EFFECTS AND INTERACTIONS OF DIETARY CHROMIUM AND MANGANESE IN STREPTOZOTOCIN-INJECTED RATS

By

CAROL RENEE JARRETT

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1989

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1995

EFFECTS AND INTERACTIONS OF DIETARY CHROMIUM AND MANGANESE IN STREPTOZOTOCIN-INJECTED RATS

Thesis Approved:

Barbara J. Stoecker
Thesis Advisor

Andrea B. Argentt

Christa F. Hanson

Thomas C. Collins
Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Drs. Christa Hanson and .Andrea Arquitt for serving on my graduate committee and for providing me with guidance, laughter, and gentle nudging. They have been wonderful to me and I miss them. I wish to express love and gratitude to my dear friend and mentor, Dr. Bernice Adeleye. She has always shown me wisdom and grace. What she has taught me is invaluable. My deepest gratitude to my major advisor, Dr. Barbara Stoecker. for teaching me to take it "bird by bird". She has been my inspiration to complete this project.

I wish to thank all the members of my family, those related and those held captive. All of whom have provided me with encouragement and support. In particular , I would like to thank my sisters Linda Kay, for providing emotional and vehicular support, and Keely, for technical support and treats that make life a little easier. I also would like to thank my colleague and office mate, Becky Porter, for giving me an empathetic ear each and every morning and Peggy Cook, medical librarian at Hillcrest Medical Center in Tulsa, Oklahoma, for her endless pursuits of research articles at a moment's notice.

I would like to express my deepest love and gratitude to my husband, Chris Cauthon. He has made it possible for me to reach my goals. He is my best friend.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

RESEARCH PROBLEM

Introduction to Topic

Streptozotocin (STZ) is an antibiotic used to induce diabetes in experimental animals. STZ produces an immediate and highly specific toxic effect on the insulin-secreting beta cells of the islets of Langerhans which is followed by a chronic diabetic state (Rerup 1970). The mechanism by which STZ induces diabetes is controversial and undefined. The nutritional status of the experimental animal and the metabolic status of the pancreatic beta cells may influence the animal's response to injected STZ (Preston 1985).

Chromium is an essential trace element for animals and humans (Anderson et al. 1985). The primary known role of chromium is the potentiation of insulin. Diabetic-like symptoms, including impaired glucose tolerance and elevated insulin, were observed in rats fed diets low in chromium (Mertz and Schwarz 1959, Mertz et al. 1965, Schroeder 1965, Schroeder 1966). In chromium deficiency a higher dose of insulin was required to elicit the same metabolic response seen in chromium adequacy (Mertz et al. 1965, Mertz et al. 1961, Roginski 1969, Schroeder 1966). Striffler et al. studied the effects of dietary chromium on insulin secretion in perfused rat pancreata. First phase insulin release was decreased by 40 to 50% in chromium deficient rats as compared to controls (1993). These data suggest that chromium may play a role in preservation of pancreatic beta cells.

Manganese is essential for all species. Rats fed manganese-free diets exhibited poor growth, testicular atrophy, and sterility (Kemmerer et al. 1931, Orent et al. 1931). Defects in carbohydrate metabolism have been reported in manganese-deficient rats and

mice. Everson and Shrader reported impaired glucose tolerance and pancreatic pathology in severely manganese-deficient newborn guinea pigs (1968). Other studies of manganese-deficient animals have reported aplasia and/or hypoplasia of pancreatic cells (Zidenberg-Cherr 1990). Decreased pancreatic insulin synthesis, enhanced intracellular insulin degradation, and decreased insulin secretion have been observed in manganese-deficient animals (Baly et al. 1985). Manganese deficiency is also associated with abnormal lipid metabolism and alterations in cellular integrity. Zidenberg-Cherr et al.(1983) reported decreased manganese dependent superoxide dismutase (MnSOD) activity and increased lipid peroxidation in manganese-deficient rats. Tissue MnSOD levels were influenced by dietary manganese intake (Davis et al. 1990).

Significance of the Problem

It has been postulated that STZ acts as an oxidant resulting in changes in the redox state of the islet cell. Oxidative stress produces an acute rise in oxidized glutathione and a fall in reduced glutathione (Slonim et al. 1976, Slonim et al. 1983). Slonim et al. found that STZ administered in vivo to rats produced a rapid fall in red blood cell reduced glutathione. Copper-zinc superoxide dismutase (Cu-ZnSOD) is present in the islets of Langerhans of rats. Crouch et al. (1981) found that STZ inhibited islet Cu-ZnSOD activity in vitro and had no effect on MnSOD. Gandy et al.(1982) investigated the potential of exogenous polyethylene glycol-derivatized superoxide dismutase (PEG-SOD) to prevent STZ-induced diabetes in rats. They found that PEG-SOD injected 10 seconds or 50 minutes prior to administration of STZ prevented or significantly attenuated diabetes. In a study by Slonim et al. (1983) the administration of the anti-oxidant vitamin E prior to STZ injections in rats provided protection against the diabetogenic drug. Rats maintained on a vitamin E and selenium-deficient diet demonstrated enhanced diabetogenic response to normally nondiabetogenic doses of STZ. These data support the postulate that STZ acts as an oxidant or a free radical producer. However, the role of STZ as an oxidative stressor or

free radical generator remains controversial since other studies of the administration of exogenous SOD and Vitamin C did not find any protective effects of these anti-oxidants against the cytotoxic effects of STZ (Gold et al. 1981, Yew 1983).

As the westernized diet has become more highly processed there has been an increase in the loss of chromium (Schroeder 1968, Anderson 1987, Kozlovsky et al. 1986). The world wide trend towards a highly processed and refined diet, which is not only low in chromium but may also stimulate chromium losses, may result in marginal chromium status. Mean chromium intake in the United States and most developed countries is approximately 50 to 60% of the lower end of the estimated safe and adequate range (Anderson and Kozlovsky 1985). In addition the Food and Drug Administration's Total Diet Study (1982-86) found manganese intakes for teenage, adult, and elderly women to be slightly less than the estimated safe and adequate daily dietary intake (Pennington et al. 1989).

Chromium and manganese appear to have roles in the protection of the beta cells of the pancreas. If chromium and/or manganese intakes are suboptimal the beta cells may be increasingly susceptible to oxidative stressors and/or to the effects of diabetogenic agents such as STZ.

Objectives

The objectives of this research are to investigate the effects of two trace minerals (chromium and manganese) on glucose metabolism (as measured by glucose tolerance, insulin, and weight loss) and kidney and liver mineral concentrations in streptozotocin-injected rats.

The following hypotheses were developed for this study:

1. There will be no statistically significant independent and or interactive effect(s) of dietary chromium and/or manganese on change in body weight,

tissue weights as percent body weight, glucose tolerance, or serum insulin, frusctosamine, or corticosterone concentrations in streptozotocin-injected rats.

2. There will be no statistically significant independent and/or interactive effect(s) of dietary chromium and or manganese on liver and kidney mineral concentrations in streptozotocin-injected rats.

Limitations

This study evaluated the effects of a relatively short term chromium and manganese deficiency. Thus the effect of chronic mineral deficiencies were not evaluated. The study evaluated the effects of deprivation or adequate intakes of the two trace minerals, thus the effects of supplementation were not studied. Trace mineral deficiencies are difficult to establish; chromium is particularly problematic due to increased risk of environmental contamination. A further limitation is the intraperitoneal administration of streptozotocin which is inherently more variable than injecting streptozotocin intravenously.

Format of Thesis

Chapter III is written in manuscript form using the guide for authors for the Journal of Nutrition.

CHAPTER II

REVIEW OF THE LITERATURE

This chapter reviews the literature on chromium and manganese and their roles in glucose metabolism. The use of streptozotocin as a diabetogenic agent in laboratory animals also is reviewed.

Chromium

Chromium is an essential trace element in animals and humans (Anderson et al. 1985). It is a transition element occurring in eight oxidation states, the most common states being 0, 2+, 3+, and 6+. The trivalent state is the most stable (Anderson 1990). A biologically active form of chromium was isolated from brewers' yeast and pork kidney powder by Schwarz and Mertz in the late 1950s and termed glucose tolerance factor (1959). The exact structure of glucose tolerance factor (GTF) remains undefined and controversial, however it is believed to contain two nicontinic acid molecules per trivalent chromium atom and either amino acids or glutathione (Flatt et al. 1989, Mertz 1992). Some organic chromium complexes have considerably greater biological activity than chromium salts (Mertz, 1976).

In a review of 15 controlled studies of the effects of supplementation with defined Cr3+ compounds in subjects with impaired glucose tolerance, three studies found no effects of supplementation while twelve studies found improved glucose tolerance and efficiency of the insulin (Mertz, 1993). Ranhotra and Gelroth studied the effects of chromium supplementation, in forms of either high chromium bakers' yeast or chromium chloride, on response to a glucose load in rats fed a chromium-deficient diet for 17 weeks.

Rats supplemented with bakers' yeast had a smaller rise in blood glucose compared to those supplemented with chromium chloride (1986).

Distribution

Chromium is widely distributed in the body with no known specific concentration in any tissue or organ (Anderson 1987). There have been few definitive studies on chromium concentration in tissues of animals and humans as there are numerous problems with chromium contamination in sampling and analysis. Current sampling and analysis methods can reduce the risks of contamination but the number of studies using these methods is limited. Thus, caution should be used in viewing historic data on chromium content of tissues (Anderson 1987). Chromium concentration in tissues may be several times higher than that in the blood and thus is not in equilibrium (Anderson 1994). In general, tissue chromium concentrations tend to be lower in parts of the world where there is a higher incidence of non-insulin dependent diabetes mellitus and atherosclerosis (Anderson 1987). There is no reliable method to assess chromium status other than response to chromium supplementation (Anderson et al. 1985).

Absorption

Trivalent chromium and inorganic chromium compounds are poorly absorbed by animals and man (Donaldson and Barreras 1966, Mertz et al. 1974). Reported absorption of chromium compounds ranges from 0.5 to 3% (Anderson 1987, Stoecker 1990). Chromium absorption is rapid with the greatest absorption occurring in the midsection of the small intestine (Chen et al. 1973). Urinary chromium excretion is considered a fairly accurate estimate of absorption (Anderson 1985) and a poor indicator of chromium status (Anderson 1983). Anderson and Kozlovsky studied chromium intake and absorption in subjects consuming self-selected diets. They determined that absorption is inversely related to intake. When chromium intakes were approximately 10 ug per day, absorption was

approximately 2%. When intakes increased to approximately 40 ug per day, absorption decreased to about 0.5% (1985). There is no apparent difference in absorption of CrCl3 supplements and chromium from dietary sources (Anderson et al. 1985).

Mineral interactions and chelation affect chromium absorption. While oxalate increases absorption of chromium, phytate appears to decrease absorption by a lesser degree (Chen et al. 1973). Chromium absorption is enhanced in zinc deficiency, and absorption is decreased by zinc supplementation. Furthermore, zinc absorption is greater in chromium deficiency and zinc absorption is decreased with chromium supplementation (Hahn et al. 1975). This interaction suggests that zinc and chromium may be metabolized by a common pathway in the intestine. Chromium and iron appear to compete for absorption since iron-deficient animals absorb more chromium than iron-supplemented animals. Chromium is believed to be transported primarily by transferrin but also by albumin (Hopkins and Schwarz 1964).

In studies of the effect of carbohydrate source on absorption and tissue chromium in animals, starch appeared to increase absorption as compared to diets with carbohydrate sources of sucrose, glucose, and fructose (Seaborn and Stoecker 1989). Diets high in simple sugars appear to increase urinary excretion of chromium (Kozlovsky et al. 1986). Separate studies have reported increased urinary excretion of chromium following glucose loads regardless of chromium status (Anderson et al. 1982, Glinsmann et al. 1966, Morris et al. 1988).

Dietary Intakes

Reported dietary intakes of chromium range from 24.5 ug/d in England to 56 ug/d in Canada. Most of the earlier studies may have been plagued with errors in sampling and analysis involving contamination which may have resulted in erroneously high values (Anderson 1987). More recent intake data for the United States estimate average intakes to be approximately 15 ug per 1000 kilocalories (Anderson et al. 1985). In Anderson and

Kozlovsky's chromium intake study of subjects consuming self-selected diets, 90% of the diets analyzed contained less chromium than the estimated safe and adequate daily dietary intake range (ESADDI) (1985). The safe and adequate range has been established at 50-200 ug chromium/day (National Research Council 1989). However, at intakes of 50 ug/d, the lower end of the recommended amounts, signs and symptoms of chromium deficiency did not develop (RDA, 1989). Long term effects of intakes less than 50 ug/d have not been fully investigated. Mean chromium intake in the United States and most developed countries is approximately 60% of the lower end of the estimated safe and adequate range for males and about 50% for females (Anderson and Kozlovsky 1985). Freely chosen diets and diets designed by nutritionists contained about the same ratio of chromium to kilocalories, 15 ug chromium per 1000 kilocalories (Anderson et al. 1992).

Significant amounts of chromium are lost in milling and refining of certains foods (Schroeder 1968). White flour contains 35-55% of the chromium found in whole wheat products and unrefined sugar contains 40% less chromium than molasses. Brown and white sugar contain 24% and 8% of the chromium in the unrefined sugar (Anderson 1987). A diet high in refined sugar not only lends itself to a diet low in chromium but may also contribute to increased urinary losses of chromium (Kozlovsky et al. 1986). In general, the less refined and processed a diet, the higher the content of chromium. The world wide trend towards a highly processed and refined diet which is not only low in chromium but may stimulate chromium losses may result in marginal chromium status (Anderson and Kozlovsky 1985). Meats and whole-grain products appear to be the best sources while fruits, vegetables, and milk are quite low in chromium (RDA, 1989). There may be significant amounts of chromium added to the food supply during preparation and processing particularly with the use of stainless steel in an acid environment during which chromium is leached out of the stainless steel (Offenbacher and Pi-Sunyer 1983).

Chromium and Glucose Homeostasis

The roles of chromium in nutrition have been studied for several decades. The primary role is the potentiation of insulin. Evans, Roginski, and Mertz studied the interaction of GTF, extracted from brewers' yeast, and insulin and their effects on glucose metabolism in epididymal fat tissue. They found that GTF bound to insulin and produced greater glucose uptake into epididymal tissue than did insulin alone (1973). Diabetic-like symptoms, including impaired glucose tolerance and elevated insulin, were observed in rats maintained in a typical laboratory environment and fed rations and diets low in chromium. A decreased rate of glucose removal was observed although there was no difference in growth rates compared to rats fed standard rations. Low glucose removal rates were reversed with the addition of chromium to the diet in the form of brewers' yeast or pork kidney powder (Mertz and Schwarz 1959). Overt deficiency may not have been established in this study secondary to environmental chromium contaminants. When experimental animals were raised in a strictly controlled environment designed to decrease sources of chromium contamination and fed a low chromium diet, more severe symptoms of deficiency were apparent such as depressed growth and survival in addition to glucose intolerance, fasting hyperglycemia, and glycosuria (Mertz et al. 1965). Dietary chromium supplementation of 5 ppm helped to reverse these trends. Young rats fed a low chromium torula yeast diet exhibited decreased growth rates, increased early mortality, and glucosuria (Schroeder 1965, Schroeder 1966). These data support the essentiality of chromium in animals.

More recently chromium supplementation in patients on long term total parenteral nutrition (TPN) eliminated the need for exogenous insulin and helped to normalize blood glucose values (Brown et al. 1986, Freund et al. 1979, Jeejeebhoy et al. 1977). One patient had been on TPN for 3 years and developed severe diabetic-like symptoms including weight loss, glucose intolerance, and peripheral neuropathy (Jeejeebhoy et al. 1977). Chromium supplementation to TPN solutions corrected the metabolic aberrations.

A similar syndrome was seen in a patient who had been on TPN for 5 months (Freund et al. 1979). Chromium by itself is not a hypoglycemic agent. Insulin must be present for biological activity to occur. Furthermore, chromium supplementation is beneficial only if a deficiency is present (Mertz et al. 1965, Glinsmann and Mertz 1966).

The primary biochemical lesion of chromium deficiency is proposed to be the decreased insulin sensitivity of peripheral tissues (Mertz et al. 1965). Chromium has been hypothesized to be essential in the assembly of insulin and its receptor sites in cell membranes (Govindaraju et al. 1989). In chromium deficiency a greater dose of insulin is required to elicit the same metabolic response seen in chromium adequacy (Mertz et al. 1965, Mertz et al. 1961, Roginski 1969, Schroeder 1966). In a study of the effects of dietary chromium on insulin secretion in perfused rat pancreata, first phase insulin release was decreased by 40 to 50% in chromium deficient rats as compared to their controls. These data suggest that chromium may play a role in preservation of the beta cells of the pancreas (Striffler et al. 1993).

In studies evaluating the benefits of chromium supplementation in hypoglycemic subjects, there were significant improvements in hypoglycemic glucose values and symptoms following supplementation (Clausen 1988). Anderson et al. studied the effects of chromium (200 ug/d) supplementation on response to a glucose challenge in 76 free-living human subjects. Subjects with pre-supplementation fasting glucose values greater than or equal to 100 mg/dl had decreased 90 minute post challenge glucose values after supplementation. Subjects with pre-supplementation 90 minute post-challenge glucose values less than fasting levels had a 90 minute blood glucose greater than fasting levels after supplementation. There was no effect of chromium supplementation in subjects with 90 minute glucoses nearly equal to fasting values (1983). Thus, it appears that chromium supplementation may be beneficial not only in regulating blood glucose in the hyperglycemic state but in the hypoglycemic state as well.

Not all studies have shown a beneficial response to chromium supplementation. It must be remembered that supplementation is helpful only in a deficient state and that not all cases of glucose intolerance are secondary to a chromium deficiency (Mertz 1976).

Manganese

Manganese is a transition element occurring in 11 oxidation states from -3 to +7 with the most common states being +2, +4, and +7. Divalent manganese is the dominant form in biological structures. Mn^{3+} is the oxidative state in manganese superoxide dismutase (MnSOD) and is the form which binds to transferrin. It is probably the oxidative state which interacts with Fe^{3+} (Keen and Zidenberg-Cherr 1994).

Manganese is essential is all species. Essentiality of manganese was first reported in 1931. Kemmerer et al. reported poor growth in mice fed a manganese- free diet (1931). Male rats raised on manganese-free diets developed testicular atrophy and sterility (Orent et al. 1931). Essentiality for humans was established in 1972 by Doisy (1972). A male subject fed a purified diet in a vitamin K deficiency study inadvertently developed manganese deficiency when the mineral was accidently omitted from the diet. The symptoms, which were not consistent with those associated with vitamin K deficiency, included weight loss and hypocholesterolemia (Doisy 1972).

Distribution

Manganese is widely distributed in tissues and fluids without any notable concentration in any one location. There is little variation among species, organs, or age (Keen and Zidenberg-Cherr 1990). There is a greater concentration of manganese in mitochondria than in cytoplasm or other cellular components (Keen and Zidenberg-Cherr 1990). Muscle has the lowest concentration of manganese while bones contain about 25% of the total body content. There is a lack of practical methods to assess manganese status. In a study by Keen et al. blood manganese levels appeared to reflect body manganese

concentration in rats fed either manganese-deficient or manganese-adequate diets (1983). This finding has not been useful in humans as the results of human studies have not reproducible (RDA, 1989).

Absorption

Manganese absorption is variable, ranging from 2 to 15% of intake in studies using 54Mn labeled test meals (Keen and Zidenberg-Cherr 1990). Freeland-Graves et al. (1988) reported greater than 17% of dietary manganese absorbed in a metabolic balance study of young men. Sources of variation might be due to variability in absorption among subjects, individual body stores of manganese, and composition of the diet. Davis et al. (1992) determined that the efficiency of manganese absorption increased when rats were fed manganese-deficient (0.9 ug/g) diets. The percent absorbed tended to be greater when fed diets with adequate manganese (48 ug/g) than when fed diets high (188 ug/g) in dietary manganese. Manganese is absorbed quickly and equally throughout the small intestine and is excreted in the feces (Thompson et al 1978). Data from Garcia-Amanda et al. (1983) suggests that intestinal manganese absorption is a rapidly saturable process and decreases linearly with time. Absorption of manganese has not commonly been thought to be under homeostatic control (Thompson 1971). However, Davis et al. (1992) investigated the effects of varying levels of iron and manganese on gut absorption and endogenous losses of manganese by rats and concluded that control of gut absorption did appear to be the major mechanism of homeostatic control.

Manganese and iron are thought to compete for absorption in animals and humans. Iron supplementation inhibits manganese absorption and vice versa (Davis et al. 1990, Davis et al. 1992, Gruden 1988, Thomson et al. 1971). Davis et al. (1992) suggested that iron depressed manganese absorption by inhibiting manganese uptake into the mucosal cells. Although the actual mechanism is undefined, it may involve a common transportation site as both manganeses and iron are carried by transferrin (Davidsson

1989). Diets high in dietary fiber, calcium, phytate, and phosphorous increase the need for manganese due to probable formation of insoluble complexes in the gut which decrease the total manganese available for uptake (Davidsson 1988, Davies et al. 1975). The significance of this in humans is undetermined.

Nearly all manganese is excreted through the intestinal wall by several interdependent routes which makes for an efficient homeostatic control of tissue levels (Britton et al. 1966, Davidsson et al. 1991). Under normal conditions bile is the primary excretory route. There is also some excretion through pancreatic juices and sloughed intestinal cells (Davis et al. 1992). Very little manganese is lost through urine (Keen and Zidenberg-Cherr 1990). Many studies have determined that variable manganese excretion is the major point of homeostatic control (Britton et al. 1966, Davidsson et al. 1991). However, the recent study by Davis et al. (1992) proposing that true absorption is the major mechanism of homeostatic control of tissue manganese levels warrants careful consideration and further research in this area.

Dietary Intake

The 1989 Recommended Dietary Allowances (National Research Council 1989) included a estimated safe and adequate daily dietary intake (ESADDI) of manganese for adults of 2.0 to 5.0 mg per day. The Food and Drug Administration's Total Diet Study (1982-86) found manganese intakes for young children, teenage boys, adult and elderly males to be within the ESADDI ranges. Infants' intakes were found to be greater than the ESADDI and teenage girls and adult and elderly females' intakes were slightly less than the recommended ranges (Pennington et al. 1989). Good sources of manganese include tea, whole grains, nuts, dried fruits, and leafy vegetables. Poorer sources are dairy products, meats, fish, and poultry (RDA, 1989).

Manganese Deficiency

Manganese deficiency has been demonstrated in most species (Keen and Zidenberg-Cherr 1994). In experimental animals manganese deficiency affects endocrine and exocrine functions (Chang et al. 1990). Symptoms of deficiency include impaired growth and skeletal abnormalities secondary to reductions in proteoglycan synthesis resulting from decreased manganese glycosyl-transferase activity (Baly et al. 1985). The reduction in this manganese dependent enzyme also adversely affects the development of otoliths in the offspring of manganese-deficient animals. Impaired otolith formation results in severe and irreversible ataxia characterized by a lack of coordination and equilibrium and head retraction (Keen and Zidenberg-Cherr 1990). Manganese deficiency also has been reported to alter reproductive performance (Orent et al. 1931).

Defects in carbohydrate metabolism have been reported in manganese deficient rats and mice. In a study of newborn guinea pigs with severe manganese deficiency, Everson and Shrader reported pancreatic pathology and impaired glucose tolerance. The manganese-deficient guinea pigs displayed elevated fasting blood glucose and responded to a glucose challenge with a diabetic-like glucose tolerance curve. Manganese supplementation for two months normalized the guinea pigs' response to glucose administration (1968). Other studies of manganese-deficient animals have reported aplasia and/or hypoplasia of pancreatic cells with a smaller number of islet cells, and fewer and less granulated beta cells (Zidenberg-Cherr 1990).

In a study of the effects of manganese deficiency on insulin-binding and glucose transport and metabolism in rat adipocytes, it was found that second generation manganese deficient rats fed a diet low (1 ug Mn/g diet) in manganese had fewer insulin receptors per cell, a decreased glucose transport velocity in adipocytes, and decreased insulin stimulated glucose oxidation. These data suggest manganese deficiency decreases the number of glucose transporters in the adipose tissue of manganese-deficient rats (Baly et al. 1990).

Minor changes in gluconeogenesis and glycogenolysis have been observed in deficient animals. The most remarkable changes are decreased pancreatic insulin synthesis, enhanced intracellular insulin degradaton, and decreased insulin secretion (Baly et al. 1985). In a study of second generation manganese deficient rats, Baly et al (1984) evaluated insulin stores and release from isolated perfused pancreata. Decreased stored insulin or impaired release of the hormone in the deficient rats as compared to controls contributed to the diabetic-like response to a glucose load. The effects of manganese deficiency on insulin are essentially unknown although insulin mRNA levels are reduced in manganese-deficient animals (Keen and Zidenberg-Cherr 1990). Werner et al.(1987) reported decreased hepatic and pancreatic manganese content in manganese-deficient rats. The decrease was more pronounced in the liver than in the pancreas which may suggest a homeostatic mechanism to conserve manganese in tissues where it is needed most.

Manganese deficiency is also associated with abnormal lipid metabolism with alterations in cellular integrity secondary to increased membrane lipid peroxidation. This lipid peroxidation may be related to the observed decrease in manganese dependent superoxide dismutase (MnSOD) activity in manganese-deficient animals (Thompson et al. 1992, Zidenberg-Cherr et al. 1983). Zidenberg-Cherr et al. investigated MnSOD activity and lipid peroxidation in the manganese-deficient rat. Decreased MnSOD activity resulted in increased lipid peroxidation by free radicals, and might have contributed to the observed damage to mitochondrial membranes. Thompson et al. (1992) studied the effects of manganese deficiency on tissue anti-oxidant status in streptozotocin (STZ) induced diabetic rats. Decreased kidney and heart MnSOD activity was observed in the STZ-injected rats and in their controls. The decreased kidney MnSOD was more pronounced in the STZ-injected rats than in the controls. These STZ-injected rats also had an increase in liver MnSOD and increased lipid peroxidation. These data demonstrate an interaction between manganese deficiency and STZ-induced diabetes resulting in an amplification of tissue and

antioxidant changes seen with either condition alone. They also support an antioxidant role for manganese.

Manganese and Glucose Metabolism

Manganese functions as a component of metalloenzymes and as an enzyme activator. Manganese metalloenzymes include arginase, pyruvate carboxylase, and manganese superoxide dismutase (MnSOD) (Baly et al. 1983, Keen and Zidenberg-Cherr 1994). Arginase is essential in the formation of urea. In manganese-deficient rats, the arginase concentration is 50% less than that of controls, but the functional significance is unclear. In experimental diabetes liver and kidney manganese concentrations can be elevated in addition to an increase in arginase activity. Whether or not diabetes results in an increased need for manganese in has not been determined (Keen and Zidenberg-Cherr 1990).

Pyruvate carboxylase catalyzes the conversion of pyruvate to oxalacetate, a key step in gluconeogenesis. There is a slight decrease in activity of this enzyme in manganese-deficient animals (Baly et al. 1985). However there gluconeogenesis is not inhibited in manganese-deficient animals, presumably because magnesium readily replaces manganese in this step of gluconeogenisis (Fahim et al. 1990).

Superoxide dismutase (SOD) is a scavenger of superoxide radicals and protects the integrity of cell membranes (Gandy et al. 1983). STZ catalyzes the disproportionation of O2- to H2O2 + O2 (Davis and Greger 1992, Thompson et al. 1992). All SODs are metalloenzymes (Cu, Zn, Mn, or a combination thereof). Tissue MnSOD levels are influenced by dietary manganese intake (Davis et al. 1990). Davis and Greger studied longitudinal changes in MnSOD activity in response to iron and manganese supplementation in women. Manganese supplementation tended to increase lymphocyte MnSOD activity (1992). Manganese-deficient animals have decreased MnSOD activity in tissues including liver, brain, heart, pancreas, and kidney (Davis et al. 1990, Keen and

Zidenberg-Cherr 1990, Thompson et al. 1992). This reduction is functionally significant in that increased levels of tissue lipid peroxidation have been observed in manganese-deficient rats compared to controls (Thompson et al. 1992). Zindenberg-Cherr et al. studied the activities of MnSOD, copper-zinc SOD (CuZnSOD), and lipid peroxidation in the livers of manganese sufficient and deficient rats from birth to 60 days of age. In both groups MnSOD activity increased with age, however the activity in the manganese-deficient group was approximately half that of the sufficient group. There was also a nearly two-fold greater increase in lipid peroxidation in the manganese-deficient rats as compared to peroxidation in controls by day 60 (1983). This may explain the increased membrane damage in manganese deficient animals.

There are numerous manganese-activated enzymes including hydrolases, kinases, decarboxylases, and transferases. Many are not manganese specific. For example in a manganese-deficient state, pyruvate carboxylase can be catalyzed by magnesium (Fahim et al. 1990). A decrease in phosphoenolpyruvate (PEPCK) activity has been reported in manganese-deficient animals. PEPCK, like pyruvate carboxylase, is a gluconeogenic enzyme but is manganese specific (Baly et al. 1985). Manganese specifically activates glycosyltransferase (Baly et al. 1985). Skeletal and otolithic pathologies induced by manganese deficiency have been demonstrated and are related to decreased glycosyltransferase activity (Baly et al. 1985).

Streptozotocin as a Diabetogenic Agent

Streptozotocin (STZ) is a diabetogenic agent used to induce experimental diabetes in laboratory animals (Dulin et al 1983, Mordes and Rossini 1981, Rerup 1970). STZ is a broad spectrum antibiotic produced by Streptomyces achromogenes and has 4 major biological properties: antibacterial, antifungal, oncogenic, and diabetogenic (Preston 1985, Rerup 1970). STZ injected into animals produces an immediate and highly specific toxic

effect on the beta cells of the islets of Langerhans which is followed by a chronic diabetic state (Rerup 1970).

Chemically, STZ consists of a 1-methyl-1-nitrosourea linked to position C2 of a D-glucose (Rerup 1970). The nitrosourea moiety is thought to elicit the cytotoxic effect while the deoxyglucose moiety facilitates entry into the beta cell (Dulin et al. 1983). The alpha anomer of STZ demonstrates higher potency than the beta anomer which suggests involvement of beta cell membrane glucoreceptors which have a greater affinity for the alpha anomer of D-glucose (Dulin et al. 1983, Mordes and Rossini 1981). Removal of the glucose moiety makes STZ much less specific to beta cells (Mordes and Rossini 1981, Preston 1985).

Once in the beta cell STZ is thought to decrease levels of cellular nicotinamide adenine dinucleotide (NAD) and impair synthesis of NAD from nicotinamide (Mordes and Rossini 1981, Dulin 1983, Rerup 1970). In studies by Maldonato et al., STZ inhibited proinsulin synthesis and insulin release in rat islets in a time and concentration dependent manner (1976). The role of STZ as a free radical generator is controversial since studies using vitamin C and exogenous superoxide dismutase (SOD) did not act as significant protective agents (Gold et al. 1981, Yew 1983). The potential to develop STZ-induced diabetes may be influenced by the nutritional status of the experimental animal and by the metabolic status of the pancreatic beta cells (Preston 1985). Vitamin E and nicotinamide inhibit the diabetogenic effects of STZ as they prevent the depletion of islet cell NAD (Mordes and Rossini 1981, Rerup 1970, Slonim et al. 1983).

STZ and other beta cytotoxins have been reported to inhibit SOD activity in the retina, islets, and erythrocytes (Dalin et al. 1983, Gandy et al. 1983). Gandy et al. evaluated the attenuation of STZ diabetes with copper (II) (3,5-Diisopropyl-salicylate)2 which has SOD-like bioactivity (1983). Administration of these compounds before STZ injection appeared to attenuate the severity of the induced diabetes as measured by glucose tolerance and glycosuria. This attenuation may represent a

partial protection of the beta cells with the copper containing SOD-like complex (Gandy et al. 1983)

STZ-induced diabetes is a widely accepted experimental model (Wright and Lacy 1988). Models for non-insulin dependent diabetes mellitus (NIDDM) have been established using neonatal rats injected with the antibiotic between days 0 and 2 after birth (Levy et al. 1984, Portha et al. 1982, 1989, Weir et al. 1981). In neonatal rats injected with STZ on day 2 there was initially a transient period of hyperglycemia lasting 2 to 4 days. Then plasma glucose concentrations remained normal for approximately 6 weeks after which frank hyperglycemia develops with a basal plasma glucose concentration ranging from 200 to 350 mg/dl and decreased basal plasma insulin values (Levy et al. 1984, Portha et al. 1982, 1989, Weir et al. 1981). There were no significant changes in weight. This model appears appropriate for long-term studies as the low insulin response is steady and chronic (Portha et al. 1982).

Experimental diabetes was induced in adult male and female rats by injecting STZ (55-60 mg/kg/i.p.) (Miller et al. 1988). The diabetic rats presented symptoms of frank diabetes including polyuria, glycosuria, and ketonuria within 24-48 hours. Blood glucose values measured between 300-800 mg/dl (Miller et al. 1988). A milder form of diabetes was induced in adult rats with a smaller dose of STZ (45mg/kg/i.v.). The diabetic rats exhibited elevated basal non-fasting plasma glucose of about 300 mg/dl without frank ketosis or insulin dependence (Tancrede et al. 1982).

There is a relationship between the dose of STZ injected and the severity of diabetes induced in the rat (Tancrede et al. 1983). Tancrede et al. studied the effects of varying doses (0, 25, 35, 45, 55, 65, and 100 mg/kg/i.v.) of STZ on glucose tolerance in male Wistar rats. Intravenous glucose tolerance tests were performed at one, four, and sixteen weeks after the STZ injection. Tancrede et al. noted that fasting glucose values were significantly different from controls 1 week after injection of doses greater than or equal to 55 mg/kg. Elevation of fasting glucose in rats receiving less than or equal to 45 mg/kg

became significantly different than controls only at 16 weeks after STZ injection. Decreases in plasma insulin levels became significantly different from controls only at doses of 65 mg/kg and 100 mg/kg (Tancrede et al. 1983).

While caution must be used in extrapolating data derived from experimental diabetes in animals to diabetes in humans, the usefulness and promise of these animal models to illustrate the various etiological and pathogenic mechanisms inherent in diabetes is critical to further study of the relationship of nutrition and nutrients to this disease. Diabetes research in human subjects is complicated by obvious ethical problems. Inducing diabetes in humans is not permissible; thus, new treatments and medications must be tested in animals. Experimental diabetes in animals also provides the opportunity to study the effects of chronic disease and long term usage of therapeutics.

CHAPTER III

EFFECTS OF DIETARY CHROMIUM AND MANGANESE IN STREPTOZOTOCIN-INJECTED RATS

Jarrett, C.R., Adeleye, B.A., Stoecker,
B.J., and Davis-Whitenack, M.L.
Department of Nutritional Sciences, Oklahoma State
University, Stillwater, OK 74078

ABSTRACT

Using a two by two factorial design, thirty-seven male weanling rats (Sprague-Dawley) were randomly assigned to one of four casein-based diets low in chromium and manganese (-Cr -Mn) or supplemented with 1 ppm chromium as chromium chloride (+Cr) and 3 (-Mn) or 55 ppm (+Mn) manganese as manganese carbonate. Experimental diets and deionized water were available ad libitum. After seven weeks on the experimental diets, the rats were injected with streptozotocin (STZ) (45 mg/kg body weight, in citrate buffer, pH 4) on two consecutive days. One week after STZ injections, a glucose tolerance test was performed using a 1 g/kg body weight glucose load, and blood was collected at 0, 60, 120, and 180 minutes. Four weeks after STZ injection, rats were fasted for 12 hours and given a 1 g/kg body weight glucose load two hours before being anesthetized and exsanguinated. Blood samples were collected from the tail prior to the glucose load and by cardiac puncture at necropsy. No significant differences were observed in glucose tolerance tests, fructosamine, plasma insulin, or autopsy glucose concentrations. Manganese and chromium did have a significant interactive effect on corticosterone concentrations at

autopsy ($p<0.05$). Rats fed (+Cr +Mn) diets has significantly lower autopsy corticosterone concentrations than did rats fed all other diets.

INDEXING KEY WORDS; Rats, chromium, manganese, streptozotocin, glucose metabolism

Streptozotocin (STZ) is a antibiotic used to induce diabetes in experimental animals. STZ produces an immediate and highly specific toxic effect on the insulin secreting beta cells of the islets of Langerhans which is followed by a chronic diabetic state (Rerup 1970). The nutritional status of the experimental animal and the metabolic status of the pancreatic beta cells may influence the animal's response to injected STZ (Preston 1985). The mechanism by which STZ induces diabetes is controversial and undefined.

STZ may act as an oxidant resulting in changes in the redox state of the islet cell. Copper-zinc superoxide dismutase (Cu-ZnSOD) is present in the islets of Langerhans of rats. STZ inhibited islet SOD activity in vitro yet had no effect on manganese-dependent SOD (MnSOD). Exogenous polyethylene glycol derivatized superoxide dismutase (PEG-SOD) injected 10 seconds or 50 minutes prior to STZ injections in rats prevented or significantly attenuated the resulting diabetes (Crouch et al. 1981). Slonim et al. (1983) found that vitamin E injected prior to STZ provided protection against the diabetogenic drug and that animals maintained on a vitamin E and selenium-deficient diet showed enhanced diabetogenic response to normally nondiabetogenic doses of STZ. Gold et al. (1981) studied the effects of exogenous SOD, and Yew (1983) studied the protective effects of vitamin C on the response to STZ. Neither found protective effects of these anti-oxidants.

Chromium and manganese are essential for normal glucose metabolism. Diabetic-like symptoms were observed in rats fed diets low in chromium (Mertz and Schwarz 1959, Mertz et al. 1965, Schroeder 1965, Schroeder 1966). Chromium's primary role is the potentiation of insulin. In chromium deficiency a higher dose of insulin is required to elicit the same metabolic response seen in chromium adequacy (Mertz et al. 1965, Mertz et al.

1961, Roginski 1969, Schroeder 1966). First phase insulin secretion of perfused rat pancreata is decreased by 40 to 50% in chromium deficient rats as compared to controls (Striffler et al. 1993). Chromium may play a role in the preservation of pancreatic beta cells.

Defects in carbohydrate metabolism have been reported in manganese-deficient rats and mice. Everson and Shrader reported impaired glucose tolerance and pancreatic pathology in severely manganese-deficient newborn guinea pigs (1968). Decreased pancreatic insulin synthesis and secretion and enhanced intracellular insulin degradation have been observed in manganese deficient animals (Baly et al. 1985). Zidenberg-Cherr et al reported decreased Mn-SOD activity and increased lipid peroxidation in manganese-deficient rats (1983). Tissue MnSOD levels were influenced by dietary manganese intake (Davis et al. 1990).

The world-wide trend towards a highly processed and refined diet which is not only low in chromium but may also stimulate chromium losses may lead to marginal chromium status. Mean intake for most developed countries is 50 to 60% of the lower end of the estimated safe and adequate range (Anderson and Kozlovsky 1985). The Total Diet Study found manganese intakes for teenage, adult, and elderly women to be slightly less than the estimated safe and adequate daily dietary intake (Pennington et al. 1989). Chromium and manganese appear to have roles in the protection of pancreatic beta cells. If chromium/and or manganese intakes are suboptimal, the beta cells may be increasingly susceptible to oxidative stressors and/or to the effects of diabetogenic agents such as STZ. The present study was designed to study the effects of chromium and manganese adequacy and deficiency on glucose metabolism in streptozotocin injected rats.

MATERIALS AND METHODS

Animals and diets. Thirty-seven male weanling Sprague Dawley rats (Sasco, Inc., Omaha, NE) were housed in pairs in plastic cages with plastic gratings. Experimental diets

and deionized water were available ad libitum. All animals were acclimatized to the conditions of the animal care facility before use. The rats were fed the semi-purified American Institute of Nutrition (AIN-76) diet for mice and rats modified to be low in chromium (<400 ug/kg) for the first three weeks. (Table I).

Using a two by two factorial design the animals were randomly assigned to casein-based diets low in chromium and manganese (-Cr -Mn) or supplemented with 1 ppm chromium as chromium chloride (+Cr) and 3 (-Mn) or 55 ppm (+Mn) manganese as manganese carbonate (Cochran and Cox 1957).

Streptozotocin injections. After seven weeks on the experimental diets, the rats were injected on two consecutive days with 45 mg/kg body weight STZ in citrate buffer, pH 4. One week following the STZ injections a glucose tolerance test was performed using a 1g/kg body weight glucose load. Approximately 0.5 ml of blood was collected from the tail at 0, 60, 120, and 180 minutes. The blood samples were centrifuged and serum samples stored in ice for subsequent enzymatic analysis of glucose. (Procedure #510, Sigma Chemical Co., St. Louis, MO)

Necropsy. Four weeks after STZ injections the animals were fasted for 12 hours in metabolic cages, given a 1g/kg body weight glucose load, and two hours later anesthetized with ketamine HCl (30 mg/kg) and xylazine (2.2 mg/kg) and exsanguinated. Blood samples were collected from the tail prior to the glucose load and by cardiac puncture at necropsy. All blood samples were centrifuged and serum was frozen for subsequent analysis (glucose, corticosterone, fructosamine, and insulin). Tissues were removed, trimmed, weighed and frozen for subsequent mineral analysis. Analytical methods. Glucose was determined enzymatically using serum samples (Procedure #510, Sigma Chemical Co., St. Louis, MO). Insulin and corticosterone were determined by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Fructosamine was determined using the ROTAG method (Roche Diagnostic Systems, Inc., Nutley, NJ).

For dietary chromium and manganese analyses, samples of the diets (200-250 mg) were weighed into acid-washed borosilicate glass tubes and dried for 24 h at 100 degrees C. Samples were then ashed in a muffle furnace with no exposed metal heating elements (Lindberg, Watertown, WI) with a dry ashing temperature of 375 degrees C. Dry ashing was alternated with wet ashing with hydrogen peroxide and concentrated nitric acid (Hill et al. 1986). After ashing, samples were diluted with a 0.5% nitric acid (double-distilled; GFS Chemicals, Powell, OH) and analyzed at 357.9 nm on a Perkin Elmer 5100PC atomic absorption spectrophotometer with a graphite furnace and Zeeman background correction (Perkin Elmer Corp., Norwalk, CT). Glass knives were used to cut the tissues for mineral analysis. Drying and ashing procedures for tissues were identical to diet those used for the analysis.

Statistical analysis. Data were analyzed using the generalized linear model (GLM) procedure in the Statistical Analysis System (SAS). Differences among means were identified using the least significant difference test. Repeated measures analysis was used for glucose tolerance curves and repeated fasting glucose values (SAS Institute Inc. 1985).

RESULTS AND DISCUSSION

There was a significant ($p<0.02$) interactive effect between chromium and manganese affecting hepatic manganese concentrations (Table 2). In animals fed low chromium diets, adequate dietary manganese increased hepatic manganese concentrations. In animals fed diets adequate in chromium, dietary manganese did not affect hepatic manganese concentrations. Bond et al. reported significantly elevated hepatic manganese concentrations in STZ-injected rats as compared to controls (1983).

There tended to be an interactive effect ($p<0.10$) of chromium and manganese on renal manganese concentration. Mean renal manganese concentrations of rats fed low chromium diets were 2.31 Ò 0.25 ug/g and 3.44 Ò 0.41 ug/g in rats fed low and adequate manganese, respectively. Mean renal manganese concentrations of rats fed diets adequate

in chromium were 2.43 Ò 0.19 ug/g and 2.60 Ò 0.24 ug/g in rats fed low and adequate manganese, respectively. Mean renal manganese concentration of rats fed the low chromium and adequate manganese was significantly greater than that of rats fed all other diets.

Although not significant ($p<0.07$), adequate dietary chromium tended to have an effect on hepatic chromium concentrations. In rats fed either low or adequate manganese diets, mean hepatic chromium concentrations were greater in rats fed diets adequate in chromium than in rats fed low chromium diets. There no observed dietary effects of either mineral on renal chromium concentration.

Failla and Gardell reported significantly elevated liver and kidney manganese concentrations in spontaneously diabetic BB Wistar rats as compared to their non-diabetic controls (1985). Raz and Havivi studied the long term effects of STZ-induced diabetes and found that renal and hepatic chromium concentrations of STZ-injected rats did not significantly differ from their age matched controls 180 days after injection (1988). Seaborn and Stoecker found no effect of dietary chromium on hepatic chromium concentration in lean and obese mice (1989).

Based on the data from this study, varying dietary trace mineral concentrations affects tissue concentrations of selected minerals in STZ-injected rats. It is possible that a chronic diabetic state, as is seen with STZ-injected rats, influences trace mineral concentrations differently in some organs, possibly depleting excessive amounts of a mineral from one organ and allowing accumulation in another.

The mean change in weight, from time of STZ injection to time of autopsy, varied from a weight loss of 23 +-16 g in the rats fed low manganese and adequate chromium diet to a weight gain of 6 +-20 g in rats fed diet low in chromium and manganese (Table 3). There were no independent or interactive effects of dietary manganese or chromium on change in body weight ($p<0.20$).

There was an significant ($p<0.01$) interactive effect of dietary chromium and manganese on spleen weight, expressed as percent body weight (Table 3). Rats fed a diet adequate in chromium and manganese had a greater mean spleen weight as percent body weight than did rats fed all other diets.

Seminal vesicle weights, expressed as percent body weight, of animals fed diets adequate in chromium tended to be greater than that of animals fed diets low in chromium ($p<0.06$) (Table 3). Anderson and Polansky reported decreased sperm counts and impaired fertility in male rats raised on a chromium deficient diet (1981). There were no independent or interactive effects of dietary manganese or chromium seen in the following tissues, expressed as percent body weight: kidney, pancreas, epididymal fat pad, or liver (Table 3).

When applying repeated measures analysis of changes in fasting serum glucose concentrations, from the day before STZ injection to four weeks after STZ injection (Figure 1), there was a significant ($p<0.04$) interactive effect of dietary chromium and manganese. Rats fed a diet adequate in chromium and manganese had a smaller change in mean fasting serum glucose concentration than did rats fed all other diets. The change in mean fasting serum glucose concentrations varied from -0.7 mmol/L in the rats fed the +Cr +Mn diet to +5.7 mmol/L in rats fed the diet low in chromium and adequate in manganese.

When repeated measures analysis is applied to the glucose tolerance curves (Figure 2), the glucose tolerance curve of animals fed the diet adequate in chromium and manganese tended to be lower than glucose tolerance curves of animals fed all other diets ($p<0.12$). There was no significant effect of dietary chromium or manganese on mean serum glucose concentrations at fasting or any of the individual one hour intervals following the administration of the glucose load (Appendix A).

At autopsy the mean serum glucose concentrations of all treatment groups were elevated (Table 4). This may be a combined effect of the stress of anesthesia and the

glucose load. There was no significant difference in mean autopsy serum glucose concentrations 120 minutes after a glucose load. ($p<0.17$).

There tended to be an interactive effect ($p<0.08$) of dietary chromium and manganese on serum fructosamine concentrations at autopsy. Rats fed diets adequate in chromium and manganese had lower mean serum fructosamine concentrations than did animals fed all other diets. In a study of glycation of blood proteins in pregnant and lactating rats, fructosamine followed a similar pattern of glucose control (Preston et al. 1992). Because fructosamine is a measurement of intermediate (one to three weeks) glucose control, the data from this study provide support for the improved glucose tolerance seen in rats fed diets adequate in chromium and manganese as compared to other experimental groups. Fructosamine appears to be a reliable indicator of glucose status as it is not as affected by stress or dietary intake as is serum or blood glucose Preston 1986).

Although no significant differences in mean serum insulin concentrations were observed ($p<0.20$), animals fed the diet low in chromium and manganese had a greater mean serum insulin concentration that did animals fed all other diets. These data do not show any significant effect of manganese on serum insulin concentration. However, Baly et al. reported decreased circulating insulin in second generation manganese-deficient rats as compared to their controls. First generation manganese deficient rats did not have significantly different glucose tolerance from their controls (1984). It may be necessary to induce the manganese deficiency in utero to have an effect on insulin synthesis.

There was a significant interactive effect of dietary manganese and chromium on serum corticosterone concentrations ($p<0.008$). Rats fed the diet adequate in chromium and manganese had lower mean serum corticosterone levels than did rats fed all other diets. Corticosterone is the primary stress hormone in the rat. In a study of the effects of immobilization stress on plasma corticosterone concentrations in rats, plasma corticosterone concentrations of stressed rats were significantly greater than that of their controls (Srivastava et al. 1993). In a study of the effects of STZ-induced chronic diabetes in rats,

serum corticosterone concentrations were elevated in the STZ-injected rats as compared to their controls (Shires et al. 1981). This provided further support for STZ acting as an oxidative stressor. The data from this study indicate a decrease in stress in the rats fed the diet adequate in chromium and manganese. If STZ is an oxidative stressor, the lower serum glucose and fructosamine concentrations may possibly be explained by dietary chromium and manganese having in interactive protective effect against the diabetogenic action of STZ.

In conclusion, feeding STZ-injected rats diets adequate in chromium and manganese appeared to have some protective effect on the diabetogenic actions of streptozotocin. Compared to the other treatement groups, they exhibited enhanced glucose tolerance, less weight loss, and less stress as measured by serum corticosterone concentrations. Rats fed diets adequate in chromium and manganese also had lower fructosamine concentrations which indicated lower serum glucose for the one to three weeks prior to autopsy.

TABLE 2

Group means of mineral concentrations of kidney and liver in STZ-injected rats fed diets low and adequate in chromium and manganese[1,2]

	Manganese (ug/g)		Chromium (ng/g)	
	Kidney	Liver	Kidney	Liver
-Cr -Mn	2.31 ± 0.25	3.74 ± 0.62	31.3 ± 5.3	17.09 ± 5.6
-Cr +Mn	3.44 ± 0.41	7.57 ± 0.45	25.9 ± 5.1	32.9 ± 12.8
+Cr -Mn	2.43 ± 0.19	5.36 ± 0.10	31.6 ± 8.2	43.8 ± 14.3
+Cr +Mn	2.60 ± 0.24	5.56 ± 0.40	34.6 ± 6.5	51.8 ± 13.7
Source of variation				
Cr	0.22	0.78	0.50	0.07
Mn	0.03	0.008	0.86	0.33
Cr x Mn	0.10	0.016	0.52	0.75

[1] Means ± SEM
[2] N = 8-10

TABLE 3

Group means of change in weight, from time of injection to autopsy, and tissue weights, expressed as percent body weight, in STZ-injected rats fed diets low and adequate in chromium and manganese[1,2]

	Change in weight	Spleen	Seminal Vesicle	Kidney	Pancreas	Epididymal fat pad	Liver
	grams	%	%	%	%	%	%
-Cr -Mn	+6 ± 20	0.18 ± 0.01	0.22 ± 0.02	0.92 ± 0.07	0.20 ± 0.02	0.86 ± 0.13	3.30 ± 0.14
-Cr +Mn	-21 ± 20	0.18 ± 0.01	0.21 ± 0.02	1.02 ± 0.06	0.22 ± 0.02	0.64 ± 0.13	3.31 ± 0.12
+Cr -Mn	-23 ± 16	0.17 ± 0.02	0.25 ± 0.02	0.99 ± 0.05	0.23 ± 0.01	0.70 ± 0.14	3.32 ± 0.13
+Cr +Mn	-4 ± 14	0.25 ± 0.01	0.26 ± 0.03	0.93 ± 0.06	0.22 ± 0.02	0.84 ± 0.05	3.31 ± 0.19
Source of variation							
Cr	0.73	0.04	0.06	0.88	0.40	0.86	0.95
Mn	0.82	0.005	0.95	0.71	0.77	0.75	1.00
Cr x Mn	0.20	0.004	0.61	0.15	0.33	0.15	0.94

[1]Mean ± SEM
[2]N = 9-10

TABLE 4

Group means of autopsy blood and serum parameters of STZ-injected rats fed diets low and adequate in chromium and manganese[1,2]

	Glucose, fasting (mmol/L)	Glucose, necropsy[3] (mmol/L)	Fructosamine (mmol/L)	Insulin (pmol/L)	Corticosterone (nmol/L)
-Cr -Mn	10.39 ± 2.43	21.34 ± 1.66	1.89 ± 0.12	9.13 ± 1.69	109.2 ± 12.9
-Cr +Mn	11.82 ± 2.57	23.33 ± 1.42	1.94 ± 0.09	6.22 ± 0.66	274.4 ± 79.7
+Cr -Mn	10.68 ± 2.65	21.89 ± 1.58	1.99 ± 0.11	6.66 ± 1.46	210.6 ± 55.8
+Cr +Mn	5.35 ± 1.00	18.71 ± 2.48	1.69 ± 0.05	6.96 ± 0.75	89.8 ± 16.6
Source of variation					
Cr	0.18	0.27	0.42	0.49	0.41
Mn	0.40	0.75	0.21	0.30	0.66
Cr x Mn	0.15	0.17	0.07	0.20	0.008

[1] Mean ± SEM
[2] N = 9-10
[3] 120 minutes after 1 g/kg body weight glucose load

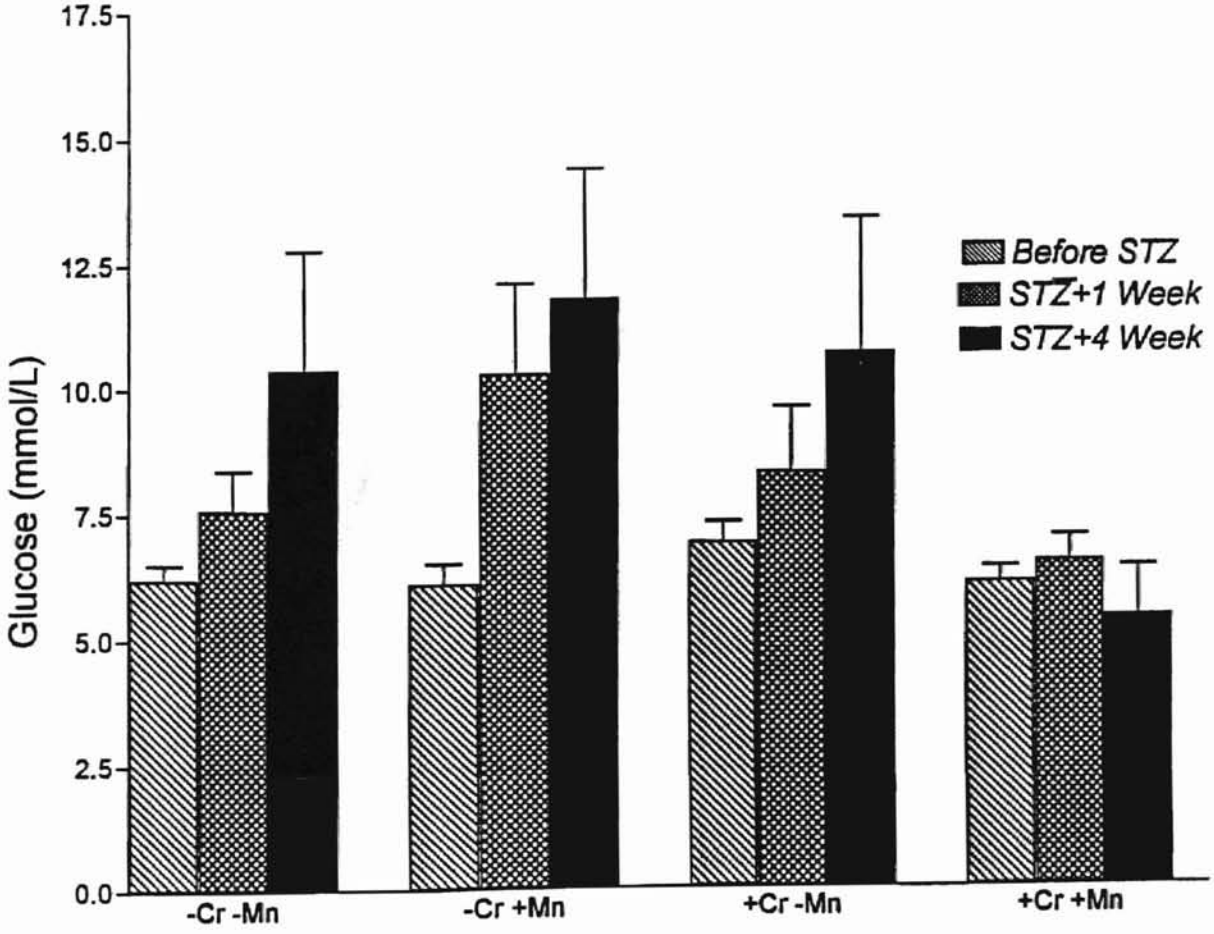

Figure 1: Fasting serum glucose concentrations before and in response to STZ injections

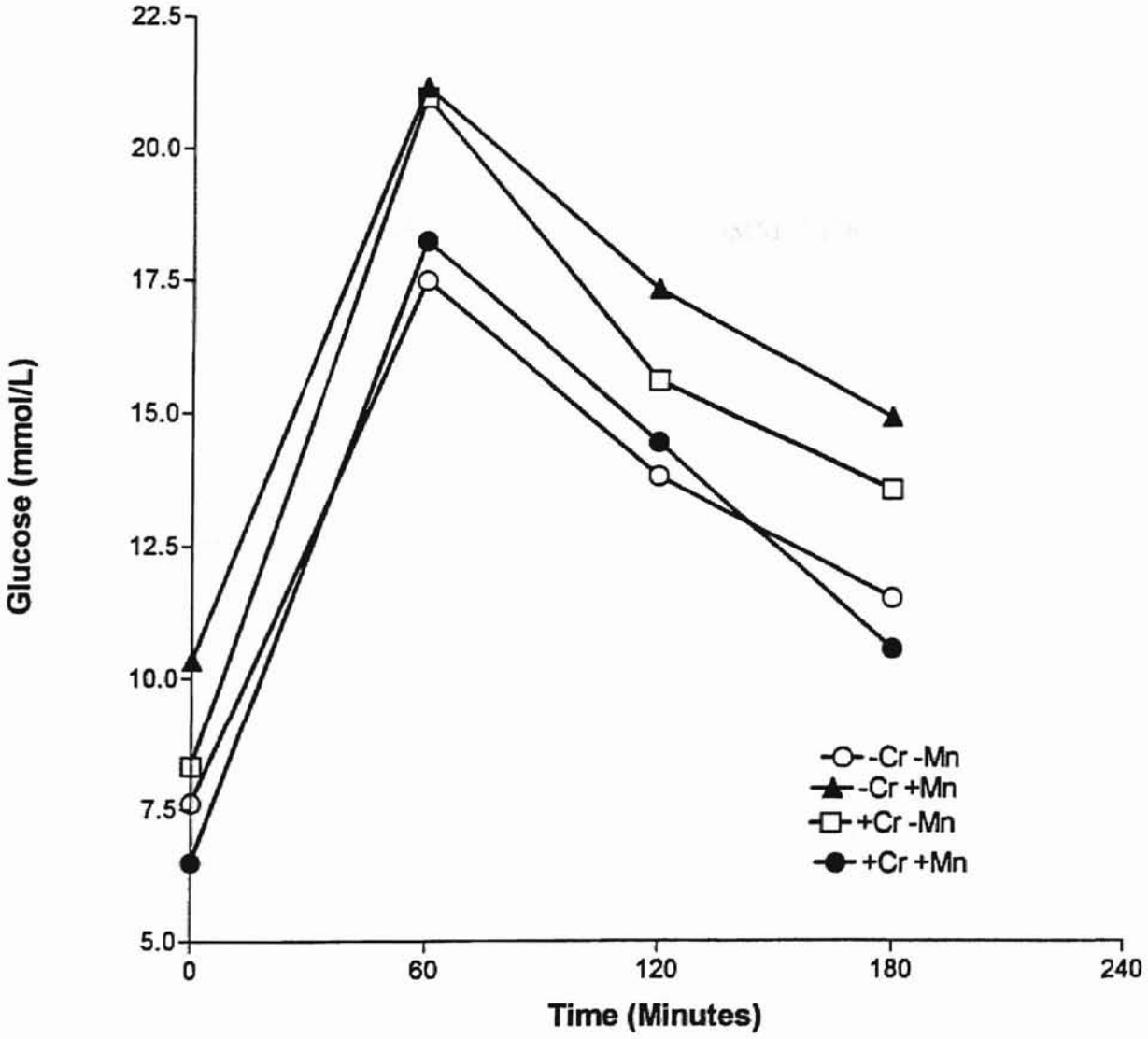

Figure 2: Mean serum glucose concentrations at fasting and at one hour intervals during a glucose tolerance test (1g/kg body wt) in STZ-injected rats fed diets low or adequate in chromium and manganese

CHAPTER IV

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

Thirty seven male weanling rats were randomly assigned to one of four experimental diets varying only in amount of chromium and manganese. After seven weeks on the experimental diets, animals were injected with STZ on two consectutive days (45 mg/kg body weight, in citrate buffer, pH 4). One week after STZ injections, a glucose tolerance test was performed. Four weeks after STZ injections, animals were fasted for 12 hours, and given a glucose load (1 g/kg body weight). Blood samples were collected before and two hours after the glucose load. The animals were then killed and selected tissues were collected for subsequently analyzed for paramters reflecting carbohydrate metabolism.

Two hypotheses were listed in the introduction of this thesis. The first hypothesis stated that there would be no statistically significant independent and/or interactive effect(s) of dietary chromium and/or manganese on change in body weight, tissue weights as percent body weight, glucose tolerance, or serum insulin, fructosamine, or corticosterone concentrations in streptozotocin-injected rats. Dietary chromium and manganese significantly decreased corticosterone concentrations, increased spleen weights as percent body weight, and decreased STZ-induced changes in fasting glucose. Therefore, we reject the first null hypothesis.

The second hypothesis stated there would be no statistically significant independent and/or interactive effects(s) of dietary chromium and/or manganese of liver and kidney

mineral concentrations in streptozotocin-injected rats. Adequate dietary manganese did increase renal manganese concentrations and did increase hepatic manganese concentrations when rats were fed a low chromium diet. Thus we reject the second null hypothesis.

Conclusions

Adequate dietary chromium and manganese increased spleen weight, as percent body weight ($p<0.005$), tended to decrease serum fructosamine concentrations ($p<0.08$), and decreased corticosterone concentrations ($p<0.008$). Rats fed diets adequate in chromium and manganese had smaller STZ-induced changes in fasting glucose ($p<0.04$).

Recommendations

To better assess the nature of the cytotoxic effects of STZ, further research may include an examination of pancreatic physiology and measurements of pancreatic SOD activity and thiobarbituric acid-reacting products as indicators of lipid peroxidation and oxidative stress. To investigate the effects of manganese deficiency it may be necessary to use a second generation manganese-deficient rat or feed a diet with no added manganese. It would be worthwhile to evaluate the effects of higher levels of supplemental chromium and manganese on glucose metabolism in STZ-injected rats.

LITERATURE CITED

Anderson, R.A. (1987). Chromium. In: Trace Elements in Human and Animal Nutrition (Mertz, W., ed.), 5. pp. 225-244. Academic Press, Inc., New York.

Anderson, R.A. (1989). Essentiality of chromium in humans. Sci. Total Envir., 86: 75-81.

Anderson, R.A. (1994). Nutritional and toxicologic aspects of chromium intake: An overview. In: Risk assessment of essential elements (Mertz, W., Abernathy, C.O., Olin, S.S., eds.), pp. 187-196. ILSI Press, Washington, D.C..

Anderson, R.A., Bryden, N.A., and Polansky, M.M. (1985). Serum chromium of human subjects: effects of chromium supplementation and glucose. Am. J. Clin. Nutr., 41: 571-577.

Anderson, R.A., Bryden, N.A., and Polansky, M.M. (1992). Dietary chromium intake: Freely chosen diets, institutional diets, and individual foods. Biol. Trace Elem., Res.32: 117-121.

Anderson, R.A. and Kozlovsky, A.S. (1985). Chromium intake, absorption and excretion of subjects consuming self-selected diets. Am. J. Clin. Nutr., 41: 1177-1183.

Anderson, R.A. and Polansky, M.M. (1981). Dietary chromium deficiency: Effect on sperm count and fertility in rats. Biol. Trace Elem., Res.3: 1-5.

Anderson, R.A., Polansky, M.M., Bryden, N.A., and Canary, J.J. (1991). Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. Am. J. Clin. Nutr., 54: 909-916.

Anderson, R.A., Polansky, M.M., Bryden, N.A., Patterson, K.Y., Veillon, C., and Glinsmann, W.H. (1983). Effects of chromium supplementation of urinary Cr excretion of human subjects and correlation of Cr excretion with selected clinical parameters. J. Nutr., 113: 276-281.

Anderson, R.A., Polansky, M.M., Bryden, N.A., Roginski, E.E., Mertz, W., and Glinsmann, W. (1983). Chromium supplementation of human subjects: effects on glucose, insulin, and lipid variables. Metabolism, 32: 894-899.

Anderson, R.A., Polansky, M.M., Bryden, N.A., Roginski, E.E., Patterson, K.Y., Veillon, C., and Glinsmann, W. (1982). Urinary chromium excretion of human subjects: Effects of chromium supplementation and glucose loading. Am. J. Clin. Nutr., 36: 1184-1193.

Baly, D., Curry, D.L., Keen, C.L., and Hurley, L.S. (1985). Dynamics of insulin and glucagon release in rats: influence of dietary manganese. Endocrinology, 116: 1734-1740.

Baly, D.L., Curry, D.L., Keen, C.L., and Hurley, L.S. (1984). Effect of manganese deficiency on insulin secretion and carbohydrate homeostasis in rats. J. Nutr., 114: 1438-1446.

Baly, D.L., Keen, C.L., Curry, D.L., and Hurley, L.S. (1985). Effects of manganese deficiency on carbohydrate metabolism. Proc Fifth Symp, 254-261.

Baly, D.L., Keen, C.L., and Hurley, L.S. (1985). Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase activity in developing rats: effect of manganese deficiency. J Nutr., 115: 872-879.

Baly, D.L., Schneiderman, J.S., and Garcia-Welsh, A.L. (1990). Effects of manganese deficiency on insulin binding, glucose transport and metabolism in rat adipocytes. J. Nutr., 120: 1075-1079.

Bond, J.S., Failla, M.L., and Unger, D.F. (1983) Elevated manganese concentration and arginase activity in livers of streptozotocin-induced diabetic rats. J. Biol. Chem., 258: 8004-8009.

Britton, A.A. and Cotzias, G.C. (1966). Dependence of manganese turnover on intake. Am. J. Physiol., 211: 203-206.

Brown, R.O., Forloines-Lynn, S., Cross, R.E., and Heizer, W.D. (1986). Chromium deficiency after long-term total parenteral nutrition. Dig.Dis.Sci., 31: 661-664.

Chen, N.S.C., Tsai, A., and Dyer, I.A. (1973). Effect of chelating agents on chromium absorption in rats. J. Nutr., 103: 1182-1186.

Clausen, J. (1988). Chromium induced clinical improvement in symptomatic hypoglycemia. Biological Trace Element Research, 17: 229-236.

Crouch, R.K., Gandy, S.E., Kimsey, G., Galbraith, R.A., Galbraith, G.M.P., and Buse, M.A. (1981). The inhibition of islet superoxide dismutase by diabetogenic drugs. Diabetes, 30: 235-241.

Davidsson, L., Cederblad, A., Lonnerdal, B., and Sandstrom, B. (1989). Manganese retention in man: a method for estimating manganese absorption in man. Am. J. Clin. Nutr., 49: 170-79.

Davidsson, L., Cederblad, A., Lonnerdal, B., and Sandstrom, B. (1991). The effect of individual dietary components on manganese absorption in humans. Am. J. Clin. Nutr., 54: 1065-1070.

Davies, N.T. and Nightingale, R. (1975). The effects of phytate on intestinal absorption and secretion of zinc, and whole-body retention of zinc, copper, iron and manganese in rats. Brit. J. Nutr., 34: 243-258.

Davis, C.D. and Greger, J.L. (1992). Longitudinal changes of manganese-dependent superoxide dismutase and other indexes of manganese and iron status in women. Am. J. Clin. Nutr., 55: 747-752.

Davis, C.D., Ney, D.M., and Greger, J.L. (1990). Manganese, iron and lipid interactions in rats. J Nutr., 120: 507-513.

Davis, C.D., Wolf, T.L., and Greger, J.L. (1992). Varying levels of manganese and iron affect absorption and gut endogenous losses of manganese by rats. J. Nutr., 122: 1300-1308.

Doisy, E.A., Jr. (1972). Micronutrient controls on biosynthesis of clotting proteins and cholesterol. In: Trace Substances in Environmental Health-VI (Hemphill, D.D., ed.), 6. pp. 193-199. Universityof Missouri, Columbia, MO.

Donaldson, R.M. and Barreras, R.F. (1966). Intestinal absorption of trace quantities of chromium. J.Lab.Clin.Med., 68: 484-93.

Dulin, W.E., Gerritsen, G.C. & Chang, A.Y. (1983). Experimental and spontaneous diabetes in animals. In: Diabetes Mellitus Theory and Practice (Ellenberg, M., Rifkin, H., eds.), 3. pp. 361-408. Medical Examination Publishing Co., Inc., New Hyde Park, NY.

Evans, G.W., Roginski, E.E., and Mertz, W. (1973). Interaction of the glucose tolerance factor (GTF) with insulin. Biochem. Biophys. Res. Comm., 50: 718-722.

Everson, G.J. and Shrader, R.E. (1968). Abnormal glucose tolerance in manganese-deficient guinea pigs. J. Nutr., 94: 89-94.

Fahim, F.A., Morcos, N.Y.S., and Esmat, A.Y. (1990). Effects of dietary magnesium and/or manganese variables on the growth rate and metabolism of mice. Ann. Nutr. Metab., 34: 183-192.

Failla, M.L. and Gardell, C.Y.R. (1985). Influence of spontaneous diabetes on tissue status of zinc, copper, and manganese in the BB wistar rat. Proc.Soc.Exp.Biol.Med., 180: 317-322.

Flatt, P.R., Juntti-Berggren, L., Berggren, P.O., Gould, B.J., and Swanston-Flatt, S.K. (1989). Effects of dietary inorganic trivalent chromium (Cr 3+) on the development of glucose homeostasis in rats. Diabete & Metabolisme, 15: 93-97.

Freeland-Graves, J.H., Behmardi, F., Bales, C.W., Dougherty, V., Lin, P.H., Crosby, J.B., and Trickett, P.C. (1988). Metabolic balance of manganese in young men consuming diets containing five levels of dietary manganese. J. Nutr., 118: 764-73.

Freund, H., Atamian, S., and Fischer, J.E. (1979). Chromium deficiency during total parenteral nutrition. J.Am.Med.Assoc., 241: 496-498.

Gandy, S.E., Buse, M.G., and Crouch, R.K. (1982). Protective role of superoxide dismutase against diabetogenic drugs. J. Clin. Invest., 70: 650-658.

Gandy, S.E., Buse, M.G., Sorenson, J.R.J., and Crouch, R.K. (1983). Attenuation of streptozotocin diabetes with superoxide dismutase-like Copper(II)(3,5-Diisopropylsalicylate)2 in the rat. Diabetologia, 24: 437-440.

Garcia-Aranda, J.A., Wapnir, R.A., and Lifshitz, F. (1983). In vivo intestinal absorption of manganese in the rat. J. Nutr., 113: 2601-2607.

Glinsmann, W.H., Feldman, F.J., and Mertz, W. (1966). Plasma chromium after glucose administration. Science, 152: 1243-1245.

Glinsmann, W.H. and Mertz, W. (1966). Effect of trivalent chromium on glucose tolerance. Metabolism, 15: 510-520.

Gold, G., Manning, M., Heldt, A., Nowlain, R., Pettit, J.R., and Grodsky, G.M. (1981). Diabetes induced with multiple sub-diabetogenic doses of streptozotocin. Lack of protection by exogenous superoxide dismutase. Diabetes, 30: 634-638.

Govindaraju, K., Ramasami, T., and Ramaswamy, D. (1989). Chromium (III)-insulin derivatives and their implication in glucose metabolism. J. Inorg. Biochem., 35: 137-147.

Gruden, N. (1988). The effect of parenteral iron administration upon manganese absorption in young rats. Nutr.Rep.Int., 37: 57-62.

Hahn, C.J. and Evans, G.W. (1975). Absorption of trace metals in the zinc-deficient rat. Am. J. Physiol., 228: 1020-1023.

Hill, A.D., Patterson, K.Y., Veillon, C., and Morris, E.R. (1986). Digestion of biological materials for mineral analyses using a combination of wet and dry ashing. Anal. Chem., 58: 2340-2342.

Holloway, D.E., Peterson, F.J., Prigge, W.F., and Gebhard, R.L. (1981). Influence of dietary ascorbic acid upon enzymes of sterol biosynthesis in the guinea pig. Biochem. Biophys. Res. Comm., 102: 1283-1289.

Hopkins, L.L., Jr. and Schwarz, K. (1964). Chromium (III) binding to serum proteins, specifically siderophilin. Biochim. Biophys. Acta., 90: 484-491.

Jacobson, G.R., Poy, F., and Lengeler, J.W. (1990). Inhibition of streptococcus mutans by the antibiotic strreptozotocin: mechanisms of uptake and the selection of carbohydrate-negative mutants. Infect. Immun., 58: 543-549.

Jeejeebhoy, K.N., Chu, R.C., Marliss, E.B., Greenberg, G.R., and Bruce-Robertson, A. (1977). Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. Am. J. Clin. Nutr., 30: 531-538.

Keen, C.L., Clegg, M.S., Lonnerdal, B., and Hurley, L.S. (1983). Whole-blood manganese as an indicator of body manganese. N Engl J Med, 30: 1230

Keen, C.L., Zidenberg-Cherr, S. (1990). Manganese. In: Present Knowledge in Nutrition (Brown, M.L., ed.), 6. pp. 279-285. International Life Sciences Institute, Washington D.C..

Keen, C.L., Zidenberg-Cherr, S. & Lonnerdal, B. (1994). Nutritional and toxicological aspects of manganese intake: An overview. In: Risk assessment of essential elements (Mertz, W., Abernathy, C.O., Olin, S.S., eds.), pp. 221-235. ILSI Press, Washington, D.C..

Kemmerer, A.R., Elvehjem, C.A., and Hart, E.B. (1931). Studies on the relation of manganese to the nutrition of the mouse. J. Biol. Chem., 92: 623-630.

Kergoat, M., Guerre-Millo, M., Lavau, M., and Portha, B. (1991). Increased insulin action in rats with mild insulin deficiency induced by neonatal streptozoticn. Am. J. Physiol., 260: E561-E567.

Kozlovsky, A.S., Moser, P.B., Reiser, S., and Anderson, R.A. (1986). Effects of diets high in simple sugars on urinary chromium losses. Metabolism, 35: 515-518.

Levy, J., Gavin, J., III, Fausto, A., Gingerich, R.L., and Avioli, L.V. (1984). Impaired insulin action in rats with non-inslulin-dependent diabetes. Diabetes, 33: 901-906.

Maldonato, A., Trueheart, P.A., Renold, A.E., and Sharp, G.W.G. (1976). Effects of streptozotocin in vitro on proinsulin biosynthesis, insulin release, and ATP content of isolated rat islets of langerhans. Diabetologia, 12: 471-481.

Mertz, W. (1976). Chromium and its relation to carbohydrate metabolism. Med. Clin. N. Am., 60: 239-244.

Mertz, W. (1992). Chromium: History and nutritional importance. Biol. Trace Elem., Res.32: 3-8.

Mertz, W. (1993). Chromium in human nutrition: A review. J. Nutr., 123: 626-633.

Mertz, W., Roginski, E.E., and Schroeder, H.A. (1965). Some aspects of glucose metabolism of chromium-deficient rats raised in a strictly controlled environment. J. Nutr., 86: 107-112.

Mertz, W., Roginski, E.E., and Schwarz, K. (1961). Effect of trivalent chromium complexes on glucose uptake by epididymal fat tissue of rats. J. Biol. Chem., 236: 318-322.

Mertz, W. and Schwarz, K. (1959). Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. Am. J. Physiol., 196: 614-618.

Mertz, W., Toepfer, E.W., Roginski, E.E., and Polansky, M.M. (1974). Present knowledge of the role of chromium. Fed. Proc., 33: 2275-2280.

Miller, L.L., Izzo, M.J., and Wemett, D. (1988). Persistent grossly elevated plasma immunoglobulin A levels in untreated STZ-induced diabetic rats. Diabetes, 37: 177-184.

Mordes, J.P. and Rossini, A.A. (1981). Animal models of diabetes. Am. J. Med., 70: 353-360.

Morris, B.W., Griffiths, H., and Kemp, G.J. (1988). Correlations between abnormalities in chromium and glucose metabolism in a group of diabetics. Clin. Chem., 34: 1525-1526.

Morris, B.W., Griffiths, H., and Kemp, G.J. (1988). Effect of glucose loading on concentrations of chromium in plasma and urine of healthy adults. Clinical Chemistry, 34: 1114-1116.

Offenbacher, E.G. and Pi-Sunyer, F.X. (1983). Temperature and pH effects on the release of chromium from stainless steel into water and fruit juices. J. Agric. Food Chem., 31: 89-92.

Orent, E.R. and McCollum, E.V. (1931). Effects of deprivation of manganese in the rat. J. Biol. Chem., 92: 651-678.

Pennington, J.A.T., Young, B.E., and Wilson, D.B. (1989). Nutritional elements in U.S. diets: results from the total diet study, 1982 to 1986. J. Am. Diet. Assoc., 89: 659-664.

Portha, B., Blondel, O., Serradas, P., McEvoy, R., Giroix, M.-H., Kergoat, M., and Bailbe, D. (1993). The rat models of non-insulin dependent diabetes induced by neonatal streptozotocin. Diabete & Metabolisme, 15: 61-75.

Portha, B., Giroix, M.H., and Picon, L. (1982). Effect of diet on glucose tolerance and insulin response in chemically diabetic rats. Metabolism, 31: 1194-1199.

Preston, A.M. (1985). Modification of streptozotocin-induced diabetes by protective agents. Nutr. Res., 5: 435-446.

Preston, A.M. and Gonzalez, M.J. (1986). Glycated hemoglobin in streptozotocin-induced diabetic rats as measured by affinity chromatography. Nutr. Res., 6: 1371-1377.

Preston, A.M., Munoz, Y.B.M., LaBoy, L.L., and Rodrigues, M.B. (1992). Glycation of blood proteins during pregnancy and lactation in the rat. Puerto Rican Hlth. Sci. J., 11: 69-71.

Ranhotra, G.S. and Gelroth, J.A. (1986). Effects of high-chromium bakers' yeast on glucose tolerance and lipids in rats. Cereal Chem., 63: 411-413.

Raz, I. and Havivi, E. (1988). Influenceof chronic diabetes on tissue and blood cell status of zinc, copper, and chromium in the rat. Diabetes Research, 7: 19-23.

Rerup, C.C. (1970). Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol. Rev., 22: 485-518.

Roginski, E. and Mertz, W. (1969). Effects of chromium(III) supplementation on glucose and amino acid metabolism in rats fed a low protein diet. J. Nutr., 97: 525-530.

Schroeder, H.A. (1965). Diabetic-like serum glucose levels in chromium deficient rats. Life Sci., 4: 2057-2062.

Schroeder, H.A. (1966). Chromium deficiency in rats: a syndrome simulating diabetes mellitus with retarded growth. J. Nutr., 88: 439-445.

Schroeder, H.A. (1968). The role of chromium in mammalian nutrition. Am. J. Clin. Nutr., 21: 230-244.

Schwarz, K. and Mertz, W. (1959). Chromium(III) and the glucose tolerance factor. Arch. Biochem. Biophys., 85: 292-295.

Seaborn, C.D. and Stoecker, B.J. (1989). Effects of starch, sucrose, fructose, and glucose on chromium absorption and tissue concentrations in obese and lean mice. J. Nutr., 119: 1444-1451.

Shires, R., Teitelbaum, S.L., Bergfeld, M.A., Fallon, M.D., Slatopolsky, E., and Avioli, L.V. (1981). The effect of streptozotocin-induced chronic diabetes mellitus on bone and mineral homeostasis in the rat. J Lab Clin Med., 97: 231-240.

Slonim, A.E., Fletcher, T., Burke, V., and Burr, I.M. (1976). Effect of streptozotocin on red-blood-cell-reduced glutathione: modification by glucose,nicotinamide, and epinephrine. Diabetes, 25: 216-222.

Slonim, A.E., Surber, M.L., Page, D.L., Sharp, R.A., and Burr, I.M. (1983). Modification of chemically induced diabetes in rats by vitamin E. Supplementation minimizes and depletion enhances development of diabetes. J. Clin. Invest., 71: 1282-1288.

Srivastava, R.K., Taylor, M.F., and Mann, D.R. (1993). Effect of immobilization stress on plasma luteinizing hormone, testosterone, and corticosterone concentrations and on 3b-hydroxysteroid dehydrogenase activity in the testes of adult rats. Proc. Soc. Exp. Biol. Med., 204: 231-235.

Stoecker, B. (1990). Chromium. In: Present Knowledge in Nutrition (Brown, M., ed.), 6. pp. 287-293. International Life Sciences Institute, Nutrition Foundation, Washington, D.C..

Striffler, J.S., Polansky, M.M., and Anderson, R.A. (1993). Dietary chromium enhances insulin secretion in perfused rat pancreas. J. Trace Elem. Exp. Med., 6: 75-81.

Tancrede, G., Rousseau-Migneron, S., and Nadeau, A. (1982). Beneficial effects of physical training in rats with a mild streptozotocin-induced diabetes mellitus. Diabetes, 31: 406-409.

Tancrede, G., Rousseau-Migneron, S., and Nadeau, A. (1983). Long-term changes in the diabetic state induced by different doses of streptozotocin in rats. Brit J Exp Path, 64: 117-123.

Thompson, K.H., Godin, D.V., and Lee, M. (1992). Tissue antioxidant status in streptozotocin-induced diabetes in rats: Effects of dietary manganese deficiency. Biol. Trace Elem. Res., 35: 213-224.

Thomson, A.B.R., Olatunbosun, D., and Volberg, L.S. (1971). Interrelation of intestinal transport system of manganese and iron. J Lab Clin Med., 78: 642-655.

Vanderslice, J.T., Maire, C.E., and Beecher, G.R. (1981). B6 vitamer analysis in human plasma by high performance liquid chromatography: a preliminary report. Am. J. Clin. Nutr., 34: 947-50.

Walker, R.C., Bachorik, P.S., and Kwiterovich, P.O., Jr. (1982). Evaluation of five enzymic kits for determination of triglyceride concentrations in plasma. Clin. Chem., 28: 2299-2305.

Weir, G.C., Clare, T., Zmachinski, C.J., and Bonner-Weir, S. (1981). Islet secretion in a new experimental model of non-insulin dependent diabetes. Diabetes, 30: 590-595.

Werner, L., Korc, M., and Brannon, P.M. (1987). Effects of manganese deficiency and dietary composition on rat pancreatic enzyme content. J. Nutr., 117: 2079-85.

Wright, J.R., Jr. and Lacy, P.E. (1988). Synergistic effects of adjuvants, endotoxin, and fasting on induction of diabetes with multiple low doses of streptozotocin in rats. Diabetes, 37: 112-118.

Yew, M.S. (1983). Ascorbic acid supplementation and induction of diabetes in rats. Nutr. Rep. Int., 27: 297-302.

Zidenberg-Cherr, S., Keen, C.L., Lonnerdal, B., and Hurley, L.S. (1983). Superoxide dismutase activity and lipid peroxidation in the rat: developmental correlations affected by manganese deficiency. J. Nutr., 113: 2498-2504.

APPENDIX

APPENDIX A

Group means of serum glucose values at fasting and one hour intervals during glucose tolerance tests in STZ-injected rats fed diets low and adequate in chromium and manganese[1,2]

	Glucose (mmol/L)			
	0 minutes	60 minutes	120 minutes	180 minutes
-Cr -Mn	7.62 ± 0.81	17.49 ± 1.99	13.82 ± 2.05	11.50 ± 2.01
-Cr +Mn	10.34 ± 1.78	21.16 ± 2.07	17.31 ± 2.51	14.91 ± 2.62
+Cr -Mn	8.32 ± 1.34	20.97 ± 1.46	15.60 ± 2.22	13.55 ± 2.70
+Cr +Mn	6.49 ± 0.50	18.24 ± 1.97	14.44 ± 1.82	10.55 ± 1.38
Source of variation				
Cr	0.21	0.88	0.80	0.61
Mn	0.72	0.80	0.59	0.93
Cr x Mn	0.07	0.10	0.29	0.17

[1] Means ± SEM
[2] N = 9-10

VITA

Carol Renee Jarrett

Candidate for the Degree of

Master of Science

Thesis: EFFECTS AND INTERACTIONS OF DIETARY CHROMIUM AND MANGANESE IN STREPTOZOTOCIN-INJECTED RATS

Major Field: Nutritional Sciences

Biographical:

Personal Data: Born in Coalgate, Oklahoma, August 5, 1959, the daughter of Milt and Phyllis McLean Jarrett

Education: Graduated from Norman High School, Norman, Oklahoma, in May 1977; attended the University of Tulsa from 1977 to 1981; received Bachelor of Science degree in Food, Nutrition, and Institution Administration from Oklahoma State University in May 1989; completed requirements for the Master of Science degree at Oklahoma State University in May 1995

Professional Experience: Graduate Research and Teaching Assistant, Nutritional Sciences Department, Oklahoma State University 1989-1991, Registered and Licensed Clinical Dietitian, Hillcrest Medical Center, Tulsa, Oklahoma

Professional Organizations: American Dietetic Association, Oklahoma Dietetic Association, Tulsa Dietetic Association, American Society of Parenteral and Enteral Nutrition