pickles (½ cup)						
Sweet peppers (½ cup)						
Asparagus (½ cup)						
Jalapeno peppers (1/4 cup)						
Potato salad (½ cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
EGGS, MEAT, ECT.						
Eggs (2)						
Chicken or turkey, roasted or broiled with skin (3-4 oz)						
Chicken or turkey, roasted or broiled skinless (3-4 oz)						
Chicken, fried with skin (3-4 oz)						
Bacon (2 slices)						
Hot dogs (2)						
Low fat hot dogs (2)						
Sausage (2 patties or 2 links)						
Bologna (1 slice)						
Other processed luncheon meat (1 slice)						
Liver, chicken or beef (3-4 oz)						
Hamburger, regular (1 patty, 3-4 oz)						
Hamburger, lean (1 patty, 3-4 oz)						
Meat loaf (3-4 oz)						
Pork, chops, roasts (3-4 oz)						
Lamb (3-4 oz)						
Beef, roast, steak (3-4 oz)						
Beef stew with vegetables (1 cup)						
Ham (3-4 oz)						
Tuna (3-4 oz)						
Tuna salad (½ cup)						
Fish, baked or broiled (3-4 oz)						
Fish, fried or fish sandwich (3-4 oz)						
Shrimp, Lobster, Scallops						
Pizza (2 slices)						
Mixed dishes with cheese (1 cup)						
Lasagna or meat pasta dishes (1 cup)						
Pot pie (1 each)						
Egg beaters (½ cup)						
Pork ribs (3 ribs)						
Deli meats (3 oz)						
Ground turkey (3 oz)						
Chicken salad (½ cup)						
Chili (1 cup)						

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
BREADS, CEREALS, STARCHES						
Cold breakfast cereal (1 cup)						
Cold breakfast cereal—fortified (1 cup)						
Cooked oatmeal (1 cup)						
Other cooked breakfast cereal (1 cup)						
White bread (1 slice)						
Pita bread (1 piece)						
Dark bread (1 slice)						
English muffin (1)						
Bagel (1)						
Dinner roll (1)						
Hamburger or hotdog bun (1)						
Muffin (1)						
Biscuit (1)						
Corn bread, corn muffin (1)						
Brown rice (1cup)						
White rice (1cup)						
Spaghetti noodles (1 cup)						
Macaroni noodles (1 cup)						
Other pasta noodles (1 cup)						
Bulgar, kasha, couscous (1 cup)						
Pancakes or waffles (2)						
Potatoes, french fries or fried (½ cup)						
Potatoes, baked or boiled (1)						
Mashed potatoes (1 cup)						
Potato chips or corn chips (small bag or 1 oz)						
Saltine crackers (5)						
Saltine crackers, low sodium (5)						
Saltine crackers, fat free (5)						
Other crackers (5)						
Other crackers, low fat (5)						
Tortilla (1 medium)						
Graham cracker (3 medium)						
Pretzels (15 small)						
Trail mix (1 cup)						

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

Type of Food (Medium Serving)	How Often			Size		
	Day	Week	Rarely/ Never	S	M	L
SWEETS, BAKED GOODS, MISC.						
Sorbet (½ cup)						
Granola bar (1 small bar)						
Chocolate (1 small bar or 1 oz)						
Candy bar (1 small bar)						
Candy without chocolate (1 oz)						
Cookies, home baked (2)						
Cookies, ready made (2)						
Brownies (2)						
Doughnuts (2)						
Cake, home baked (1 slice)						
Cake, ready made (1 slice)						
Sweet roll, coffee cake, or other pastry ready made (1 serving)						
Sweet roll, coffee cake, or other pastry home baked (1 serving)						
Pie, homemade (1 slice)						
Pie, ready made (1 slice)						
Jam, jelly, preserves, syrup, or Honey (1 Tbsp)						
Peanut butter (1 Tbsp)						
Popcorn (1 cup)						
Popcorn, air popped (1 cup)						
Nuts (small packet or 1 oz)						
Bran, added to food (1 Tbsp)						
Wheat germ (1 Tbsp)						
Chowder or cream soup (1 cup)						
Oil and vinegar dressing (1 Tbsp)						
Soy sauce (1 tsp)						
Mayonnaise or other creamy salad dressing (1 Tbsp)						
Mustard, dry or prepared (1 tsp)						
Salt (1 shake)						
Pepper (1 shake)						

VITA \

Joshua Andrew Phelps

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF CHROMIUM SUPPLEMENTATION ON INDICATORS OF TYPE 2 DIABETES IN HYPERGLYCEMIC MEN

Major Field: Nutritional Sciences

Biographical:

Personal Data: Born in Pontiac, Michigan, on February 4, 1970, the son of Thomas and Adelaide Phelps.

Education: Attended Oakland Community College, Union Lake, Michigan from September 1988 to December 1989; received Bachelor of Art degree in Anthropology from the University of Michigan, Ann Arbor, Michigan in December 1992; attended Oklahoma State University undergraduate school from January 1994 to May 1994 and from August 1996 to May 1998 and from January 1999 to May 1999. Completed the requirements for the Master of Science degree with a major in Nutritional Sciences at Oklahoma State University in May 2001.

Professional Experience: Research Assistant for Dr. Elizabeth Droke in the Nutritional Sciences Department at Oklahoma State University from August 1999 to December 1999; Research Assistant for Dr. Barbara Stoecker in the Nutritional Sciences Department at Oklahoma State University from August 1999 to December 1999; Research Assistant for Dr. Janice Hermann in the Nutritional Sciences Department at Oklahoma State University from January 2000 to May 2001; Research Assistant for Dr. Kathyrn Keim in the Nutritional Sciences Department at Oklahoma State University from January 2001 to May 2001.