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CHROMIUM STATUS: EFFECTS OF DEMOGRAPHIC CHARACTERISTICS, ORAL CONTRACEPTIVE AGENTS AND CHROMIUM SUPPLEMENTATION

The University of Nebraska - Lincoln PH.D. 1985

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106

CHROMIUM STATUS: EFFECTS OF DEMOGRAPHIC CHARACTERISTICS, ORAL CONTRACEPTIVE AGENTS AND CHROMIUM SUPPLEMENTATION

by

Hinda-Rose Bizem

A Dissertation

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Interdepartmental Area of Nutrition

Under the Supervision of Professor Constance V. Kies
Lincoln, Nebraska

June, 1985

TITLE

CHROMIUM STATUS: EFFECT OF DEMOGRAPHIC CHARACTERISTICS, ORAL CONTRACEPTIVE AGENTS AND CHROMIUM SUPPLEMENTATION

BY

HINDA-ROSE BIZEM

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GRAD 52002 300 5-85(T)

CHROMIUM STATUS: EFFECT OF DEMOGRAPHIC CHARACTERISTICS, ORAL CONTRACEPTIVE AGENTS, AND CHROMIUM SUPPLEMENTATION

Hinda-Rose Bizem

University of Nebraska, 1985

Advisor: Constance V. Kies

Chromium (Cr) nutriture was investigated in three related projects. A retrospective Cr assessment study was conducted utilizing dietary records, urine, and blood serum from young adults and pregnant adolescents. Average daily Cr intakes for all subjects was 37.0 µg. Differences in chromium status were not demonstrated in subjects from different geographic locations. Pregnant adolescents had marginal Cr intakes (48 µg/day) and non-detectable levels of Cr in urine. Pregnancy may divert maternal Cr stores to the fetus.

Nortestosterone-derived oral contraceptive agents have the greatest capacity to alter glucose tolerance in women. Retired breeding female mice were fed either an oral contraceptive preparation or a Cr supplement in a 2x2 factorial arrangement of treatments. All mice gained weight regardless of experimental treatment. In addition, treatments did not affect percentage carcass fat or moisture. Glucose tolerance tests were conducted. Chromium supplementation appeared to moderate the glucose response at 40 minutes post-glucose injection as compared to non-Cr supplementation.

Organic Cr as a component of the glucose tolerance factor found in brewer's yeast is considered to be the most biologically available form

of Cr. A five week study was employed to examine differences in bioavailability of inorganic and organic Cr supplements in midwestern women. Each woman serving as her own control randomly received the basal diet alone, basal diet + brewer's yeast, and basal diet + chelated Cr. Mean urinary Cr excretion was similar for subjects consuming the basal diet alone or with the brewer's yeast supplement (0.16 µg/day). However, the pattern of urinary excretion was different. Brewer's yeast supplementation decreased variability in Cr excretion in comparison to values when either the basal diet was fed alone or supplemented with chelated Cr. Chelated Cr led to significantly higher urinary Cr excretion ($P<0.03$) than did either the basal diet alone or with brewer's yeast supplementation. This suggests that either the Cr supplied by chelated Cr was better absorbed than was that supplied by yeast, although both were apparently poorly absorbed in comparison to the Cr supplied by the diet, or that the absorbed chelated Cr was more poorly utilized than was the Cr from yeast. Blood serum Cr concentrations were not significantly changed throughout the experimental treatments, although the numerically highest mean blood serum Cr level was found when the brewer's yeast was fed.

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ACKNOWLEDGEMENTS

Much appreciation goes to Dr. Kies. Without her support to allow me to explore my research interests, I would not have accomplished this achievement.

Many things are extended to Dr. Fox for her advise on teaching and serving on my reading committee, to Dr. Kinder for his help in the design of my mouse study, to Dr. Knoche for answering my chromium and glucose questions, and to Dr. Lowry for expert statistical advice.

My award winning performance of Clair de Lune by Ravel really made me appreciate Mrs. Arlone Miller. Her patience and understanding in the past three years made everything else more bearable.

Jane Clarke, Miss Mouse, Cherly Witte, Barb Clement, and Phyllis Todd were wonderful friends. Antonio Monroy gave me constant encouragement: "All it takes is guts."

Mom, Dad and Grandma were always there for either emotional or financial support when needed. I am the first Ph.D. in the family. I hope future family members also find the rewards of advanced education.

HRB

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INTRODUCTION

During the past 30 years, interest in chromium (Cr) as an essential micronutrient has broadened. At first Cr was identified as an essential trace mineral in the prevention of impaired carbohydrate metabolism in rats (Mertz et al., 1965). Chromium deficiency in humans was first demonstrated in a patient receiving total parenteral nutrition (Jeejeebhoy, et al., 1977). Chromium supplements are now being given to some people with non-insulin dependent diabetes with subsequent improvements in carbohydrate metabolism (Offenbacher and Pi-Sunyer, 1980). Current research is focusing on Cr and lipid interactions and Cr and other trace element interactions (e.g., vanadium) (Polansky et al., 1985; Oladut et al., 1985; Wang et al., 1985).

Gestational diabetes affects only 1-2% of pregnant women (Paige, 1983). In most cases abnormal blood glucose concentrations return to normal post partum. During the first trimester of pregnancy, the demand for insulin decreases but responsiveness increases. Insulin antagonism can occur in the second trimester in addition to increased glucose transport to the fetus. Glucose utilization declines during the third trimester due to increased degradation of insulin by placental enzymes and increased peripheral resistance to insulin binding (Paige, 1983). Women secreting low, but normal, levels of insulin may be at risk for gestational diabetes.

Oral contraceptives produce metabolic changes similar to those of pregnancy, especially, delayed glucose clearance and increased insulin resistance. Nortestosterone-derived oral contraceptives have the most

potent effects on glucose tolerance. Once oral contraceptive therapy is discontinued, blood glucose concentrations return to normal.

Marginal or deficient Cr nutriture is thought to characterize the United States population. Limited success of Cr supplementation in Type II diabetics (Offenbacher and Pi-Sunyer, 1980) suggests that some women with gestational diabetes or altered glucose tolerance from oral contraceptive usage may be Cr deficient and might benefit from Cr supplementation.

LITERATURE REVIEW

CHROMIUM NUTRITION

Since the 1950's chromium (Cr) has been regarded as an essential nutrient for mice and rats. Torula yeast supplementation experiments conducted in environments rigidly controlled to prevent trace mineral contamination resulted in impaired glucose tolerance. Chromium was identified as the missing element in the yeast preparations (Mertz et al., 1964; Schroeder et al., 1965). Other Cr deficiency symptoms observed in rats included symptoms of diabetes mellitus. In particular, symptoms of hyperglycemia, glycosuria, and retarded growth were recorded (Schroeder, 1966; Hambidge, 1974; Schroeder et al., 1965). The aberrations in carbohydrate metabolism could be corrected by Cr (III) supplementation. Low protein, low Cr diets fed to rats lead to the development of corneal lesions. Chromium supplementation prevented further development of the lesion but did not reverse developed lesions (Underwood, 1977).

The first documented case of Cr deficiency in humans was observed in a woman being maintained on total parenteral nutrition (TPN). After three years of TPN therapy, symptoms of diabetic neuropathy developed which could not be resolved by supplemental energy, insulin, or zinc. Chromium at a level of 250 µg/day was administered for two weeks. Then, daily infusions of 29 µg/day were given. After four months all symptoms of diabetic neuropathy had disappeared (Jeejeebhoy et al., 1977). Chromium deficiency found in both animals and humans is a result of inadequate Cr intake.

Chromium nutriture is considered to be dependent on geographical location (Schroeder, 1968). American populations have marginal Cr

intakes when compared to populations from Africa and the Near East. The elderly, pregnant women, women receiving oral contraceptive preparations, and Type II diabetics are particularly at risk with respect to adequate Cr nutrition. Chromium intakes in the range of 50-200 µg/day are considered safe and sufficient (National Research Council, 1980).

CHROMIUM BIOCHEMISTRY

Chromium is a transition element with an electron orbital configuration of $3s^2\ 3p^6\ 3d^5\ 4s^1$ which accounts for the numerous oxidation states of Cr compounds. These oxidation states range from 0 to +6 with +3 and +6 being the most common (Kutsky, 1981). Chromium has been shown to be a stable component of RNA and DNA. Chromium and other transition elements are thought to aid in the maintenance of nucleic acid configurations (Wacker and Vallee, 1959). Cr is a component of the glucose tolerance factor. Inorganic Cr or Cr found in GTF is considered to be an essential nutrient (Mertz et al., 1965). GTF functions as a potentiator of insulin.

Polarographic studies were conducted to examine the interactions of Cr, insulin, and mitochondria (Christian et al., 1963). These researchers indicated that insulin and Cr (III) react with mitochondria via sulfhydryl groups. Chromium (III) was thought to contribute to the formation of a sulfhydryl-disulfide linkage by forming an intermediary ternary complex. A minor criticism of this study was the concentrations of reactants were at a non-physiological level.

The effects of physiological concentrations of insulin on swelling of mitochondria from rat livers was studied to determine the effects of Cr (III) supplementation on insulin bioactivity (Campbell and Mertz,

1963). Two levels of zinc-insulin, $1.6x10^{-3}$mM and $1.4x10^{-4}$mM and two levels of Cr (III), $6x10^{-5}$mM and $6x10^{-3}$mM were added to the mitochondria. Although the mitochondria swelled in the control solution, the addition of $1.6x10^{-3}$mM of insulin significantly increased the degree of swelling. The $1.4x10^{-4}$mM concentration of insulin eliminated the swelling effect. In the absence of insulin, $6x10^{-5}$mM Cr (III) had no effect on mitochondrial swelling. A concentration of $6x10^{-4}$mM insulin plus $6x10^{-5}$mM Cr (III) led to a significant increase in mitochondrial swelling. These results signify the potentiating effects of Cr at low concentrations of insulin.

The structural identification of GTF as well as the synthesis of compounds which have the same insulin potentiating effects as GTF has been elusive. To determine GTF activity in brewer's yeast and synthetic compounds, an <u>in vitro</u> procedure was developed. In this procedure the stimulation of [1-^{14}C] glucose oxidation by epididymal adipose tissues from Cr-deficient rats in the presence of adequate insulin concentrations (Mertz et al., 1961; Mertz et al., (1974) was observed.

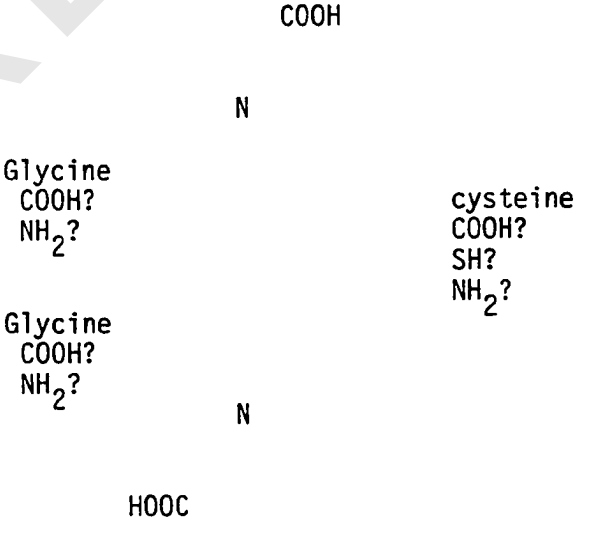

Figure 1: Hypothetical Sketch of GTF (Mertz, 1974)

Figure 1 is a tentative model of GTF structure. The amino acids identified in the figure are thought to stabilize the structure to prevent olation. The structural formula which interacts with insulin may be a tetra-aquo-dinicotinate compound. Mertz's theory (1974) is as follows: if the two acid ligands (nicotinic acid) were connected to basic groups on the insulin and tissue receptor, respectively, bringing insulin closer to its active site, the four coordinated water molecules could exchange with the four sulfur atoms involved in the disulfide exchange reaction. To date, no further information on GTF structural configuration has been reported.

Simple Cr compounds have biologically different responses than does GTF. These differences encompass the following points: 1) GTF is an organic, low molecular weight, dialyzable, ethanol extractable compound, 2) less GTF than Cr salts is needed to repair impaired glucose tolerance in rats and response time to GTF administration is rapid as compared to the lag phase from Cr salts, 3) GTF crosses placental membranes, 4) GTF has access to other body pools which are non-accessible to Cr salts, and 5) GTF is found in foods and is better absorbed from the gastrointestinal tract (Mertz, 1975).

The effects of insulin are widespread throughout most body tissues, as are the effects of Cr. The following table will clarify this point.

Table 1: POSSIBLE OR DEMONSTRATED PHYSIOLOGICAL FUNCTIONS OF CHROMIUM

Circulatory - serum cholesterol homeostasis*
Excretory - urinary sugar homeostasis*
Respiratory - increased cellular CO_2 output*
Digestive - glucose, amino acid absorption*
Nervous - neuronal homeostasis*
Reproductive - homeostasis of fetal carbohydrate metabolism
Special sensory - corneal clarity
Muscular - energy for contraction*
Skeletal - component of bones
Integument - component of hair
Immune - infection resistance of lung*
Metabolic - glucose, lipid, protein metabolism*
Detoxification - liver detoxification functions

* insulin dependent activities

(Kutsky,1981)

BODY CHROMIUM DISTRIBUTION

The distribution of Cr within body tissues has been studied over the past twenty years with no conclusive results. The greatest impedence to identifying Cr distribution is current methodologies used in Cr analysis. Schroeder and coworkers (1965) fed four groups of rats (male: <2 years and >2 years, female: <2 years and >2 years) control or Cr supplemented diets (as Cr acetate). Tissues from the kidney, liver, heart, lung, and spleen were analyzed. Heart, lung, and spleen tissues contained higher Cr concentrations than kidney and liver tissues. Lower tissue Cr values were found in breeding female rats than in younger non-breeding female rats. Infant, stillborn, and embryonic rats contained Cr in their tissues but maternal liver Cr was negligible. More recently Verch and associates (1983) analyzed Cr concentrations in the following

tissues from adult male rats: liver, pancreas, kidney, testis, lung, heart, spleen, and bone. Tissues from the pancreas, testis, heart, spleen, and bone contained greater Cr concentrations than tissues from liver, kidney, and lung.

Distribution of Cr in dam and fetus has been reexamined by Wallach and Verch (1984). Two groups of rats of similar age were used. One group was pregnant for the first time and the other group was composed of non pregnant controls. On the 17th day of gestation ^{51}Cr was injected into both groups of rats. All rats were sacrificed three days later. The results were similar to those reported by Schroeder. Chromium quickly accumulated in fetal tissues. After fetal components were removed from the rat, very little ^{51}Cr was found suggesting post-partum Cr depletion may be common.

Schroeder (1968) reported tissue Cr concentrations in wild and domestic animals (cow, lamb, pig, wild rats, squirrels, fox, beaver, woodchuck, and deer) as well as humans. Highest Cr concentrations were found in lung and spleen tissue and in lung and placental tissue of humans. Liver, kidney, heart, spleen (human), muscle, and stomach tissues were lower in Cr concentration.

A tentative model for Cr (III) kinetics has recently been developed (Lim et al., 1983). After injecting 100 μCi of $^{51}CrCl_3 \cdot 6H_2O$, blood samples were drawn periodically over 60 days. The distribution of ^{51}Cr was measured by a computerized rectilinear whole-body scanner. Scanning was done periodically up to 84 days post IV injection of ^{51}Cr. The following diagram was developed to illustrate a Cr pathway: