CHROMIUM AND MANGANESE: DEPLETION EFFECTS IN GENETICALLY OBESE (ob/ob) MICE

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1978

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
December, 1993

OKLAHOMA STATE UNIVERSITY

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CHAPTER I

INTRODUCTION

Abnormalities in carbohydrate and lipid metabolism are characteristic complications of non-insulin dependent diabetes mellitus (NIDDM). Hyperglycemia, hyperinsulinemia, and hypercholesterolemia are among the contributing factors to the long term complications of the disease. Trace minerals are known to participate in the metabolism of both carbohydrates and lipids and deficiencies of certain trace minerals may induce symptoms similar to those of NIDDM.

The essentiality of chromium in animals was established in the 1950's when chromium was identified as the active component in glucose tolerance factor (Mertz et al. 1974). Sufficient evidence to support its essentiality in humans resulted in the addition of chromium to the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) of the Recommended Dietary Allowances since 1980 (National Research Council 1989).

Manganese has been found to be essential in all animal species studied. Defects in carbohydrate and lipid metabolism are among the complications common to the offspring of manganese-deficient animals. Known functions of manganese include enzyme activation and serving as a

constituent of metalloenzymes such as pyruvate carboxylase (Hurley & Keen 1987).

Previous research in this laboratory involved the effects of low chromium and low manganese diets on rats with streptozotocin (STZ) induced diabetes. Four weeks post injection of STZ, only those animals fed adequate amounts of both chromium and manganese displayed normal fasting glucose levels.

The purpose of this study was to investigate the effects and interactions which may occur in animals fed diets lacking in adequate amounts of chromium and manganese. In order to eliminate the possible effects of trace minerals on the efficacy of STZ, this study used the genetically obese mouse. This mouse, C57BL/6J (ob/ob) is characterized by obesity, hyperglycemia and hyperinsulinemia making it a good model to examine the effects of chromium and manganese depletion.

Hypothesis

Low and adequate levels of dietary chromium and manganese will have significant effects on plasma glucose, insulin, corticosterone, and cholesterol on glycosylated hemoglobin and on tissue concentrations of trace minerals in genetically obese mice.

CHAPTER II

REVIEW OF LITERATURE

Chromium

Essentiality

The essentiality of chromium in animals was first documented by Mertz et al. (1961) in the late 1950's. Trivalent chromium was identified as the active agent in glucose tolerance factor (GTF) helping to maintain normal glucose removal rates from the bloodstream. Diets containing low amounts of GTF resulted in impaired glucose tolerance in rats. The impaired glucose tolerance was subsequently corrected with supplementation by trivalent chromium complexes.

Glinsmann and Mertz (1966) showed improved glucose tolerance in three out of six diabetic subjects and suggested that chromium was essential for optimal utilization of glucose in the human. Total parenteral nutrition (TPN) solutions containing no added chromium resulted in glucose intolerance and peripheral neuropathy. When the patient was subsequently supplemented with 250 ug chromium per day, these symptoms disappeared (Jeejeebhoy, et al. 1977).

It has been suggested that essentiality must meet four criteria: the essential element must be in living matter; it must interact with living systems; a dietary deficiency must consistently and specifically reduce a biologic function to suboptimal; and, the reduced biologic function must be preventable or curable by physiologic amounts of the nutrient. Using these criteria, it has been suggested by some authors that chromium meets some but not all standards of essentiality. The lack of response to chromium supplementation in apparent chromium-deficient neuropathy and the failure of serum chromium levels to rise in response to chromium supplementation in one case have been cited as questions in determining essentiality (Anonymous 1988). Anderson et al. (1985) however, demonstrated a significant increase in serum chromium levels following supplementation reflective of chromium intake but determined that serum chromium concentration is not a meaningful indicator of chromium status.

The exact role of GTF in the potentiation of insulin is as yet undefined. Further, there is no evidence for chromium as a component of the insulin receptor either as part of insulin binding or in mediating the effect of insulin on cells. Despite questions about its essentiality in humans, the inclusion of an estimated safe and adequate daily intake (ESADDI) for chromium in the 1980 and 1989 editions of the Recommended Dietary Allowances (National

Research Council 1989) supports the view of essentiality in humans.

Carbohydrate Metabolism

Trivalent chromium was identified as the active agent in glucose tolerance factor (GTF) by Schwarz and Mertz (1959). Although isolation of the compound has proved difficult, it is thought to contain nicotinic acid, glutamic acid, glycine, and a sulfur containing amino acid (Mertz et al. 1974, Mertz 1993, Stoecker 1990). This complex is released from storage in a body pool in response to insulin and it subsequently acts to potentiate the action of insulin in peripheral tissues. Chromium deficiency is thought to decrease the sensitivity of peripheral tissue to insulin; much higher levels of insulin are required to maintain desirable serum glucose levels at suboptimal levels of chromium. In vitro, glucose catabolism to carbon dioxide was shown to respond similarly when insulin levels were increased as when insulin was held constant and biologically active chromium was added (Anderson 1984).

Chromium increases the effectiveness of the insulin but is not a substitute for insulin. Chromium functions in the presence of insulin; no effects of chromium supplementation occur in the absence of insulin (Mertz 1993). Mertz showed a 67% increase in glucose uptake by epididymal fat tissue in rats fed a GTF-deficient diet supplemented with chromium (III) as compared to tissue from unsupplemented controls.

The effect of the chromium was found to be dependent on the presence of small amounts of insulin (Mertz 1976). In a review of chromium research spanning thirty-four years in humans as well as in animals and in vitro, Mertz (1993) concludes that chromium deficiency results in insulin resistance which can be improved with chromium supplementation.

Hubner et al. (1989) studied the effects of chromium supplementation on serum insulin levels in pregnant rats and their offspring. Pregnant Wistar rats were supplemented for fifty days with 1 mg $CrCl_3.6H_20$ by stomach tube beginning on day one of gestation; control rats received the same diet but unsupplemented water by stomach tube. No effects of chromium supplementation were noted on glucose tolerance but significant reductions in serum insulin were observed 30 minutes after a glucose load. Among newborn pups from the supplemented mother group, basal concentrations of insulin were significantly decreased in comparison to controls. The lack of effect on glucose tolerance was anticipated as the animals were not fed a chromium-deficient diet. The authors suggested that the reduced level of circulating insulin was due to an increased efficiency of utilization of available insulin in response to chromium supplementation. They further suggested that the amount of insulin produced by the whole pancreas was reduced due to a lower demand for insulin among the chromium treated rats. Evans et al. (1973) found a significantly greater uptake of glucose in epididymal

tissue of chromium-deficient rats using insulin reacted with GTF from brewers yeast than the effect on the same tissue using native insulin. Their results suggest possible binding sites for GTF with certain amino groups of the insulin molecule.

Other research has shown less conclusive results. Flatt et al. (1989) questioned the effect of trivalent chromium on glucose homeostasis. They found no difference in effects of chromium supplemented (1 mg Cr/kg diet) or chromium depletion diets (0.03 mg/kg) fed for 32 days to Wistar rats on food intake, body weight, glycosylated hemoglobin, plasma glucose or plasma insulin at 53 days of age. Tissue chromium likewise was not significantly different between the two groups except for the pancreas which showed a 44% decrease in chromium stores in the rats fed deficient diets as compared to chromium supplemented rats. The authors conceded that they could not rule out the possibility that saturation of the tissues could have occurred prior to the onset of the deficient diet but cited other studies of longer duration with similar effects to back up their conclusions. Donaldson et al. (1985) fed low chromium (0.06 - 0.1 mg Cr/kg diet) and chromium-supplemented (5 mg/kg), high sucrose, high cholesterol diets to male Sprague-Dawley rats from weanling to eighteen months. Rats in the low chromium group showed significantly higher one hour postprandial plasma glucose at four and eight months but the significance disappeared at twelve

months and did not reoccur at fourteen or sixteen months. Tissue chromium concentrations, with the exception of kidney tissue, did not differ significantly between the two groups.

Berggren and Flatt (1985a) examined tissue concentrations of chromium of genetically diabetic and lean mice fed a standard diet. No significant differences were observed. Further, they determined that urinary chromium was not an accurate indicator of tissue chromium status. While tissue chromium concentrations between the two groups were comparable, "defective handling at the cellular level leading to impaired metabolism of GTF and resultant glucose intolerance" could not be ruled out. In a follow-up study, Berggren and Flatt (1985b) reported that tissue chromium concentrations of obese hyperglycemic mice were not significantly different from lean mice but that injection of trivalent chromium intraperitoneally (2 mg/kg body weight) resulted in significantly higher tissue concentrations among the lean mice. The authors suggest that the diabetic state of the obese mouse is associated with rapid excretion and/or an inability to utilize the trivalent chromium rather than an actual deficiency. The positive effects of addition of GTF may indicate an inability of the obese mouse to convert trivalent chromium to active GTF.

Preston et al. (1976) failed to find a correlation between glucose tolerance, peak glucose concentrations and serum cholesterol between normal, pregnant and lactating guinea pigs fed chromium supplemented or depleted diets.

They did see an increase in mortality among the pregnant-depleted diet group and suggested that chromium may provide some level of protective effect against the stresses associated with mating and pregnancy in the guinea pig. Their diet was also noted to contain less protein than recommended by the National Research Council (17% vs. 25 -30%). The authors also suggested that results may be reflective of variations in chromium requirements between species noting that guinea pig insulin exhibits different properties than that of other rodents or the bovine.

Studies with human subjects have verified a relationship between chromium and carbohydrate metabolism. Morris et al. (1992) found a significant inverse relationship between postprandial plasma insulin and plasma chromium levels. The relationship did not occur between plasma chromium and plasma glucose. The decrease in plasma chromium could not be correlated with an increase in urinary chromium indicating a change in uptake or binding of chromium within insulin sensitive tissues.

The effect of a low chromium diet on glucose, insulin, glucagon and urinary chromium losses was examined by Anderson et al. (1991). Subjects were divided into hyperglycemic (> 5.56 mmol glucose/L, and < 11.1 mmol/L at 90 minute after a 1 g/kg body weight glucose challenge) and control groups. All individuals with diabetes or subjects with abnormal blood or urine profiles were excluded from the

study. Dietary intakes were controlled to maintain chromium in the lowest quartile of normal intake for adults. In this double blind study, subjects received either a supplement (200 ug Cr tablet ($CrCl_3$)) or a placebo. Hyperglycemic subjects receiving chromium supplementation showed significant improvement in glucose tolerance, circulating insulin and glucagon when compared to controls. The authors suggest that diets low in chromium similar to those consumed by 25% of the U.S. population can lead to detrimental effects on glucose tolerance, circulating insulin and glucagon in hyperglycemic persons. A study of elderly Canadian women (Martinez et al. 1985) verified the results that chromium supplementation among those subjects with moderate glucose intolerance resulted in a significant decrease in two hour glucose levels and an increase in insulin sensitivity within the same group. In a six month trial, subjects with hyperglycemia, insulin-dependent diabetes mellitus (IDDM), NIDDM, and controls were supplemented with 218 ug chromium as high potency yeast. The only statistically significant results occurred among the group with hyperglycemia for whom blood glucose control improved (Vinson & Bose 1984). Fasting blood glucose levels dropped significantly in some diabetic subjects given a chromium supplement (~600 ug) for 2-4 months (Mossop 1983). Glinsmann and Mertz (1966) found improved glucose tolerance in 50% of diabetics given trivalent chromium supplementation; normal glucose tolerance remained

unaffected. Offenbacher and Pi-Sunyer (1980) studied the effects of chromium on glucose tolerance among elderly subjects using brewers yeast and torula yeast. Results indicated that chromium rich brewers yeast improved glucose tolerance and insulin sensitivity with no changes seen in the control (torula yeast) group.

A follow-up study by Offenbacher et al. (1985) found no significant difference in glucose tolerance or insulin levels among groups supplemented with chromium chloride, brewers yeast or placebo. Uusiptupa et al. (1983) failed to find any correlation between supplemental chromium and glucose tolerance or serum insulin in NIDDM subjects. Abraham et al. (1992) found beneficial effects on blood glucose levels to be transitory, reverting to pretreatment status in longer periods of supplementation.

Rabinowitz et al. (1980) found an inverse correlation between plasma chromium and fasting plasma glucose. In a follow-up study, Rabinowitz et al. (1983) found a significant increase in postprandial insulin release in NIDDM subjects following supplementation of brewers yeast. A study by Riales and Albrink (1981) found that supplementation of trivalent chromium (200 ug in 5 ml of water) improved insulin sensitivity in subjects displaying insulin resistance but normal glucose tolerance.

A study was conducted by Anderson et al. (1987) to examine possible effects of chromium supplementation in patients with reactive hypoglycemia. Eight female patients

with previously diagnosed hypoglycemia were randomly assigned to either a supplemented group receiving 200 ug chromium as chromium chloride or a placebo in a double-blind crossover design. Each test period lasted twelve weeks with testing at six and twelve weeks during each period. Following a glucose load, blood samples were obtained at 30, 60, 90, 120, 180, 240, and 300 minutes; urine samples were obtained at 120 and 300 minutes. Significant results included a decrease in hypoglycemic area (defined as the area of the glucose tolerance curve which falls below fasting levels), an increase in insulin binding to red blood cells, an increase in number of insulin receptors and a reduction in hypoglycemic symptoms. Control subjects with normal glucose tolerance showed no change in glucose tolerance, receptor number, or receptor affinity but did show increases in serum chromium.

Lipid Metabolism

Evidence from animal and human studies indicates an alteration in lipid metabolism in the presence of chromium deficiency affecting the development of atherosclerosis and serum concentrations of cholesterol and triglycerides. Chromium deficiency has been linked to an increase in serum cholesterol and aortic plaque in animals. In a study reviewed by Anderson, chromium supplementation in a low-chromium diet (1 ug/ml or 5 ug/ml) decreased the serum cholesterol level in rats and reversed the tendency toward

increasing cholesterol levels with advancing age. In addition, chromium-supplemented animals had 17% less aortic plaque than the control animals at the end of their natural lives (Anderson 1987). Abraham et al. (1982) fed high-cholesterol diets to rabbits to induce atherosclerotic plaques. Subsequent daily administration of 20 ug of potassium chromate intraperitoneally led to a decrease in aortic cholesterol concentration and a reduction of aortic plaques.

However, Donaldson et al. (1985) found no significant differences between rats fed for twelve months either chromium-depleted (60 to 100 ug/kg) diet or chromium-supplemented (5000 ug/kg diet) for plasma glucose, total cholesterol, or triglycerides. A similar finding was reported by Preston et al. (1976) studying the effect of chromium supplemented diets (0.5 ppm or 50 ppm as $CrCl_3.6H_2O$) on non-pregnant, pregnant, and lactating guinea pigs. Their findings stated "....serum cholesterol appeared to be more affected by pregnancy and generation of guinea pigs than by the level of dietary chromium."

Human studies have shown improvements in serum lipid parameters when subjects were supplemented with trivalent chromium. Riales and Albrink (1981) showed a significant increase in HDL cholesterol levels among subjects consuming 200 ug chromium as $CrCl_3.6H_2O$ in 5 ml of water five days a week for twelve weeks. Triglyceride levels were also significantly reduced in the experimental group when

compared to the control at 12 weeks. Abraham et al. (1992) studied the effects of oral supplementation of 250 ug chromium as chromium chloride in 5 ml of water on patients with and without non-insulin dependent diabetes mellitus (NIDDM) for 7 to 16 months. Findings included a significant decrease in serum triglycerides and a significant increase in HDL cholesterol with no change in total cholesterol or glucose. The findings were not significantly different between diabetic and non-diabetic subjects. The extended length of study was designed to overcome any transitory changes due to supplementation. A Zimbabwean study of thirty-nine diabetic subjects supplemented with ~600 ug trivalent chromium in water for three months found a consistent increase in HDL cholesterol levels in the supplemented group and a decrease in HDL cholesterol among the control group (Mossop 1983). A study of 24 elderly subjects (including eight with NIDDM) found a 12% decrease in serum cholesterol among the experimental group fed 9 grams of brewers yeast daily for eight weeks. Among subjects with hypercholesterolemia (> 300 mg/dl), an 18% decrease in total cholesterol and an average 20% decrease in serum triglycerides were noted. Total lipids declined in all experimental subjects with the greatest decline among the group with hypercholesterolemia (Offenbacher & Pi-Sunyer 1980).

Uusitupa et al. (1983) found no significant differences in serum lipid parameters among volunteers with NIDDM

supplemented with 200 ug trivalent chromium daily for 6 weeks. These findings may help to substantiate the concept that chromium supplementation is only effective in chromium depleted subjects. In a double blind crossover study, Anderson and co-workers (1983) found no effect on any serum lipid levels for either sex when normoglycemic subjects were supplemented for three months with 200 ug chromium as chromic chloride.

Tissue Chromium Concentration

Chromium concentrations of tissues in normal and genetically diabetic (db/db) mice were compared by Berggren and Flatt (1985a). Tissue chromium concentrations in kidney, muscle and exocrine pancreas were not significantly different than that of the endocrine pancreas in diabetic mice. In a subsequent study, Berggren and Flatt (1985b) found an increase in non-pancreatic tissue chromium concentrations in lean mice 96 hours after intraperitoneal injection of 2 mg trivalent chromium/kg body weight. This administration of exogenous chromium failed to affect tissue accumulations in the obese mouse indicating either a rapid chromium excretion or an inability to utilize chromium (III) rather than a deficiency per se.

Tissue concentrations of rats fed chromium (III) deficient (0.03 mg Cr/kg) diet for three weeks were not different from controls fed 1 mg Cr/kg diet with the exception of significantly lower (44%) pancreatic chromium.

Possible explanations for these results included tissue saturation prior to the onset of the deficient diet, an increase in absorption among those animals fed the chromium deficient diet, or variation among species between mice and rats (Flatt et al. 1989).

Donaldson et al. (1985) compared the effects of high sucrose, high cholesterol, low chromium diets to similar chromium supplemented diets. Sprague-Dawley rats fed the low-chromium diets for eighteen months had significantly lower kidney chromium concentrations than those fed the chromium-supplemented diet. Seaborn and Stoecker (1989) studied the effect of various sources of carbohydrate on chromium absorption and tissue concentration. Obese mice had higher chromium concentration in testes, bone and spleen when compared to lean mice. Kidney concentration was not affected by obesity. Lean mice showed greater effects of diet on liver chromium concentration than did the obese model. Animals fed starch showed greater uptake of ^{51}Cr from $^{51}CrCl_3$ and generally greater tissue chromium than did animals fed sucrose, glucose or fructose, indicating that dietary carbohydrate affects chromium absorption and retention.

Variations Among Studies

Results of both animal and human studies on chromium have been equivocal. Variations in methods, initial glycemic or lipid status of subjects, animal model

selection, length of study, dietary intake, source of chromium, amount of supplementation, and sources of contamination may affect the outcome of the research and may help to explain the inconsistent results among similar experiments.

Manganese

Essentiality

Manganese has been found to be essential in all animal species studied; deficiency has been documented in mice, rats, guinea pigs, poultry, pigs, and cattle (Hurley & Keen 1987). Symptoms of deficiency shared by the offspring of all species include impaired growth, skeletal malformations, decreased reproductive function, neonatal ataxia, and defective lipid and carbohydrate metabolism. Severity of symptoms vary with degree and duration of the deficiency and with the developmental period coinciding with the deficiency (Hurley & Keen 1987).

Manganese deficiency in humans has not been clearly established; the widespread availability of manganese in the food supply in relation to need and the tendency of the body toward homeostatic control of manganese concentrations in the tissues may be partial explanations. Manganese is known to function as an activator for a variety of metalloenzymes including hydroxylases, kinases, decarboxylases, and transferases. Activation of these enzyme systems in vitro

may not be specific to manganese. Manganese may be replaced by a number of other divalent cations, primarily magnesium, thereby reducing or eliminating potential deficiency signs stemming from these systems.

Carbohydrate Metabolism

Defects in carbohydrate metabolism are common among the offspring of manganese-deficient animals. The varied role of manganese in carbohydrate metabolism has provided several possible explanations for alterations observed in the manganese-deficient animal. Suggested mechanisms include the impairment of insulin synthesis and secretion, a decrease in insulin sensitivity, destruction of pancreatic beta cells, and a decrease in gluconeogenesis (Keen & Zidenberg-Cherr 1990).

Everson and Shrader (1968) studied the effects of manganese deficiency in guinea pigs. Fasting glucose of deficient animals averaged 40 mg/dl higher than controls. Results of glucose tolerance tests showed elevated blood glucose among deficient animals. Blood glucose remained elevated throughout the test period and in some cases exceeded fasting values by more than 50 mg/dl after four hours. Control animals exhibited more normal tolerance curves with blood glucose returning to fasting levels within two to three hours. Subsequent supplementation of the deficient animals with adequate dietary intakes of manganese reversed the impaired glucose utilization.

Based on Everson and Shrader's work, Baly et al. (1984) hypothesized that "manganese deficiency might result in altered insulin secretion." Reduced insulin synthesis, secretion or sensitivity were cited as possible mechanisms of manganese action. Male and female weanling Sprague-Dawley rats were fed diets containing either 45 ug Mn/g (control) or 1 ug Mn/g beginning at 21 days of age. At three months, the females were mated with stock fed males; males were used for comparative effects with the 2nd generation offspring in carbohydrate studies. Both first and second generation of manganese-deficient rats showed lower fasting blood insulin levels than controls. In second generation rats, increasing blood glucose levels failed to stimulate adequate insulin secretion. No differences were noted between first generation manganese-deficient rats and controls. The authors concluded that the lack of effect of manganese-deficiency on the first generation rats suggests that the abnormal glucose tolerance is developmental and that deficiency must "be present while the animal is in utero or during its suckling period, or both." They attribute the deficiency effects to abnormalities in pancreatic development, beginning around the twelfth day of embryonic development. Other potential causes cited included damage or destruction of pancreatic beta cells by free radicals due to reduced manganese superoxide dismutase (MnSOD) activity in manganese deficiency. Initial release of preformed insulin was only 76% that of controls in second

generation rats. Secondary release, including preformed and newly synthesized insulin, was also significantly lower in the offspring of deficient rats. The degree of inhibition in insulin secretion increased as glucose stimulation was extended, suggesting an impaired biosynthetic mechanism.

Manganese functions as part of the metalloenzymes pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK), both key enzymes in gluconeogenesis. Blood glucose levels of the neonatal rat are dependent upon gluconeogenesis during the first days postpartum due to the low carbohydrate, high fat concentrations of rat milk. Baly et al. (1985) investigated the relationship between the activity of PEPCK, PC and blood glucose levels in the neonates of manganese-deficient rats and assessed pancreatic function in the adult offspring of manganese-deficient rats. Results of the studies included the following: impaired gluconeogenesis in the neonatal period, possibly related to decreased PEPCK activity; decreased glucose tolerance; low plasma insulin levels; and depressed insulin output and synthesis.

In a follow-up study (Baly et al. 1985b), the effect of glucose on insulin biosynthesis and secretion in the presence of manganese deficiency was investigated. A glucose concentration of 300 mg/dl stimulated the production of 19.4 ug insulin per gram of pancreas compared to 3.4 ug/g produced by the pancreas of manganese-deficient rats. In addition, no degradation of insulin occurred in the control

animals but 7.8 ug of insulin per gram of pancreas was degraded by the deficient pancreata after 80 minutes. These results were significant in that they were the first to demonstrate a role for manganese in insulin biosynthesis and degradation.

A study by Baly et al. (1990) examined insulin binding and glucose transport in adipose tissue of manganese-deficient and control rats. Both basal and insulin stimulated glucose uptake were found to be impaired in the manganese-deficient rats. A lower maximal transport velocity in these animals suggests a reduction in the number of transporters available compared to control rats. Also noted was a decrease in the number of insulin receptors in the manganese-deficient rats suggesting a loss of insulin sensitivity in the peripheral tissues.

Lipid Metabolism

A variety of defects in lipid metabolism related to manganese deficiency have been documented. Fahim et al. (1990) studied the effects of diets varying in manganese and magnesium content on total cholesterol levels in Swiss albino mice. Normal (control) diets contained 335 mg magnesium and 53 mg manganese/kg and deficient diets contained 110 mg magnesium and 1 mg manganese/kg. Mice were fed the experimental diets for five weeks. Only the animals fed the diet deficient in both magnesium and manganese showed a significant increase in serum cholesterol

($p < 0.001$) when compared to controls. Groups deficient in only manganese or only magnesium showed no significant difference in total cholesterol. The authors also found that the combined effects of dietary manganese and magnesium deficiency caused a 46% increase in liver lipid content when compared to controls.

Baly et al. (1990) studied the effect of manganese deficiency on the conversion of glucose to triglycerides in the adipocytes of offspring of Sprague-Dawley rats. No difference between basal rate of conversion of glucose to triglyceride was observed between manganese-deficient and control animals. However, insulin stimulated conversion was significantly lower in the deficient animal averaging 26% less than the conversion rate of controls.

Research on the relationship between manganese and lipid metabolism has been reviewed by Hurley and Keen (1987). One study showed that a manganese-deficient diet may produce excessive fat deposition in mice. Addition of manganese to rat liver in vitro resulted in a pronounced increase in cholesterol synthesis. Another study cited found that male weanling Sprague-Dawley manganese-deficient rats displayed lower levels of cholesterol in plasma and liver than control animals. Similar findings were reported where a manganese-deficient diet in human adult men was related to an increased incidence of hypocholestrolemia.

Tissue Concentration

Tissue concentrations of manganese are uniformly distributed throughout the body with little variation between species. Keen et al. (1985) studied the effect of intraperitoneal injections of manganese on tissue mineral concentrations. After two weeks on standard laboratory diet, Sprague-Dawley rats were injected with 2.5 - 4.0 mg $MnCl_2$/kg body weight and sacrificed at various times post-injection. Tissues analyzed included liver, kidney, brain, and pancreas. All tissues were found to have a significant increase in manganese concentration; the increase was dose dependent and maximal levels were found to occur between 30-60 minutes post injection. Davis et al. (1992) analyzed tissue concentrations in rats fed ^{54}Mn and in rats injected intraportally with ^{54}Mn after being fed varying levels of manganese and iron for seven weeks. Animals fed ^{54}Mn retained significantly less of the isotope after 4 days than those receiving ^{54}Mn intraportally regardless of the dietary manganese and iron levels. Manganese-deficient animals retained more of the isotope regardless of whether it was fed or injected. Dietary manganese significantly affected tissue distribution of the isotope with an increased proportion appearing in the liver and a lower proportion appearing in pancreas, kidney, bone and muscle of deficient animals than in animals fed adequate or high levels of manganese. The authors concluded that the

mucosal cell was the likely site of control of manganese homeostasis.

Kennedy et al. (1986) found that the obesity of the C57BL/6J (ob /ob) mice is associated with decreased concentrations of manganese, zinc, copper, and iron in several tissues and these alterations are independent of the animals' sex. Tissue manganese concentrations were not significantly different between obese and control at five weeks but by twenty-two weeks, concentrations in liver, small intestine and bone were significantly reduced in the obese mice. Tissue manganese concentrations of first and second generation rats fed manganese-deficient diets were significantly lower in liver, kidney, heart, and pancreas than those of controls. No significant differences were seen between copper, zinc or liver iron concentrations of the groups (Baly et al. 1990).

Variations in the Obese (ob/ob) Model

The obese (ob/ob) mouse provides an interesting model for attempting to understand similar interactions that occur in human diabetes. These mice display characteristics similar to those found in the human experience. Characteristics of the obese mouse include hyperinsulinemia, hyperphagia, and hyperplasia and hypertrophy of pancreatic beta cells. Secondary characteristics include hyperglycemia, obesity and severe diabetes (Coleman 1982).

A longitudinal hormonal profile of the ob/ob mouse model by Garthwaite et al. (1980) concluded that hyperinsulinemia and hyperadrenocorticism were characteristic throughout the life span while plasma glucose levels were elevated at 5-20 weeks and again at 62 weeks of age. Insulin resistance was also found to improve after 20 weeks of age.

The obese mouse is further characterized by an increase in both size and number of adipocytes. There is an increase in the activity of insulin-dependent enzymes and the rate of lipogenesis in the liver and adipose tissue is more than twice that of lean models. It has been suggested that the progressive hyperglycemia with an inverse number of active insulin receptors may protect the obese model from potentially fatal hypoglycemia should circulating insulin levels be fully effective. The loss of insulin receptors is thought to be associated with the increase in gluconeogenesis which contributes to the hyperglycemic state of the obese mouse model (Coleman 1982).

Studies have shown that obese mice may consume as much as 75% more food per day than lean controls when food is available ad libitum (Kennedy et al. 1986). Despite the dramatic increase in intake, tissue levels of some trace elements remain below the level of the control animals indicating "alterations in the tissue distribution and metabolism of endogenous micronutrients" (Kennedy et al. 1986).

CHAPTER III

CHROMIUM AND MANGANESE: DEPLETION EFFECTS IN GENETICALLY OBESE (ob/ob) MICE

ABSTRACT

Forty male weanling genetically obese mice (C57BL/6J-OB) (ob/ob) and their lean littermates were assigned to one of four experimental diets. Groups included low chromium - low manganese (-Cr/-Mn), low chromium - adequate manganese (-Cr/+Mn), adequate chromium - low manganese (+Cr/-Mn), or adequate chromium - adequate manganese (+Cr/+Mn).

Animals were maintained on the diets for a period of thirty-five days. Body weight and weight gain were increased by obesity with weight gain also showing significant effects due to manganese and an interaction between obesity and chromium. Plasma glucose, insulin, corticosterone, cholesterol, and glycosylated hemoglobin were all elevated in obese animals compared to lean. Chromium supplementation resulted in significantly higher serum cholesterol levels. Glycosylated hemoglobin was significantly higher among +Mn animals. Liver concentrations of manganese, iron, zinc, calcium, and

magnesium were significantly lower in obese mice than in controls. Manganese supplementation resulted in higher liver manganese concentrations ($p < 0.001$) in lean and obese groups. Kidney manganese and zinc were significantly lower in the obese animals; kidney copper increased ($p < 0.0005$) in the obese animals. Animals supplemented with manganese had significantly higher concentrations of kidney manganese and lower concentrations of zinc and calcium. Overall, no significant effects of dietary chromium and/or manganese were observed on selected parameters of carbohydrate or lipid metabolism in the obese mouse. Further research is warranted.

Introduction

Abnormalities in carbohydrate and lipid metabolism are characteristic complications of non-insulin dependent diabetes mellitus (NIDDM). Hyperglycemia, hyperinsulinemia, and hypercholesterolemia are among the contributing factors to long term complications of the disease. Trace minerals are known to participate in the metabolism of both carbohydrate and lipid and studies have shown that deficiencies of certain trace minerals may induce symptoms similar to those of NIDDM (Mertz 1993).

The essentiality of chromium in animals was established in the 1950's when chromium was identified as the active component in glucose tolerance factor (Schwarz & Mertz 1959). An Estimated Safe and Adequate Daily Dietary Intake

(ESADDI) for chromium was added to the Recommended Dietary Allowances in 1980 (National Research Council 1989). A recent review examined fifteen studies evaluating the effects of chromium supplementation. Conclusions of the review suggested that chromium deficiency results in insulin resistance which can be improved with chromium supplementation and that such deficiencies do occur in the United States and elsewhere and may be an important cause of insulin resistance in those populations (Mertz 1993).

Manganese has been found to be essential in all animal species studied. Defects in carbohydrate and lipid metabolism are among the complications common to the offspring of manganese-deficient animals (Hurley & Keen 1987). The varied role of manganese in carbohydrate metabolism has provided several possible explanations for the alterations seen in the manganese-deficient animal. Suggested mechanisms include the impairment of insulin synthesis and secretion, a decrease in insulin sensitivity, destruction of pancreatic beta cells, and a decrease in gluconeogenesis (Keen & Zidenberg-Cherr 1990). Manganese is known to function as an activator for a variety of metalloenzymes including hydroxylases, kinases, decarboxylases, and transferases (Hurley & Keen 1987). Activation in vitro is not specific to manganese. Manganese may be replaced by a number of other divalent cations, primarily magnesium, thereby reducing or eliminating

potential deficiency signs stemming from these systems (Hurley & Keen 1987).

Previous research into the relationship between trace minerals and altered metabolism of carbohydrate and lipid in diabetes has not included studies of the combined effects of chromium and manganese depletion. Prior research in this laboratory involved the effects of low chromium and low manganese diets on rats with streptozotocin (STZ) induced diabetes. Four weeks post injection, only those animals fed adequate amounts of both chromium and manganese displayed normal fasting glucose levels. In order to eliminate the possible effects of trace minerals on the efficacy of STZ, this study used the genetically obese mouse. This genetically obese model C57BL/6J (ob/ob) is characterized by obesity, hyperglycemia, hyperinsulinemia, and hyperadrenocorticism (Coleman 1982, Garthwaite 1980) making it a good model to examine the effects of chromium and manganese depletion.

Materials and Methods

Forty weanling male genetically obese (ob/ob) mice (C57BL/6J-OB) and their lean littermates were received and held for a three-week adaptation period on the American Institute of Nutrition (AIN) diet for mice and rats with the AIN mineral mix modified in our laboratory to have no added chromium and to be low in manganese. The diet contained 50% dextrose, 20% casein, 15% cornstarch, 5% celufil, 5% corn

oil, 3.5% modified AIN mineral mix, 1.0% AIN vitamin mix, 0.3% DL-methionine, and 0.2% choline bitartrate. Diet and deionized water were provided ad libitum. Animals were housed in plastic cages and fed from ceramic cups.

After the adaptation period, animals were assigned to one of four diet groups for six weeks. Groups included low chromium - low manganese (-Cr/-Mn), low chromium - adequate manganese (-Cr/+Mn), adequate chromium - low manganese (+Cr/-Mn), or adequate chromium - adequate manganese (+Cr/+Mn). Low chromium diets were analyzed by atomic absorption spectrophotometer and found to contain < 20 ug Cr/kg diet chromium. Basal diet with adequate chromium added contained 2 mg Cr/kg diet as $CrCl_3.6H_20$; low and adequate manganese diets were supplemented with 3.0 or 55 mg Mn/kg diet as $MnCO_3$ respectively.

Prior to autopsy, animals were food-deprived for ten hours and then weighed. The mice were anesthetized with Metofane (methoxyflurane) and were exsanguinated by cardiac puncture using heparinized syringes; samples were chilled and centrifuged to obtain plasma samples. Plasma was frozen for subsequent analyses. Liver, kidney, spleen, heart, testes, lung, epididymal fat pad and thymus tissues were removed carefully to avoid chromium contamination, wrapped and frozen for mineral analysis.

Insulin was assayed using Equate RIA Insulin Double Antibody kits (Binax, Inc. South Portland, ME) (Crowley & Garbien 1974). Glucose was analyzed using the glucose

oxidase method (Procedure No. 510, Sigma Chemical, St. Louis, MO) as described by Kadish et al. (1967). Corticosterone was determined by radioimmunoassay using Coat-A-Count Rat Corticosterone kit (Diagnostic Products Corp., Los Angeles, CA). Glycohemoglobin was determined using the Isolab GlycAffin columns. Cholesterol was analyzed by the enzymatic method described by Allain et al. (1974).

Tissues were analyzed using the method described by Hill et al. (1986). After ashing, samples were diluted with 0.5% nitric acid and analyzed at 357.9 nm using a Perkin-Elmer Model 5000 atomic absorption spectrophotometer with a graphite furnace and Zeeman background correction.

The Statistical Analysis System (SAS) was used to evaluate treatment effects. The generalized linear model (GLM) was used for analysis of variance and least squares means determination. Data were analyzed using a split-plot design with a 2 X 2 factorial subplot. Obesity was the main effect and dietary chromium and manganese levels were subplot factors.

Results

Body weight was significantly greater in obese mice when compared to lean (Table 1). Mean body weight for the obese group was 44.3 g compared to a mean of 29.8 g among lean mice. Mean weight gain among obese mice was 15.6 g; mean weight gain among lean mice was 7.2 g. Mice fed a -Mn

diet showed a greater weight gain than did those fed the +Mn diet. A significant interaction between the obese gene and dietary chromium was observed in mean weight gain. Chromium supplementation resulted in lower total weight gain among obese mice. Similar results were not observed among lean animals.

Obesity significantly increased serum levels of glucose, glycosylated hemoglobin, corticosterone, insulin, and cholesterol (Table 2). Manganese significantly affected glycosylated hemoglobin with the higher levels occurring among +Mn animals. Cholesterol levels were higher ($p < 0.05$) in the +Cr group.

Liver and kidney tissues were analyzed for mineral concentrations. All liver mineral concentrations analyzed except chromium were significantly reduced among obese animals (Table 3). Liver chromium concentrations tended to be lower in obese animals ($p < 0.07$). Significant interactions were observed between the obesity gene and dietary manganese. Low manganese diets resulted in significantly lower concentrations of liver manganese in both obese and lean mice. Liver iron concentrations reached near significance ($p < 0.07$) due to manganese with the higher concentrations of iron occurring in the -Mn group. No other significant effects of dietary treatments or obesity were observed.

Chromium, iron and magnesium concentrations were not significantly affected by obesity or dietary treatments in

kidney (Table 4). Kidney manganese was significant for both obesity and manganese, the higher concentrations occurring among lean and +Mn respectively. Kidney zinc concentrations were significant with respect to obesity and manganese, zinc levels were higher among lean and manganese-deprived animals. Copper levels were significantly higher among obese mice. Calcium concentrations were lower among +Mn animals but showed no significance due to obesity.

Discussion

Final body weights for lean and obese animals were 29.8g and 44.3g respectively. No significant effects of dietary chromium and manganese on body weight were observed. Similar findings by Baly et al. (1990) showed no significant difference in body weight between manganese-deficient and control groups of Sprague-Dawley rats.

Manganese-supplemented animals showed a lower total weight gain than -Mn animals. In contrast, Fahim et al. (1990) showed a 29% reduction in weight gain among manganese-deficient Swiss albino mice when compared to controls. Diets containing normal (0.053 g Mn/kg) dietary amounts of manganese showed no significant effect on weight gain.

Chromium supplementation resulted in a significant ($p < 0.03$) reduction in weight gained in the obese group compared to control. No differences in weight gain were observed by Flatt et al. (1989) in Wistar rats fed chromium

deficient (0.03 mg/kg) or chromium supplemented (1 mg/kg) diets.

Plasma cholesterol levels were higher ($p < 0.0001$) in obese mice. Chromium supplementation resulted in higher ($p < 0.05$) plasma cholesterol levels. These findings contrast with findings of elevated cholesterol with increasing age among rats fed low chromium diets (Anderson 1987). Abraham et al. (1992) showed reduced aortic plaque in rabbits given trivalent chromium by intraperitoneal injection. Other studies using rats and guinea pigs (Evans et al. 1973, Preston et al. 1976) have shown no relationship between chromium intakes and serum cholesterol.

No effect was found of dietary manganese on plasma cholesterol. These results were similar to the findings of Fahim et al. (1990). Swiss albino mice showed no effect in serum cholesterol levels when manganese levels in the diet were decreased to 0.001 gram Mn/kg diet for five weeks. In another study reviewed by Hurley and Keen (1987), a decrease in serum cholesterol occurred among male Sprague-Dawley rats fed a diet deficient in manganese.

Serum glucose and glycosylated hemoglobin concentrations were significantly elevated among the obese mice compared to lean. Elevated levels of glucose are consistent with known characteristics of the ob/ob mouse model (Coleman 1982, Garthwaite et al. 1980). Glucose levels were not affected by chromium or manganese. Glycosylated hemoglobin was not affected by chromium;

however a significant ($p < 0.02$) effect due to manganese was observed with +Mn animals showing higher concentrations. In contrast are findings of Everson and Shrader (1968) in which higher fasting glucose levels were found among guinea pigs fed a low manganese diet which reverted to normal levels by supplementation with adequate dietary manganese. Research has shown defects in carbohydrate metabolism to occur predominantly in second generation manganese-deficient animals.

Serum insulin was higher ($p < 0.0001$) in obese mice. This finding is consistent with other studies of the obese mouse model (Coleman 1982, Garthwaite et al. 1980). In our study, manganese had no effect on serum insulin concentrations. In contrast, Baly found lower circulating insulin levels among both first and second generation manganese-deficient rats when compared to controls. The second generation rats were unable to secrete adequate insulin in response to increasing blood glucose levels, a response not seen in the first generation test animals (Baly et al. 1984).

Our results concur with those of Flatt et al. (1989) who found no relationship between dietary chromium intakes and body weight, glycosylated hemoglobin, plasma glucose or plasma insulin in Wistar rats fed a low chromium (0.03 mg/kg) diet for 32 days post-weanling when compared to controls.

Corticosterone levels were higher ($p < 0.0001$) among the obese mice. These findings are consistent with other research involving the C57BL/6J ob/ob mouse model. Elevated levels of adrenocorticotropic hormone (ACTH) in young obese mice were reported by Garthwaithe et al. (1980). This early onset elevation of ACTH correlates with the elevated insulin and glucose levels also seen in the obese model. Corticosterone levels were not affected by the variation of dietary manganese and chromium in our study.

All liver mineral concentrations except chromium ($p < 0.07$) were significant with respect to obesity, lower concentrations occurring among the obese animals. This may be a result of increased excretion, failure to store or both. Similar results were found by Kennedy et al. (1986) where lower concentrations of zinc, copper, iron and manganese were found in the liver of the obese mouse when compared to the controls at 22 weeks of age. No significant differences were noted in tissue concentrations between the two groups at five weeks indicating a progression of tissue depletion with age among obese animals.

Liver chromium concentrations were not affected by dietary chromium or manganese. Liver chromium stores tended to be lower ($p < 0.07$) in obese animals. Berggren and Flatt (1985a) found no significant difference between liver chromium concentrations of genetically diabetic-obese mice and their lean littermates. Similar findings in a follow-up study using ob/ob mice found no difference in chromium

levels of the liver or kidney between obese and lean mice (Berggren & Flatt 1985b).

Liver iron concentrations tended to be lower ($p < 0.07$) with respect to manganese, lower tissue stores occurring in the +Mn group. Interactions between iron and manganese have been investigated in several species. Results indicate that the two minerals compete for binding sites in the intestinal mucosa affecting absorption rates of both minerals (Hurley & Keen 1987). Davis et al. (1992) showed that high intakes of iron significantly decreased liver manganese concentrations by inhibiting the uptake of manganese by mucosal cells and thereby affecting total absorption.

Liver manganese levels were lower in the obese -Mn group of animals ($p < 0.0001$). Liver manganese stores for obese -Mn mice were 29% of the lean -Mn group and 14% of the lean +Mn group. Liver manganese levels in lean -Mn mice were not different when compared to obese +Mn. Dietary manganese levels had a significant effect on liver manganese concentrations with +Mn animals showing significantly higher concentrations than the -Mn group. Similar results were found by Davis et al. (1992) who found an increase in liver manganese concentrations as dietary manganese levels were adjusted from deficient to adequate to high intake (0.9, 48 or 188 ug Mn/g diet respectively) in Sprague-Dawley rats. Liver manganese concentrations were not significantly affected by chromium.

Kidney mineral concentrations were not as consistent as the liver with respect to obesity; manganese, zinc and copper showed the only significant effects. Kidney manganese and zinc levels were lower in obese than control mice; kidney copper concentrations were greater in obese mice. Chromium, iron, calcium and magnesium levels were not significantly different between obese and lean mice.

Kidney manganese and zinc levels were also significant with respect to manganese supplementation. Kidney manganese levels were affected more by dietary manganese (49.23 vs 93.04 ugmol/g, $p < 0.0001$) than obesity (74.93 vs. 67.32 ugmol/g, $p < 0.03$). Animals fed the +Mn diet showed significantly higher manganese levels in the kidney in both lean and obese animals although the obese animals had significantly lower concentrations than the lean mice. Kennedy et al. (1986) found no difference in kidney manganese levels at 5 and 22 weeks between lean and obese mice fed 61 ug Mn/g dry weight diet.

Kidney zinc levels were significantly reduced in the obese group when compared to lean (1.05 vs 1.001 ugmol/g, $p < 0.02$). Animals fed the +Mn diet had significantly lower levels of zinc in the kidney when compared to the -Mn group (1.04 vs 1.00 ugmol/g, $p < 0.02$). Kidney zinc levels were not affected by chromium.

Liver, spleen, and epididymal fat pad tissue weights were significantly increased due to obesity and heart was

decreased (Table 6). No other significant effects of dietary treatment or obesity were seen on tissue weights.

In conclusion, feeding diets low in chromium and manganese to genetically obese mice and lean controls had little effect on the parameters of carbohydrate and lipid metabolism measured. Several other researchers (Anderson 1987, Baly et al. 1984, Everson and Shrader 1968, Fahim et al. 1990, Flatt et al. 1989) using different protocols have shown effects of manganese or chromium on carbohydrate and lipid metabolism. Differences may be the result of wide variability in this study, inadequate trace mineral depletion, or failure to assay the more sensitive parameters. Further investigation might include additional testing of plasma glucose, insulin, cholesterol and glycosylated hemoglobin prior to and during initiation of the feeding regimen. In addition, a glucose challenge prior to sacrifice and examination of pancreatic physiology might yield significant findings.

TABLE #1

Final body weight and weight gain of obese and lean mice fed diets with low and adequate chromium and manganese[1]

	Final Body Weight	Weight Gain
	Grams	
Main Plot Means		
OB	44.3 ± 0.5	15.6 ± 0.3
Lean	29.8 ± 0.5	7.2 ± 0.3
Subplot Means		
-Cr	37.1 ± 0.4	11.56 ± 0.3
+Cr	37.0 ± 0.5	11.17 ± 0.4
-Mn	37.5 ± 0.4	11.85 ± 0.3
+Mn	36.6 ± 0.5	10.87 ± 0.4
Source of variation	P-values	
Gene	< 0.0001	< 0.0001
Cr	0.74	0.43
Mn	0.25	< 0.05
Gene x Cr	0.53	< 0.03
Gene x Mn	0.13	0.77
Cr x Mn	0.12	0.75
Gene x Cr x Mn	0.21	0.11

[1]Values are least squares means ± SEM (Gene, N = 39-40, Cr, N = 39-40, Mn, N = 39-40)

TABLE #2

Plasma glucose, glycosylated hemoglobin (GHB), corticosterone, insulin and cholesterol of obese and lean mice fed diets with low and adequate chromium and manganese.[1]

	Glucose mmol/L	GHB %	Corticosterone nmol/L	Insulin pmol/L	Cholesterol mmol/L
Main Plot Means					
OB	11.14 ± 0.45	6.16 ± 0.24	409.91 ± 35.63	565.17 ± 46.91	6.27 ± 0.16
Lean	8.30 ± 0.48	4.08 ± 0.24	167.26 ± 39.00	92.63 ± 51.05	4.03 ± 0.20
Subplot means					
-Cr	10.10 ± 0.50	5.27 ± 0.24	321.66 ± 255.51	312.24 ± 48.60	5.00 ± 0.17
+Cr	9.32 ± 0.47	4.96 ± 0.24	255.51 ± 37.60	345.55 ± 49.41	5.40 ± 0.18
-Mn	9.78 ± 0.45	4.70 ± 0.28	246.50 ± 35.89	324.03 ± 48.00	5.29 ± 0.17
+Mn	9.64 ± 0.47	5.54 ± 0.24	330.67 ± 38.88	333.76 ± 50.83	5.02 ± 0.18
Source of variation			P - Values		
Gene	< 0.0003	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cr	0.24	0.36	0.22	0.63	< 0.05
Mn	0.083	< 0.01	0.12	0.89	0.30
Gene x Cr	0.65	0.93	0.93	0.50	0.18
Gene x Mn	0.61	0.39	0.81	0.80	0.76
Cr x Mn	0.42	0.16	0.78	0.90	0.28
Gene x Cr x Mn	0.77	0.88	0.73	0.98	0.48

[1]Values are least squares means ± SEM (Gene, N = 39-40, Cr, N = 39-40, Mn, N = 39-40)

TABLE #3

Liver mineral concentration of obese and lean mice fed diets with low and adequate chromium and manganese[1]

	Cr umol/g	Mn umol/g	Fe umol/g	Zn umol/g	Ca mmol/g	Mg mmol/g	Cu umol/g
Main plot means							
OB	0.84 ± 0.50	16.48 ± 1.10	2.23 ± 0.11	0.60 ± 0.02	1.63 ± 0.13	8.40 ± 0.45	0.12 ± 0.00
Lean	2.18 ± 0.51	42.21 ± 1.12	4.13 ± 0.11	1.20 ± 0.02	3.02 ± 0.13	13.33 ± 0.46	0.27 ± 0.00
Subplot means							
-Cr	1.76 ± 0.50	28.90 ± 1.08	3.23 ± 0.11	0.88 ± 0.02	2.38 ± 0.12	10.89 ± 0.44	0.18 ± 0.00
+Cr	1.30 ± 0.52	29.80 ±1.14	3.13 ± 0.12	0.91 ± 0.02	2.27 ± 0.13	10.81 ± 0.46	0.20 ± 0.00
-Mn	1.10 ± 0.51	17.89 ± 1.10	3.33 ± 0.11	0.90 ± 0.02	2.18 ± 0.13	11.20 ± 0.45	0.19 ± 0.00
+Mn	2.00 ± 0.50	40.80 ± 1.12	3.03 ± 0.11	0.89 ± 0.02	2.46 ± 0.13	10.50 ± 0.46	0.19 ± 0.00
Source of Variation				P - Values			
Gene	0.07	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cr	0.50	0.56	0.55	0.25	0.57	0.90	0.15
Mn	0.24	< 0.0001	0.07	0.58	0.13	0.30	0.70
Gene x Cr	0.14	0.58	0.35	0.33	0.46	0.72	0.88
Gene x Mn	0.86	< 0.0006	0.60	0.82	0.48	0.54	0.85
Cr x Mn	0.83	0.63	0.88	0.84	0.92	0.43	0.96
Gene x Cr x Mn	0.91	0.23	0.58	0.84	0.92	0.41	0.98

[1]Values are least squares means ± SEM (Gene, N = 39-40, Cr, N = 39-40, Mn, N = 39-40)

TABLE #4

Kidney mineral concentration of obese and lean mice fed diets with low and adequate chromium and manganese[1]

	Cr umol/g	Mn umol/g	Fe umol/g	Zn umol/g	Ca mmol/g	Mg mmol/g	Cu umol/g
Main Plot Means							
OB	13.14 ± 1.76	67.32 ± 2.11	5.14 ± 0.20	1.01 ± 0.01	5.68 ± 0.16	16.46 ± 0.42	0.26 ± 0.00
Lean	12.36 ± 1.80	74.93 ± 2.14	5.10 ± 0.20	1.05 ± 0.01	5.44 ± 0.16	15.48 ± 0.42	0.24 ± 0.00
Subplot Means							
-Cr	12.01 ± 1.73	70.00 ± 2.07	5.20 ± 0.20	1.01 ± 0.01	5.61 ± 0.20	15.65 ± 0.41	0.25 ± 0.00
+Cr	13.50 ± 1.82	72.25 ± 2.18	5.04 ± 0.20	1.03 ± 0.01	5.52 ± 0.20	16.28 ± 0.43	0.26 ± 0.00
-Mn	14.08 ± 1.76	49.23 ± 2.11	4.91 ± 0.20	1.04 ± 0.01	5.88 ± 0.16	15.85 ± 0.42	0.25 ± 0.00
+Mn	11.42 ± 1.80	93.04 ± 2.15	5.32 ± 0.20	1.00 ± 0.01	5.24 ± 0.20	16.08 ± 0.42	0.25 ± 0.00
Source of Variation				P - Values			
Gene	0.76	< 0.03	0.87	< 0.02	0.25	0.11	< 0.0005
Cr	0.56	0.45	0.59	0.26	0.66	0.30	0.13
Mn	0.30	< 0.0001	0.15	< 0.02	< 0.01	0.71	0.85
Gene x Cr	0.18	0.77	0.70	0.68	0.42	0.36	0.30
Gene x Mn	0.96	0.67	0.87	0.47	0.60	0.31	0.90
Cr x Mn	0.33	0.88	0.22	0.25	0.32	0.43	0.30
Gene x Cr x Mn	0.26	0.48	0.76	0.66	0.53	0.64	0.96

[1]Values are least squares means ± SEM (Gene, N = 39-40, Cr, N = 39-40, Mn, N = 39-40)

TABLE #5

Tissue weights of obese and lean mice fed diets with low and adequate chromium and manganese[1]

	Liver	Kidney	Spleen	Heart	Fat[2]	Thymus
			grams			
Main Plot Means						
OB	2.63 ± 0.05	0.32 ± 0.01	53.2 ± 1.8	0.12 ± 0.00	2.93 ± 0.04	40.3 ± 2.1
Lean	1.04 ± 0.05	0.32 ± 0.01	64.1 ± 1.8	0.13 ± 0.00	0.94 ± 0.04	42.1 ± 2.1
Subplot Means						
-Cr	1.86 ± 0.04	0.33 ± 0.01	61.0 ± 1.7	0.13 ± 0.0	2.00 ± 0.03	43.2 ± 2.0
+Cr	1.81 ± 0.05	0.31 ± 0.01	56.2 ± 1.8	0.12 ± 0.0	1.90 ± 0.03	39.2 ± 2.1
-Mn	1.84 ± 0.04	0.32 ± 0.01	58.6 ± 1.7	0.12 ± 0.0	2.00 ± 0.04	40.5 ± 2.0
+Mn	1.83 ± 0.05	0.32 ± 0.01	58.7 ± 1.8	0.13 ± 0.0	1.90 ± 0.04	42.0 ± 2.1
Source of Variation						
Gene	< 0.0001	0.4252	< 0.0006	< 0.0084	< 0.0001	0.38
Cr	0.45	0.18	0.06	0.11	0.14	0.17
Mn	0.87	0.70	0.97	0.06	0.27	0.66
Gene x Cr	0.39	0.61	0.80	0.95	0.14	0.73
Gene x Mn	0.40	0.78	0.12	0.60	0.27	0.90
Cr x Mn	0.13	0.64	0.21	0.39	0.12	0.77
Gene x Cr x Mn	0.18	0.45	0.66	0.92	0.10	0.07

[1]Values are least square means ± SEM (Gene, N = 39-40, Cr, N = 39-40, Mn, N = 39-40)

[2]Epididymal fat pad

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CHAPTER IV

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

Forty male weanling genetically obese mice and forty lean control mice were randomly assigned to one of four experimental diets varying only in the amounts of chromium and manganese. After six weeks, animals were killed and selected tissues analyzed for parameters reflecting carbohydrate and lipid metabolism. Weight gain was significantly affected by manganese and an interaction between obesity and chromium. Chromium supplemented animals had higher ($p < 0.05$) serum cholesterol levels compared to non-supplemented groups. Manganese supplementation resulted in higher levels of glycosylated hemoglobin. Manganese supplementation also resulted in higher liver manganese concentrations ($p < 0.001$) in lean and obese groups as well as higher concentrations of kidney manganese and lower concentrations of kidney zinc and calcium. Obesity significantly elevated plasma glucose, insulin, glycosylated hemoglobin, corticosterone and cholesterol while lowering concentrations of all trace minerals in the liver tissue.

Conclusions and Recommendations

The objective of the study was to investigate the effects and interactions which may occur in animals fed diets lacking in adequate amounts of chromium and manganese. Chromium showed no effect on glucose, insulin or corticosterone levels. Lack of effect may be due to the large variability within the study making significance harder to achieve. Although chromium concentrations in the diet were low, mere depletion of chromium may not be sufficient to cause an effect without an additional stressor. The lack of effect of manganese on serum glucose and insulin may also be partially explained by wide variability within the study. In addition, most pronounced effects of manganese deficiency occur in the offspring of manganese-deficient animals rather than during the first generation. Both variables may have been adversely affected by the age of the animals at autopsy.

Future research in this area might benefit from additional testing of plasma parameters of glucose, insulin, glycosylated hemoglobin and cholesterol prior to and during initiation of the feeding regimen. In addition, a glucose challenge prior to autopsy may yield significant findings with respect to plasma glucose and insulin levels. Examination of pancreatic tissue post-autopsy, although difficult in the ob/ob model, might provide interesting comparisons of possible alterations in pancreatic physiology due to chromium and manganese depletion.

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APPENDIXES

APPENDIX A

Group means of weight gain and final body weight of obese and lean mice fed diets with low and adequate chromium and manganese[1, 2]

	Final weight	Weight gain
	grams	
Lean		
-Cr -Mn	30.2 ± 0.7	5.9 ± 1.4
-Cr +Mn	28.8 ± 0.8	4.2 ± 1.4
+Cr -Mn	28.0 ± 0.7	5.3 ± 1.4
+Cr +Mn	28.7 ± 1.0	4.5 ± 1.7
Obese		
-Cr -Mn	45.4 ± 0.6	13.0 ± 2.4
-Cr +Mn	43.5 ± 0.9	12.9 ± 2.2
+Cr -Mn	45.1 ± 0.9	12.8 ± 2.0
+Cr +Mn	44.3 ± 0.6	10.0 ± 2.2

[1]Mean ± SEM

[2]N = 10

APPENDIX B

Group means of plasma parameters in obese and lean mice fed diets with low and adequate chromium and manganese[1,2]

	Glucose (mmol/L)	GHB (%)	Corticosterone (nmol/L)	Insulin (pmol/L)	Cholesterol (mmol/L)
Lean					
-Cr -Mn	8.80 ± .080	4.26 ± 0.65	150.2 ± 47.0	107.0 ± 19.2	4.34 ± 0.16
-Cr +Mn	8.26 ± 1.00	4.30 ± 0.70	248.2 ± 73.7	93.0 ± 15.0	3.66 ± 0.46
+Cr -Mn	7.82 ± 0.91	3.37 ± 1.20	119.8 ± 35.8	84.4 ± 9.4	4.02 ± 0.23
+Cr +Mn	8.31 ± 0.55	4.48 ± 0.33	169.7 ± 45.5	86.0 ± 7.7	4.23 ± 0.22
Obese					
-Cr -Mn	12.07 ± 1.44	6.01 ± 1.67	413.3 ± 69.2	516.2 ± 121.8	6.00 ± 0.35
-Cr +Mn	11.30 ± 0.86	6.66 ± 2.50	487.4 ± 84.3	533.9 ± 108.7	5.72 ± 0.33
+Cr -Mn	10.64 ± 0.50	5.24 ± 1.30	342.4 ± 66.3	602.0 ± 152.0	6.85 ± 0.31
+Cr +Mn	10.70 ± 0.50	6.87 ± 1.35	403.1 ± 99.0	625.0 ± 144.6	6.50 ± 0.50

[1]N = 10

[2]Mean ± SEM

APPENDIX C

Group means of mineral concentrations of liver in obese and lean mice fed diets with low and adequate chromium and manganese[1, 2]

	Cr umol/g	Mn umol/g	Fe umol/g	Zn umol/g	Ca mmol/g	Mg mmol/g	Cu mmol/g
Lean							
-Cr -Mn	2.50 ± 3.75	26.54 ± 8.06	4.30 ± 0.72	1.20 ± 0.10	3.04 ± 0.51	13.51 ± 2.43	0.26 ± 0.03
-Cr +Mn	3.40 ± 6.28	57.84 ± 8.40	4.21 ± 1.07	1.18 ± 0.13	3.23 ± 0.93	13.47 ± 2.56	0.26 ± 0.03
+Cr -Mn	0.55 ± 0.77	28.91 ± 6.98	4.14 ± 0.41	1.20 ± 0.11	2.85 ± 0.50	14.36 ± 5.00	0.27 ± 0.01
+Cr +Mn	2.02 ± 2.80	54.92 ± 9.00	3.89 ± 0.89	1.20 ± 0.12	3.01 ± 0.40	12.10 ± 5.06	0.27 ± 0.04
Obese							
-Cr -Mn	0.32 ± 0.35	7.57 ± 1.44	2.43 ± 0.73	0.58 ± 0.10	1.44 ± 0.27	8.43 ± 1.44	0.11 ± 0.02
-Cr +Mn	0.80 ± 1.70	23.60 ± 3.56	2.00 ± 0.50	0.56 ± 0.08	1.82 ± 1.21	8.12 ± 1.00	0.11 ± 0.01
+Cr -Mn	0.65 ± 1.01	8.24 ± 2.08	2.42 ± 0.46	0.62 ± 0.10	1.44 ± 0.32	8.56 ± 1.05	0.12 ± 0.02
+Cr +Mn	1.56 ± 2.62	26.27 ± 7.48	2.07 ± 0.34	0.60 ± 0.08	1.88 ± 1.42	8.18 ± 1.46	0.11 ± 0.01

[1]Mean ± SEM

[2]N = 10

APPENDIX D

Group means of mineral concentrations of kidney in obese and lean mice fed diets with low and adequate chromium and manganese[1,2]

	Cr umol/g	Mn umol/g	Fe umol/g	Zn umol/g	Ca mmol/g	Mg mmol/g	Cu umol/g
Lean							
-Cr -Mn	12.06 ± 5.40	50.90 ± 7.10	4.80 ± 0.67	1.03 ± 0.04	5.60 ± 1.08	14.64 ± 2.45	0.24 ± 0.01
-Cr +Mn	14.58 ± 15.14	97.63 ± 6.00	5.48 ± 0.31	1.04 ± 0.03	5.20 ± 0.27	16.23 ± 1.04	0.23 ± 0.01
+Cr -Mn	15.58 ± 2.48	53.90 ± 1.44	4.90 ± 0.19	1.08 ± 0.02	6.07 ± 0.30	15.30 ± 0.52	0.25 ± 0.01
+Cr +Mn	8.08 ± 2.72	98.57 ± 8.76	5.25 ± 0.28	1.04 ± 0.02	5.00 ± 0.35	15.79 ± 0.93	0.24 ± 0.01
Obese							
-Cr -Mn	12.17 ± 2.92	45.77 ± 4.00	4.86 ± 0.24	1.01 ± 0.03	6.05 ± 0.37	16.00 ± 1.17	0.11 ± 0.00
-Cr +Mn	9.21 ± 2.26	85.76 ± 2.35	5.68 ± 0.90	1.00 ± 0.01	5.61 ± 0.25	15.77 ± 0.60	0.26 ± 0.01
+Cr -Mn	16.65 ± 5.28	46.34 ± 2.00	5.04 ± 0.16	1.03 ± 0.02	5.85 ± 0.33	17.34 ± 0.84	0.27 ± 0.00
+Cr +Mn	13.30 ± 4.24	91.00 ± 2.68	5.00 ± 0.21	1.00 ± 0.02	5.15 ± 0.21	16.76 ± 0.38	0.11 ± 0.00

[1]Values are mean ± SEM

[2]N = 10

APPENDIX E

Group means of hematological parameters of obese and lean mice fed diets with low and adequate chromium and manganese[1, 2]

	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
	10^6/L	10^{12}/L	g/Dl	%	Fl	pg	g/L	10^9/L
Lean								
-Cr -Mn	8.45 ± 1.77	10.73 ± 0.07	163 ± 2	50.0 ± 1	46.4 ± 0.2	15.2 ± 0.1	328.20 ± 2.40	1252 ± 38
-Cr +Mn	7.87 ± 1.66	11.22 ± 0.49	170 ± 19	52.0 ± 6	46.4 ± 0.4	15.2 ± 0.1	327.00 ± 6.54	1241 ± 93
+Cr -Mn	6.17 ± 1.72	10.44 ± 0.22	158 ± 9	48.0 ± 3	46.1 ± 0.3	15.1 ± 0.1	328.00 ± 4.52	1276 ± 20
+Cr +Mn	6.82 ± 2.93	10.73 ± 0.21	162 ± 8	50.0 ± 2	46.1 ± 0.2	15.1 ± 0.2	326.77 ± 6.57	1387 ± 32
Obese								
-Cr -Mn	12.26 ± 4.13	10.61 ± 0.13	173 ± 5	51.0 ± 1	48.7 ± 0.1	16.3 ± 0.2	334.80 ± 8.70	1141 ± 37
-Cr ±Mn	13.93 ± 3.06	11.12 ± 0.20	178 ± 7	54.0 ± 2	48.6 ± 0.3	16.1 ± 0.1	330.40 ± 4.35	1245 ± 30
+Cr -Mn	12.81 ± 4.14	10.62 ± 0.13	174 ± 5	52.0 ± 2	48.6 ± 0.1	16.5 ± 0.1	338.50 ± 7.70	1207 ± 25
+Cr ±Mn	11.64 ± 4.46	10.74 ± 0.18	175 ± 8	52.0 ± 2	47.5 ± 1.1	16.4 ± 0.1	337.33 ± 6.61	1271 ± 36

[1]Mean ± SEM

[2]N = 10

APPENDIX F

Group means of tissue weights of obese and lean mice fed diets with low and adequate chromium and manganese[1, 2]

	Liver	Kidney	Spleen	Heart	Testes	Lung	Fat[3]	Thymus
	g	g	mg	g	g	g	g	mg
Lean								
-Cr -Mn	1.03 ± 0.03	0.33 ± 0.01	65.2 ± 2.3	0.13 ± 0.00	0.21 ± 0.01	0.15 ± 0.01	1.11 ± 0.08	47.1 ± 3.4
-Cr +Mn	1.06 ± 0.05	0.33 ± 0.02	67.1 ± 4.2	0.14 ± 0.01	0.21 ± 0.01	0.21 ± 0.04	0.93 ± 0.10	42.0 ± 4.0
+Cr -Mn	1.02 ± 0.04	0.31 ± 0.01	59.0 ± 4.2	0.13 ± 0.00	0.21 ± 0.01	0.20 ± 0.01	0.80 ± 0.06	36.1 ± 2.0
+Cr +Mn	1.06 ± 0.03	0.31 ± 0.01	64.5 ± 3.4	0.13 ± 0.00	0.21 ± 0.01	0.20 ± 0.01	0.92 ± 0.07	42.4 ± 2.8
Obese								
-Cr -Mn	2.81 ± 0.20	0.32 ± 0.01	60.0 ± 5.0	0.12 ± 0.00	0.18 ± 0.01	0.16 ± 0.01	3.00 ± 0.10	38.7 ± 3.1
-Cr +Mn	2.60 ± 0.11	0.34 ± 0.02	51.9 ± 2.7	0.13 ± 0.01	0.17 ± 0.14	0.14 ± 0.01	2.88 ± 0.10	44.7 ± 4.5
+Cr -Mn	2.52 ± 0.15	0.32 ± 0.01	50.3 ± 2.7	0.12 ± 0.00	0.18 ± 0.01	0.13 ± 0.01	3.00 ± 0.11	40.2 ± 4.3
+Cr +Mn	2.66 ± 0.10	0.32 ± 0.02	51.0 ± 2.5	0.12 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	2.90 ± 0.06	37.4 ± 5.5

[1]Mean ± SEM

[2]N = 10

[3]Epididymal fat pad

APPENDIX G

Hematological parameters of obese and lean mice fed diets with low and adequate chromium and manganese[1]

	WBC 10^6/L	RBC 10^{12}/L	HGB g/dL	HCT %	MCV Fl	MCH pg	MCHC g/L	PLT 10^9/L
Main Plot Means								
OB	11.97 ± 0.58	10.78 ± 0.12	176 ± 14	52 ± 5	48.3 ± 0.2	16.3 ± 0.1	335.2 ± 11.4	1290.2 ± 23.3
Lean	7.71 ± 0.58	10.76 ± 0.12	163 ± 14	50 ± 5	46.3 ± 0.2	15.1 ± 0.1	327.5 ± 11.4	1216.0 ± 23.3
Subplot Means								
-Cr	10.58 ± 0.57	10.92 ± 0.12	171 ± 14	52 ± 5	47.5 ± 0.2	15.7 ± 0.1	330.1 ± 11.1	1220.0 ± 23.0
+Cr	9.11 ± 0.58	10.62 ± 0.12	167 ± 14	50 ± 5	47.1 ± 0.2	15.7 ± 0.1	332.6 ± 11.6	1287.0 ± 23.7
-Mn	9.64 ± 0.57	10.60 ± 0.12	167 ± 14	50 ± 5	47.5 ± 0.2	15.8 ± 0.1	332.3 ± 11.2	1219.0 ± 23.0
+Mn	10.05 ± 0.60	11.00 ± 0.12	171 ± 14	52 ± 5	47.2 ± 0.2	15.6 ± 0.1	330.4 ± 11.6	1287.3 ± 24.0
Source of Variation								
Gene	< 0.0001	0.94	< 0.0001	< 0.0025	< 0.0001	< 0.0001	< 0.0001	< 0.02
Cr	0.08	0.08	0.07	< 0.03	0.14	0.50	0.11	< 0.05
Mn	0.62	< 0.04	0.04	< 0.03	0.33	0.26	0.23	< 0.04
Gene x Cr	0.36	0.45	0.07	0.34	0.60	0.11	0.10	0.52
Gene x Mn	0.86	0.90	0.72	0.86	0.26	0.54	0.62	0.63
Cr x Mn	0.91	0.35	0.40	0.36	0.50	0.56	0.61	0.52
Gene x Cr x Mn	0.52	0.86	0.93	0.84	0.37	0.68	0.62	0.21

[1]Values are least squares means ± SEM (Gene, N = 39-40, Cr, N = 39-40, Mn, N = 39-40)

VITA

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