# GLUCOSE TOLERANCE AS AFFECTED BY PREGNANCY, LACTATION, AND DIETARY CHROMIUM

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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, 2001

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## ACKNOWLEDGEMENTS

I would like to thank my major adviser Dr. Barbara J. Stoecker for her patience, guidance, encouragement and friendship during my stay at Oklahoma State University. I would also like to express my appreciation to my other committee members Dr. Bahram Arjmandi and Dr. Elizabeth Droke for their support.

I would like to express gratitude to my late cousin Mary Wakeman. She entered school and graduated and became a Registered Dietitian after raising a family. She has since succumbed to cancer after a long battle. Her strength and determination was, in large part, a motivation that kept me going when stopping would have easier.

I would also like to acknowledge my Father, John D. Porter, and my aunts, Virginia Hilsabeck and Ruth Wakeman who believed in me and were there to encourage me when it was needed.

Finally, I would like to thank my friends and comrades for their support and encouragement.

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# CHAPTER I

## INTRODUCTION

The relation that exists among chromium, insulin, and glucose has been investigated through many research projects. Because of the involvement of insulin and glucose it was natural that research expanded into the area examining diabetes mellitus. Glucose intolerance is a major component of this pathology. Understanding the nature and degree of the glucose intolerance in diabetes and pregnant mothers, in particular, has very important health considerations.

In the mid-1950's a new dietary component was isolated. This substance had the effect of improving glucose intolerance in rats. The active ingredient of this substance was shown to be trivalent chromium. Later research demonstrated that chromium was essential in rats (Mertz, et al., 1961) and humans (Jeejeebhoy, et al., 1977). Early researchers experienced difficulty in determining how much of the chromium was to be found in a biological system. It was not until the mid-1980's that the understanding of chromium being a micronutrient was established. Because chromium in biological systems is measured in parts per billion, it is easy to understand how contamination led to spurious results prior to this understanding. Since then special techniques and procedures for doing micro-mineral research have been developed.

Chromium deficiency could explain some diabetes-like symptoms in cases of diabetes; it does not explain all the cases. The concentration of glucose in the blood was shown to reflect the amount of glucose control a patient had. The inability to control the

serum glucose concentration is implicit in diabetes diagnosis. Human research has done much to examine this condition. What is yet to be devised is a simple test for determining the extent of glucose control and a way to administer such a test.

***Statement of the Problem***. A small but significant number of pregnancies involve the diagnosis of gestational diabetes mellitus (GDM). The outcomes of GDM are increased morbidity and mortality for both the mother and her offspring. Up to 40% of mothers who experience GDM go on to develop Type 2 diabetes later in life (Kjos and Buchanan, 1999; Coustan and Carpenter, 1998; CDC 1998, Mokdad, et al., 2000).

The deterioration of glucose tolerance is normal during pregnancy. In its early history GDM was only regarded as an indicator of increased risk of developing non-gestational diabetes at some point later in life (Coustan and Carpenter, 1998). About 50 years ago, it was noted that there was an increase in infant mortality and morbidity related to maternal diabetes. Unfortunately, the criteria then for a diagnosis of GDM did not take into consideration the mother's physiological response to pregnancy (Coustan and Carpenter, 1998). A problem still remaining today is that there is no one cut-off point to determine those mothers at risk from those mothers not at particular risk of increased morbidity and mortality (Coustan and Carpenter, 1998). In 1996 the US Preventative Task Force published their "Guide to Clinical Preventative Services." They state there was not enough evidence to "recommend for or against universal screening for gestational diabetes" (US Preventative Service Task Force, 1996).

Screening women to determine those at increased risk for developing GDM and administering an Oral Glucose Tolerance Test (OGTT) for those found to be at risk seems to be a reasonable step to take (Kjos and Buchanan, 1999). Aside from the human

costs of GDM, the financial cost is estimated to be $44 billion a year in direct cost and an additional $54 billion in indirect expense of disability, work loss, and early death of the mother (CDC, National Fact Sheet, 1998; Mokdad, et al., 2000). Criteria for diagnosis of GDM are listed in **Table 1**.

**Table 1**[1]

Gestational diabetes mellitus serum glucose cut-off concentrations used for human diagnosis.[2]

| | Fasting | 1 Hour | 2 Hour | 3 Hour |
|---|---|---|---|---|
| mg/dL | 95 | 180 | 155 | 140 |
| mmol/L | 5.3 | 10.0 | 8.6 | 7.8 |

[1] Based on a table by Metzger, Coustan. Summary and Recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus, 1998.
[2] Values based on a 100 oral glucose load.

A cure for this state of glucose intolerance is not available; however, in humans, treatment with a diet counseling plan, exercise, and even chromium supplementation have been shown to lessen the degree of glucose intolerance in humans (Kjos and Buchanan, 1999; Jeejeebhoy, et al., 1977). Glucose intolerance has been shown to improve in rats that use an exercise wheel and who receive chromium supplementation ( Schroeder, 1966; Wright, et al., 1983;). Improving glucose tolerance has been shown to lessen the degree of Type 2 diabetes' impact on the mother or her baby (Anderson, et al., 1991; Hod, et al., 1991). In order to diagnose this malady, a test needs to be established which is easy to administer and effective. This test must be routinely administered in the physician's office and be of relatively low cost and high predictive power. An OGTT can be done with some success but this test takes 2 to 3 hours and involves consuming a very concentrated sugar solution. Many pregnant mothers experience nausea and vomiting as a

result of ingesting this solution (Coustan and Carpenter; 1998, Kjos and Buchanan, 1999).

The purpose of this research is to evaluate the use of chromium picolinate and chromium chloride as a means of lessening glucose intolerance during pregnancy and lactation using the rat as a model. Examining whether a relation exists between fructosamine and serum glucose concentrations is another goal of this research. Fructosamine is formed by a non-enzymatic reaction between fructose and serum proteins, primarily albumins. Fructosamine, as a marker, reflects the last 2 – 3 weeks of serum glucose concentrations, whereas the OGTT gives an indication of serum glucose concentrations at the time the test is preformed.

Diabetes mellitus has been defined as four separate but related physiological manifestations of impaired glucose tolerance. The first group is made up of people with Type 1 diabetes mellitus. This group makes up about 5% of diagnosed cases of diabetes and results from a lack of insulin. When this deficiency arises from surgeries, accidents, infections and genetic syndromes, this makes up the second group that represents about 2% of diabetic (CDC, National Fact Sheet 1998; National Diabetes Data Group, 1979). The third group is made up of people with Type 2 diabetes mellitus and is by far the largest group. About ninety percent of individuals who have diabetes mellitus have this form. Risk factors include older age, obesity, family history of diabetes, a diminished glucose tolerance, certain races or ethnicity, little physical activity, and previous diagnosis of gestational diabetes mellitus (GDM). The fourth group is GDM. This form of diabetes includes 2 - 5 % of pregnancies and about 3% of the population. At the end of

pregnancy, glucose tolerance, for most mothers, returns to normal. (CDC, National Fact Sheet, 1998; Mokdad, et al.,2000).

Two problems arise when trying to test for GDM. First, the nausea problem with some mothers undergoing an OGTT. Secondly, having to wait for 2 to 3 hours to finish the test is often inconvenient and more time than the mother can spend. It would be helpful to have a test that can be done in one step. One such test is to examine the serum concentration of fructosamine (Kjos, 1999 and Buchanan; Hughes, 1995).

Chromium is involved in several aspects of metabolism including growth, glucose tolerance, lipid metabolism, interactions with nucleic acids, and the immune response (Schroeder, et al., 1971; Schroeder, 1966). The dietary form of chromium and the form which the body can best utilize have been points of major interest. For some people chromium intake through dietary choices is below the Adequate Intake (AI) established by the Food and Nutrition Board of the National Academy of Sciences (2001). Chromium absorption is low and competes with other ions and compounds and so finding the most utilizable form is desirable (Anderson et al., 1985).

Serum chromium does not reflect the body's reserves (Hopkins, 1964; Onkelinx, 1977; Mertz, 1992) and serum chromium is too low to measure as a standard clinical test. If analyses were more sensitive, measuring the serum concentration of chromium might provide a simple screening tool to reveal an at-risk condition regarding glucose tolerance. However, chromium deficiency is not the only cause of GDM. Without knowing the relation between serum chromium concentration and glucose intolerance, there has been a strong force for seeking a surrogate marker for quantifying glucose intolerance.

In the search for the simple glucose control marker, glycated proteins have been researched. Glycated hemoglobin and fructosamine are the most promising indicators. Since each of these glycated proteins reflects the serum glucose levels over differing lengths of time, each has been examined for its ability to identify diabetes.

***Limitations***. Limitations to this thesis include the general physical condition of the rat and the exact amount of time each rat experienced fasting. Other limitations include allowing the pups to remain with the mother while she was fasting before the OGTT procedure. During the pregnancy and lactation the mother rat produces and supports many pups and enough milk to support her litter for a period of three weeks. This amounts to more product than the mother's weight. Therefore, a direct comparison with humans is not realistic.

*Statement of Hypothesis.*

HO1: Serum concentrations of glucose and insulin are not significantly different during an oral glucose tolerance test on the 18th day of pregnancy between similarly aged non-pregnant rats and groups of pregnant rats receiving diets deficient in chromium, supplemented with chromium chloride, or supplemented with chromium picolinate.

HO2: Serum concentrations of glucose and insulin are not significantly different during an oral glucose tolerance test on the 19th day of lactation between similarly aged female rats and groups of lactating rats receiving diets deficient in chromium, supplemented with chromium chloride, or supplemented with chromium picolinate.

HO3: Serum concentrations of fructosamine are not significantly different on the 18th day of pregnancy between similarly aged non-pregnant rats and groups of pregnant rats receiving diets deficient in chromium, supplemented with chromium chloride, or supplemented with chromium picolinate.

HO4: Serum concentrations of fructosamine are not significantly differenttwo hours after an oral glucose load on the 19th day of lactation between similarly aged female rats and groups of lactating rats receiving diets deficient in chromium, supplemented with chromium chloride, or supplemented with chromium picolinate.

HO5: Serum concentrations of non-esterified fatty acids, triglycerides, cholesterol, and total proteins are not significantly different on the 18th day of pregnancy between

similarly aged non-pregnant rats and groups of pregnant rats receiving diets deficient in chromium, supplemented with chromium chloride, or supplemented with chromium picolinate.

HO6: Serum concentrations of non-esterified fatty acids, triglycerides, cholesterol, and total proteins are not significantly different two hours after an oral glucose load on the 19th day of lactation between similarly aged female rats and groups of lactating rats receiving diets deficient in chromium, supplemented with chromium chloride, or supplemented with chromium picolinate.

# CHAPTER II

## LITERATURE REVIEW

***Introduction.*** In this chapter a review of steps in the discovery of the essential nature of chromium will be examined. Some important studies that demonstrate the function of chromium and its effect on diabetes and glucose tolerance will be reviewed. The importance of understanding gestational diabetes and how it is diagnosed will also be reviewed briefly. Research examining the relation between chromium and glucose tolerance has mainly used the rat model. The area of gestational diabetes mellitus has largely been investigated with human subjects. Finally, a look at the utilization of fructosamine as an indicator for diagnosing gestational diabetes will be covered.

***Chromium.*** Chromium has been shown to be essential for normal glucose tolerance in many studies (Schwartz and Mertz, 1959; Mertz, et al., 1965; Schroeder et al., 1968). All of these studies utilized rats because rats' glucose and insulin metabolism is similar to humans (Flint, et al, 1981; Leturque, et al, 1984).

The quantification of chromium until the 1980's was problematic. Once the problems of background and general contamination were overcome and the development of the graphite furnace was achieved, conducting chromium research with atomic absorption spectroscopy was able to quantify the small amounts of chromium present in biological systems with reliable results (Mertz, 1992).

The form in which chromium is ingested has been demonstrated to be quite variable in its effect on cell tissue. While chromium chloride is the most typical form

used, chromium picolinate has been shown to increase the internalization of insulin and subsequent uptake of glucose in rat skeletal muscle cells. Using tritiated glucose, cellular uptake of glucose was measured relative to various chromium compounds. It was found that the picolinate form of chromium resulted in nearly double the uptake of glucose compared to the other tested compounds (chromium chloride and chromium nicotinate) (Evans and Bowman, 1992).

Research in recent years has demonstrated not only the essentiality of chromium for potentiating the function of insulin but also that chromium, in combination with different anions like chloride and picolinate, can have different effects on insulin sensitive tissues (Vincent, 2000).

More recently the actual role of chromium in glucose tolerance factor, a facilitator of insulin, was identified and the compound was called low-molecular-weight chromium binding substance (LMWCr). When the chromium was removed, the activity of this substance, which was shown to enhance glucose oxidation and lipogenesis from glucose, was nearly gone. The return of chromium reactivated the substance in its ability to increase glucose metabolism (Yamamoto, et al., 1989). This substance has also been called chromodulin. Chromodulin contains four chromium atoms and binds to insulin activated receptors and functions in an auto amplification reaction resulting in glucose being metabolized to carbon dioxide or lipids (Vincent, 2000).

*Chromium, Glucose Tolerance Factor, Essentiality.* As the current understanding of chromium evolved several important steps of discovery had to be made. Some of these discoveries follow below.

Research with rats was conducted in the mid 1950's to determine what dietary component would prevent necrotic liver degeneration (Mertz and Schwartz, 1955).They determined three components could independently retard this deterioration. Of the three dietary components, cystine, vitamin E, and Factor 3, only Factor 3 had the additional effect of reducing the circulating glucose of hyperglycemic rats to "glucose tolerance equal to that of normal controls" (Mertz and Schwartz, 1955).

A later investigation showed that one component of Factor 3 was responsible for the "normal glucose removal rates". This component was isolated and named Glucose Tolerance Factor (GTF). It was found that chromium was an essential component of GTF (Schwartz and Mertz, 1957; Schwartz and Mertz, 1959).

When rats were fed various diets, those rats consuming diets containing GTF had higher serum glucose clearance rates than rats fed commercial pelletized feed (Schwartz and Mertz, 1959). When sources of GTF, like pork kidney or brewer's yeast, were added to those diets with low clearance rates, the clearance of glucose from the animals' serum was shown to increase. This was the first test used to determine the presence of GTF (Mertz, et al., 1959). Many minerals were examined to determine which, if any of them, gave GTF its ability to restore glucose tolerance to the rats being studied. Not only was chromium discovered to be this mysterious element, but also the active form was chromium with a positive three charge. Research also showed that a deficiency of GTF left the rats with a level of glucose tolerance resembling diabetes (Schwartz and Mertz, 1959).

Once GTF was observed to affect glucose tolerance, other research was conducted to identify the extent of GTF involvement. In another study, rats that consumed a diet that

was chromium deficient developed symptoms that simulated diabetes and also retarded growth, especially in the female rats. The conclusion was that chromium was necessary for normal glucose tolerance (Schroeder, 1966).

Research was conducted with epididymal fat tissue investigating the effect of GTF on insulin efficiency. A specific amount of insulin was combined with varying amounts of the GTF, as found in brewer's yeast. Glucose was labeled with $^{14}$Carbon and combined with GTF in epididymal fat tissues and the glucose uptake by the cells was measured. When the chromium dose, as GTF, in the media increased from 0.001 $\mu$g to 0.1 µg the cellular uptake of glucose more than tripled, but at greater concentrations of chromium, glucose absorption fell off by nearly half. As the cellular uptake increased the rats response changed too. The rats with the most severe glucose intolerance experienced the greatest improvement; those rats with near normal glucose tolerance were less affected. A number of other elements were tried but only chromium had this pronounced ability to increase glucose clearance. However it was noted that manganese did exhibit a similar but lesser effect than seen with chromium. This ability to improve glucose clearance was seen only in the presence of insulin (Mertz, 1960, Mertz, et al.,1965; Glinsmann, et al., 1966).

***Chromium Metabolism.*** In one study Schroeder (Schroeder, 1968) fed a low chromium diet to 200 weanling rats to assess the effect of such a diet on serum cholesterol and glucose. These rats were divided into two series. In both of these series, rats were examined for growth and serum concentrations of glucose and cholesterol. Series one was redivided into 3 different diet groups with equal numbers of each gender. Each group of rats was fed a diet in which the sugar content was altered. The diets

contained raw sugar, which was noted by the researcher to be slightly refined, refined sugar without added chromium, or refined sugar with 5 ppm chromium added as chromium acetate to the drinking water. The sera of these rats of series one were examined at 5 and 10 months. It was found that the level of serum cholesterol increased as the age of the rat increased in those rats fed refined sugar. Those rats receiving raw sugar or chromium supplementation had lower levels of serum cholesterol. Serum glucose was higher in those rats fed refined sugar with no added chromium and lower in those receiving raw sugar. Serum glucose in the refined sugar supplemented with Cr group was lower than the group receiving plain refined sugar. Fasting serum glucose was relatively low in rats that received raw sugar and females that were fed refined sugar supplemented with chromium had lower levels than males. Series two rats were divided into two groups containing equal numbers of each gender. Group one of this series was fed a diet containing refined sugar. Group two was fed a diet containing raw sugar. The series two rats were examined at four months. Serum cholesterol and glucose concentration were found to be lower in the group fed raw sugar. The series two rats fed raw sugar were generally smaller than those of series one. Schroeder concluded was that refined sugar without chromium allowed the serum cholesterol and glucose levels to rise. Chromium supplementation lowered both cholesterol and glucose levels in the serum (Schroeder, 1968).

A study examining the effect of chromium supplementation was conducted on weanling male rats. The rats were fed a diet high in sucrose (55%) and low in chromium ($33 \pm 14$ ng Cr/g diet). The mineral mix for all rats was adjusted to try to exacerbate chromium deficiency by elevating iron and copper concentrations. Iron may compete

with chromium for absorption and transport (Striffler, et al., 1995). Copper was overtly added to "compromise functioning of the endocrine pancreas" (Striffler, et al., 1995). The concentration of the copper was reduced to 6 mg/kg diet after the first six weeks of the experiment. As an experimental treatment, five parts per million chromium was added as chromium chloride to the water of one group. The second group of rats received unsupplemented water. At the end of 12 weeks both groups were hyperinsulinemic (+Cr, 103 ± 13; -Cr, 59 ± 12 μU/ml) (normal range 19 - 21 μU/mL) and normoglycemic (+Cr, 127 ± 7; -Cr, 130 ± 4 mg/dl), which indicated a state of insulin resistance. After 24 weeks the insulin levels were normal (+Cr, 19 ± 5; -Cr, 21 ± 3 μU/ml) and the glucose level was also normal (+Cr, 124 ± 8; -Cr, 131 ± 6 mg/dl). After an intravenous glucose tolerance test (IVGTT), glucose removal was measured and was found to be lower though not significantly so in the chromium deficient group (3.58% vs 5.29%) than in the chromium supplemented group. Chromium deficiency was seen by hypersecretion of insulin by β-cells as revealed by comparing the ratio of incremental insulin area, when graphed, to the incremental area of glucose, when graphed (insulinogenic index) and also peripheral tissue insulin resistance. The tissue insulin resistance was assessed by measuring the decreased tissue levels of cyclical AMP phosphodiesterase (cAMP PDE) activity (Striffler, et al., 1995). In order for the β-cells to manufacture insulin, it is necessary for cyclic cAMP to be hydrolyzed by phosphodiesterase during the protein coding process. If activity of this enzyme is reduced, cellular insulin resistance goes up.

The many experiments trying to alter insulin resistance and influence glucose intolerance by supplementing with chromium make the question of toxicity an important consideration. Most people consume less than 50 μg chromium per day from food

(Anderson and Kozlovsky, 1985) however, both chromium chloride and chromium picolinate were found to be non-toxic at levels of 100 mg of Cr/ kg diet in rats. Toxicity was determined by examining blood and histological measurements. This study was conducted over a 20 week period (Anderson et al., 1997).

In a study conducted in 1988, findings were that trivalent chromium was not significantly related to "the maintenance of glucose homeostasis in healthy animals". In this research with two groups of weanling rats, one group was fed a chromium deficient diet containing 0.03 mg/kg diet while the other group was fed 1 mg/kg diet chromium. The chromium was in the form of chromium chloride. At the end of 53 days there was not any difference between the groups when body weight, food intake, glycosylated hemoglobin, and plasma concentrations of glucose and insulin were compared (Flatt et al., 1989).

***Essentiality of Chromium for Humans.*** After receiving total parenteral nutrition (TPN) for three and a half years, a white female patient developed a 15% weight loss with peripheral neuropathy. An intravenous glucose tolerance test (IVGTT) was administered and although her glucose clearance was very low her insulin response was near normal. Even with glucose and insulin infusions she did not maintain her weight. The insulin infusion was stopped and a 250 μg dose of chromium as chromium chloride was added daily to her TPN for the next 2 weeks. Her response to the glucose tolerance test improved. Subsequently, the patient received a maintenance addition of about 20 μg/day of chromium from her TPN. Over the next few months the insulin infusions were stopped and the glucose infusion greatly reduced since the patient was beginning to put on weight. Her nerve conduction was improved over this period. The conclusion was that

chromium deficiency caused her glucose intolerance and other pathologies (Jeejeebhoy, et al., 1977). Since the Jeejeebhoy study, other studies have been conducted to affirm those findings (Freund, et al., 1979).

***Glucose and Insulin Metabolism During Pregnancy and Lactation.*** Glucose utilization during pregnancy is a complex process. During gestation the dam has to make physiological changes to accommodate the fetus and the feto-placental unit (PFU) as well as to prepare for lactation after parturition. In a study with rats (Herrera et al., 1991), dams were seen to put on weight during the first half of pregnancy while the conceptus changed little in weight. The dams' weight increased rapidly due to the increase in liver weight and especially the accumulating fat deposits. During this period, glucose is converted to fatty acids that are primarily stored in the adipose tissue. The main portion of the weight gain in the mother is due to increased fat deposits.

In the second half of pregnancy, the weight of the dams' livers declined as the progeny grew but lipogenesis continued until day 19 of pregnancy. The dam accumulated fat due to her increased food intake and an increase in lipoprotein lipase (LPL) activity. Fatty acids accumulated through the conversion of glucose until the $20^{th}$ day. At this time there was a decrease in lipogenesis and an increase in lipolytic activity. This activity is accompanied by a reduced uptake of fatty acids by adipose tissue and a diversion of fatty acids to the liver. The LPL activity in the liver leads to increased triglyceride production. The mother's tissues shift their food needs from glucose to ketone bodies leaving the glucose for conversion by the mammary glands to lactose and fats in the milk as well as the glucose transported across the placenta for the fetus. These metabolic shifts lead to

commonly seen hypertriglyceridemia, a condition that has adaptive advantages for the progeny (Herrera, et al., 1988; Herrera, et al., 1991).

Research has shown that during pregnancy the PFU extracts large amounts of chromium from the dam's circulation. This harvesting of chromium amounted to 9 – 16% of an intravenously injected dose. The mother rats did not retain any more of the chromium injection than non-pregnant rats despite this removal of chromium by the PFU. In tissue examination, it was found that the PFU contained 25-30% of the retained chromium from that injection. This finding was suggestive of the idea that postpartum chromium deficiency for the mother rat might be common (Wallach and Verch, 1984).

Using an euglycemic hyperinsulinemia clamp it was discovered that insulin resistance in the pregnant rat is characterized by decreased sensitivity of liver and peripheral tissues (Leturque, et al., 1984, Kuhl, 1991). Glucose production was completely suppressed at insulin concentrations >1000 μU/ml in pregnant and non-pregnant rats (Leturque, et al., 1984).

A study has been conducted to investigate the effect of different carbohydrates on glucose tolerance. Pregnant rats were fed a diet containing 50% sucrose, 50% fructose or a reference diet of rat chow. There was no significant difference between the glucose fed and the reference diet fed dams when an OGTT was given on the 19$^{th}$ day of pregnancy. The rats receiving the 50% fructose had significantly higher glucose concentrations then either other group. However, after adjusting for the baseline glucose concentrations there was no significant difference between the groups (Jen, et al., 1991).

In a study examining the glucose tolerance of rats during gestation and lactation, it was discovered that the fasting basal serum glucose levels were high during early pregnancy,

and low during late pregnancy yet normal through lactation. The basal serum glucose levels fluctuated throughout pregnancy between 7.5 and 8.5 mM early and 6.0 and 5.0 mM late pregnancy and just below 7.0 mM during lactation. During lactation, insulin values varied from a high of 2.8 mM to a low of 1.0 mM at weaning. One change seen in early pregnancy is a decrease in glucose tolerance. The insulin response was greatly increased in late pregnancy. Glucose tolerance after an IVGTT was normal during pregnancy and slightly decreased during lactation. The glucose did not rise due to increased turnover of the glucose. The increased uptake of the mammary glands was thought to be responsible for the absence of a rise in the serum glucose. Insulin responses to the infusion were increased during pregnancy and decreased during lactation (Koiter, et al., 1989).

***Gestational Diabetes.*** Glucose intolerance during pregnancy in humans has been regarded as a prediabetic state since the 1940's. Initially, the morbidity and mortality rates of the mothers was the only concern but as more statistics were accumulated it was seen that gestational diabetes was more than a prediabetic state, but rather carried an impact for the health of mother and child. An increase in risk to the fetus was noticed as the maternal glycemia increased (Kjos and Buchanan, 1999). Reports documented "a perinatal mortality rate of 8% in infants delivered to mothers who subsequently developed diabetes in middle age, compared with 2% in control subjects" (Coustan and Carpenter, 1998). A large study of 3,000 mothers with glucose intolerance, but not diagnosed with gestational diabetes, was conducted. The outcomes of these women showed that as the degree of glucose intolerance increased, the incidence of cesarean

section, preeclampsia, macrosomia, and increased length of stay in the hospital following birth increased significantly (Sermer, et al., 1995).

Two to three percent of pregnant women demonstrate glucose intolerance sufficient for a diagnosis of GDM. These women tend to be older and obese (Kuhl, 1991). While some deterioration of glucose tolerance is normal during pregnancy, those women who develop GDM risk increased morbidity and mortality for themselves and their babies.

In a recent study done with women diagnosed with GDM, supplementation with chromium picolinate (CrPic) was determined to improve glucose intolerance and lower hyperinsulinemia. This study initially looked at 20 women diagnosed with gestational diabetes. This diagnosis was made by the criteria set down by the Third International Gestational Diabetes Workshop (Jovanovic, et al, 1999). All the women were at 20 – 24 weeks of pregnancy. These women were randomized into two groups. The diet of the first group was supplemented with 4 μg/kg/body weight/d of CrPic. The second group was matched for glucose intolerance and body mass index (BMI) and received an unsupplemented diet. All the women were instructed on the "euglycemic" diet that they consumed for a period of 8 weeks. After 8 weeks, three of the women in the chromium supplemented group and four in the placebo group required insulin. Because of the number of each group requiring insulin, a third group of 10 women was given twice the original supplementation, 8 μg Cr/kg body weight/d and matched against the placebo group for weight, age, and gestational age. The same placebo group of women was followed for an additional 8 weeks. This puts the gestational age near full term in both groups.

The serum $HbA_{1c}$, glucose, insulin, and C-peptide were measured at the beginning and after 8 weeks. At the end of 8 weeks the group supplemented with 4 μg Cr/kg diet had significantly ($p<0.05$) lower glycosylated hemoglobin levels ($HbA_{1c}$) compared to their baseline values [$5.6 \pm 0.4\%$ vs. $5.2 \pm 0.6\%$ (mean ± coefficient of variation)]. Those on the 8 μg CrPic/kg body weight showed no change. However, at the end of the first 8 weeks, the 4 μg Cr/kg body weight group had both significantly lower postprandial glucose and insulin levels when compared to the baseline and to the placebo group. This was also true at the end of the second 8 week period with the 8 μg Cr/kg group (Jovanovic et al., 1999).

Different approaches to diagnosing GDM have been suggested but all include an initial risk assessment. High risk factors include: obesity, diabetes in a first-degree relative, history of glucose intolerance, previous infant with macrosomia, and current glycosuria (Kjos and Buchanan, 1999). In 1964 O'Sullivan and Mahan established the first standard for administering an oral glucose tolerance test (OGTT) during pregnancy (O'Sullivan, et al., 1964). From 986 women who registered at Boston City Hospital over a period of 4 months, the 752 women who completed the OGTT were used to establish the baseline glucose concentrations that were to become the criteria used to assess a second group of 1,013 women. The women in the first group were screened using the criteria: a family history of diabetes, the birth of a baby weighing nine pounds or more, a history of fetal death or neonatal death, congenital anomaly, prematurity, toxemia in two or more previous pregnancies, and a venous blood glucose concentration ≥ 130 mg per 100 ml. They gave this group of women 100 g of glucose. Their blood glucose levels were checked at hourly intervals for 3 hours. Of the women receiving the OGTT, 20 were

in the first trimester, 339 were in the second trimester, and 393 were in the third trimester. From the data obtained during the OGTT, categories were set up representing increasing risk of subsequent development of diabetes mellitus.

The second group of 1,013 women was given a 100 g OGTT and from the outcome of this test individuals were followed to see if the OGTT categories obtained from the first group were accurate predictors of developing diabetes mellitus during the next 8 years (O'Sullivan and Mahan, 1964). The problem with this approach was that while it was predictive of 22% of future diabetes diagnoses, it did not take into account the immediate consequence to the mother and her offspring (Kjos and Buchanan, 1999; Coustan and Carpenter, 1998). This 100 g OGTT standard has persisted and was endorsed by the American Diabetes Association in a 1986 position paper. The procedure was used by more than 75% of obstetricians and gynecologists in 1990 (Coustan and Carpenter, 1998).

The World Health Organization (WHO) criterion utilizes a 75g 2 hr OGTT. This WHO test is used to diagnose diabetes outside of pregnancy. If a pregnant woman is diagnosed with diabetes, this becomes a diagnosis of gestational diabetes. In some centers, WHO diagnosis of diabetes is based on impaired glucose tolerance (IGT). In these populations there is a high prevalence of IGT and so a test is used for the general population and identifies a greater number of individuals not just pregnant women. This criterion identifies a higher proportion of pregnancies as "abnormal" (Coustan and Carpenter, 1998).

The need to make diagnosis simpler and cheaper is made especially clear by a press release from the Centers for Disease Control and Prevention in August of 2000. The

fact that diabetes mellitus represents a serious health risk for millions of Americans and the rate of diagnosis has increased 33% during the 1990's is evidence of the importance of being able to test appropriately screened women. Of those in the age group of 30 to 39, the rate of increase in the 1990's through 1998 is 72%! (CDC, 2000). As the rate of diagnosis continues to go up, that many more people will be putting an increased pressure on the medical and healthcare industry to respond.

Carbohydrate intolerance without a diagnosis of GDM was associated with increased cesarean section deliveries, preeclampsia, macrosomia, and increased hospital stay (Sermer, et al., 1995). Those mothers diagnosed with GDM had further increases in the previous maladies and additional problems for the infant, including hypoglycemia, jaundice, respiratory distress syndrome, polycythemia, and hypocalcemia (Kjos and Buchanan, et al., 1999).

***Fructosamine.*** The need for tests that are relatively inexpensive and easy to conduct as well as representing a longer glycemic history than plasma glucose resulted in tests such as glycosylated hemoglobin and fructosamine. The fructosamine test requires a small amount of serum (0.1ml) and reagents developed to take advantage of the reducing capacity of ketoamines (fructosamines). This test demonstrated clear separation between normal and diabetic populations (Johnson, et al., 1982).

Parallel examination of an OGTT and serum fructosamine in 190 asymptomatic women was conducted during their sixth week of pregnancy. The OGTT identified 10 of the 190 as having GDM but the fructosamine test failed to identify these women. Fructosamine was only effective in the most severe cases of glucose intolerance at the sixth week of pregnancy (Huter, et al., 1992).

Plasma protein glycation using the fructosamine assay in 87 diabetic patients (Type 1 and Type 2), tested the usefulness of the fructosamine assay compared to glycated hemoglobin ($HbA_{1c}$) as an indication of glucose control. The findings showed the predictive power of fructosamine was "consistent with a conservative diagnosis of diabetes" (Mosca, et al., 1987).

To compare fructosamine, $HbA_{1c}$, and other glycated plasma proteins as a measure of glycemic control, a study was conducted using 100 Type 1 diabetics and 104 Type 2 diabetics. The findings indicated fructosamine is a potential alternative to $HbA_{1c}$ but there remained the need to understand the difference in what time frame each represents (Smart et al., 1988). $HbA_{1c}$ is an indicator of glycemic control over a period of 2-3 months while fructosamine is an indicator of glycemic control over 2-3 weeks.

In 1988 a study to determine whether to use fructosamine or $HbA_{1c}$ as a glycemic control indicator was conducted with 77 mixed Type 1 and Type 2 diabetics. Their conclusion was that there was no correlation between fructosamine and $HbA_{1c}$ as indicators, and so using both may give information that using only one would miss (Dominiczak et al., 1988).

A different study looked at the ratio between fructosamine and albumin as an indicator for GDM. They looked at 96 pregnant women. All of these women screened positive (>140 mg/dL) after a 50 g glucose load. Non-pregnant and pregnant women were given a 75 or 100 g, respectively, glucose load after a 12 hour fast. These women were matched against the diagnostic criteria set down by the National Diabetes Data Group in 1979. If a woman's serum glucose equaled or exceeded 105 mg/dL after fasting, 190 mg/dL after one hour, 165 mg/dL after two hours, and 105 mg/dL after three hours she was diagnosed with GDM. They were compared against a group of 54 non-diabetic healthy women. The fructosamine concentration and the

fructosamine/albumin ratio for pregnant women with GDM were not significantly higher than for normal pregnant women (2.05 ± 0.47 mmol/L vs 1.84 ± 0.29 mmol/L and 67 ± 16 μmol/g vs 62 ± 15 μmol/g, respectively). The conclusion was that "both fructosamine and the fructosamine/albumin ratio have low sensitivity as predictors of GDM and can therefore not be used as screening tests" (Bor, et al., 1999).

Fructosamine was tested against glycated albumins and $HbA_{1c}$ in order to evaluate response to plasma glucose changes over time. Glycated albumin and fructosamine decay half-times were shown to be 17.1 ± 2.8 and 12.2 ± 4.8 days while $HbA_{1c}$ was 34.6 ± 10.1 days. The finding was that the levels of plasma glucose detected were the "weighted mean of the preceding plasma glucose level over a considerably longer period than was previously speculated". These researchers hypothesized a step-wise change in serum glucose. What was seen was a ramping change in serum glucose (Tahara and Shima., 1995).

With technical improvements, a second-generation fructosamine assay was developed. This assay allowed for differentiation among non-diabetics, diabetics with "good control" and diabetics with "poor control". This test has significant correlation ($r=0.91$, $p<0.001$) between the assay results and the measured capillary blood glucose. This second generation assay was also compared against the "original" fructosamine test and found to have a high correlation at the baseline but greatly decreased correlation at the end of the 10 week study ($r=0.78$, $p<0.001$; $r=0.34$, $p<0.09$). The researcher did not speculate about the decline in correlation between the second fructosamine assay and the first fructsosamine assay (Cefalu, et al., 1991).

The evolution of utilizing fructosamine as a marker for determining "at risk" pregnant women has changed over the years as technique and technology have improved. An

understanding of the importance of being able to make a diagnosis of GDM has become much wider spread, as witnessed by the wide geographical research being conducted.

# CHAPTER III

## MATERIALS AND METHODS

This research using rats was a longitudinal experiment using four randomly selected groups, one non-pregnant and three pregnant. The non-pregnant and one of the pregnant groups received identical diets, containing chromium chloride, $CrCl_3$. One pregnant group received a chromium deficient diet. The last group received a diet containing chromium picolinate. The diets not deficient in chromium contained 1 mg Cr/kg diet of either Chromium Picolinate (CrPic) or chromium chloride ($CrCl_3$) plus that amount of chromium found contaminating the ingredients of the diets. The amount of chromium contamination contributed by the diet was 22.53 μg Cr/ kg diet.

Forty-one female virgin Sprague-Dawley rats (215 – 250 g) were obtained from Harlan (Indianapolis, IN) at three months of age. Eight adult Sprague-Dawley male rats were obtained for breeding.

Rats were housed in individual plexiglass cages (25 X 25 X 30 cm) at 22 °C with a 12-hour light/dark cycle (0700 to 1900 hr). Flooring consisted of PVC plastic square mesh. The rats were given deionized water and a maintenance diet containing 22.53 μg chromium/kg of diet from the day of their arrival until the day they became pregnant. All diets were a modified AIN-93G (American Institute of Nutrition) (Reeves et al., 1993) rodent diet. The modifications are described in **Table 2**. This formulation provided 3.7 kcal/g of diet mix. The mineral mix in each diet was as described for the AIN-93G diet except for the chromium. The chromium was either omitted creating a chromium

deficient diet (Cr, 22.53 ppb) or was added as either chromium picolinate or chromium chloride to give 1 mg chromium / kg diet.

Each of the minerals for the mineral mix was weighed and blended together in a ball mill (US Stoneware, Mahwah, NJ) for a period of 10 hours. This initial mixing excluded chromium to allow for a diet that had no added chromium. The mineral mix needed to prepare the chromium deficient diet was removed from the blended mineral mix, as was the amount of mix needed to prepare the chromium picolinate diet. Then the $CrCl_3$ was added to the remaining mineral mix and returned to the ball mill and allowed to turn for 5 hours. The CrPic was added to the deficient mineral mix and incorporated in a manner similar to that done for the $CrCl_3$.

The diet was mixed in two major steps. Step one included the blending of those ingredients of relatively small weight and volume. An acid washed mixing bowl and clean beaters were used. Some sucrose was used to aid mixing. Each ingredient was added and hand mixed wearing vinyl, low mineral gloves. After all the ingredients were blended by hand, a small portion (1 – 2 tablespoons) of oil was added and the mixture blended for 5 minutes.

The second step was the blending of the larger volume and weight ingredients. Each ingredient was added to a large acid washed "low chromium" plastic mixing bowl. Each newly added ingredient was hand mixed into the mixture. After the major volume ingredients were blended, the mixed small volume components were added to the large bowl and thoroughly blended into the other mixture by hand and then mechanically. Once all the ingredients were mixed they were put into a labeled plastic bag, sealed and stored

in a 4 C° refrigerator. All diets were mixed and stored in such quantity as to prevent storage longer than 3 weeks.

Each diet was assigned an acid washed measuring cup, which was used for all the feedings from that diet. Cross contamination was consistently avoided. The rats were fed their powder diets in a clean glazed ceramic bowl. The bowls were emptied and wiped clean, as needed, each day before being refilled. The ceramic bowls were replaced once a week. Uneaten diet was discarded. Each rat was given 25 – 27 g of diet each day through the pre-pregnancy.

For breeding, a male rat was placed into a cage with an individual female. Each morning the cage floor was checked for a vaginal plug as an indicator that mating had occurred. If a plug was seen, the female was randomly assigned to one of the three experimental diets until thirty females were pregnant. Ten virgin females were randomly selected for the non-pregnant control group as the experiment progressed and fed the same $CrCl_3$ diet as the pregnant group. Once a vaginal plug was discovered, the male was moved to the next available female until all the pregnancies were accomplished.

Pregnant rats were fed one of three experimental diets beginning on the first day of pregnancy. One diet contained no added chromium, one diet contained 1 mg of chromium as chromium picolinate/ kg diet, and one diet contained 1 mg of chromium as chromium chloride/ kg diet. The non-pregnant control rats were also fed the diet supplemented with chromium chloride.

As each rat became pregnant and was randomly assigned to a diet, a clean diet bowl was introduced to the cage. Through pregnancy and lactation as a dam began to eat all the food, diet dispensed was increased by approximately 5 grams.

Female rats were fed their assigned diets for the 3 weeks of gestation and through 19 days of lactation. On day 18 of pregnancy, each rat was food deprived for 4 hours but deionized water was available *ad libitum*. A blood sample from the fasted rat was taken from the tip of the tail before the rat was given a 2g/kg body weight dose of glucose as a 50% solution in water by gavage. A second sample of blood was taken 2 hours after the glucose intubation. The collected blood was chilled on ice until they could all be centrifuged. Serum was aliquoted and frozen. Food was returned to the cages after the second blood sampling and the PVC flooring was removed from the cage in order to allow the female to deliver her pups on the softer ground corncob bedding. Within 24 hours of delivery the litter was reduced to 10 when there were more than 10 pups.

On the 19$^{th}$ day of lactation or the equivalent number of days for the non-pregnant rats, a blood sample from a 4-hour fasted rat taken from the tail before the 2g/kg body wt OGTT was repeated as during pregnancy. The rat was given a 0.2 ml injection of 10 : 1 mixture of ketamine and xylazine approximately 20 minutes before the 2 hour blood sampling. Next the anesthetized rat was placed on the DEXA table (Hologic QDR 4500A, Waltham, MA) and scanned using the small animal software available with this instrument. After scanning, blood was taken from the abdominal aorta and organs and tissues were collected. Blood was chilled on ice until all samples were collected. The blood was then centrifuged and the serum aliquoted and frozen for later analysis.

When all the rats had been necropsized, the frozen blood samples were thawed and selected clinical parameters were analyzed using the COBAS FARA (Roche Diagnostic Systems, Montclaire, NJ) analyzer in accordance with the protocols found in the package inserts accompanying each reagent kit (Roche Diagnostic Systems). Serum

concentrations of glucose, fructosamine, total cholesterol, proteins, and non-esterified fatty acids were examined. In order to analyze insulin, samples were diluted 1:1 because of small sample size and analyzed using the RIA protocol as provided by Linco Research Inc., specific for rat insulin determinations. This process utilizes radioactive iodine labeling of insulin and an antigen/antiserum procedure. Samples were counted for $^{125}I$ using a gamma counter.

Data were entered in SAS (Version 8.0) software in order to run statistical analysis on the variables. Means were determined through the Proc Means routine of the SAS software. This routine provided means, n, standard error, and minimum and maximum details for each group. The Proc GLM procedure was used to evaluate treatment effects and significant differences between individual means were determined by the LS Means procedure. The level of significance was set at $p < 0.05$.

# CHAPTER IV

# RESULTS

## *Gestation*

***Glucose and Insulin.*** An oral glucose tolerance test (OGTT) of 2g/kg body weight was administered on the 18$^{th}$ day of gestation. Mean serum glucose concentration in the non-pregnant rats was significantly higher than in the pregnant rat groups at baseline. These values remained significantly higher at the 2-hour blood sampling (**Table 3**). At baseline the mean serum insulin level of the non-pregnant rat group (NP+$CrCl_3$) was significantly lower than the pregnant plus chromium chloride (P+$CrCl_3$) and the pregnant without added chromium (P-Cr) group. The basal plus chromium picolinate (P+CrPic) group was not significantly different from the NP+$CrCl_3$ or either of the other pregnant groups.

The mean serum glucose of the rats fed the P+$CrCl_3$ diet was not significantly different from, and was almost identical to that of the rats fed the P-Cr diet at both the beginning and the end of the OGTT. The mean serum insulin concentrations at baseline and at the two hour blood sampling of the P+$CrCl_3$ diet group were not significantly different from the rats fed the P-Cr diet. However, the mean serum insulin concentration declined in the pregnant P+$CrCl_3$ group to a level below that of the baseline blood sampling.

***Fructosamine, Total Serum Proteins.*** There were no significant differences in the mean serum fructosamine or protein concentrations among any of the groups.

However, the amount of blood obtained from the tail was too small to measure either variable in most of the rats (**Table 4**).

***Non-esterified Fatty Acids, Triclycerides, Cholesterol.*** The NP+$CrCl_3$group had significantly lower means of serum non-esterified fatty acid (NEFA) concentrations than the P+$CrCl_3$ or the P-Cr diets (**Table 5**). The serum NEFA concentrations of the non-pregnant group were not significantly different from the pregnant rats consuming the P+CrPic diet. The NEFA concentration of the P-Cr group was significantly higher than the P+CrPic group.

The mean serum triglyceride concentration for the non-pregnant group was significantly less than the concentrations in all the pregnant groups. The P-Cr group did not show a significant difference from the P+$CrCl_3$ group or the P+CrPic group. The P+$CrCl_3$ group showed a tendency ( $p< .08$ ) toward having a greater triglyceride concentration than the P+CrPic group. There was no significant difference in mean serum concentration of cholesterol between any of the groups.

### *Lactation*

***Glucose & Insulin.*** On the 19$^{th}$ day of lactation an OGTT of 2 g glucose/kg body weight was given to each rat after a four hour fast. At the baseline of the OGTT the mean serum glucose concentration for the non-pregnant group was significantly less ($p < 0.02$) than the P-Cr and the P+CrPic groups (**Table 6**). There were no significant differences in glucose at 2 hr; however, the 2 hour serum concentration of glucose is very high and perhaps reflects an anesthesia effect in the elevated glucose levels. There were

no significant differences between the mean serum insulin concentrations among any of the groups at either time period.

***Fructosamine, Total Serum Proteins.*** Fructosamine is an indicator of serum glucose levels over the past two to three weeks. Since the OGTT takes two hours, the fructosamine concentration should be unchanged between the endpoints and so only the final concentration has been recorded (**Table 7**). No significant difference is seen between the P+$CrCl_3$ and the P-Cr groups. There was no significant difference between any of the pregnant groups due, at least in part, to the large standard error. There was no significant difference between the mean serum protein concentrations of any of these groups. There was no significant difference in the ratio of fructosamine to serum proteins among the four groups.

***Non-esterified Fatty Acids, Triglycerides, Cholesterol.*** There was no significant difference between the mean serum non-esterified fatty acids (NEFA) of any of the groups (**Table 8**). There was no significant difference among the mean serum triglyceride concentration of any of the groups. There was no significant difference in the mean serum cholesterol among the non-pregnant group and any of the lactating groups.

***Number of Pups Delivered or Carried Through Lactation.*** No significant difference was shown for the number of pups carried through lactation for the three groups (**Table 9**). Seventeen of 30 litters were reduced to 10 pups. Litter sizes ranged from 1 pup to 15 pups. No pups died more than 48 hours after birth.

*Anatomical Measures*

***Liver Weight, Kidney Weight, and Spleen Weight.*** The mean liver weight of the groups that had been pregnant and were lactating was approximately double that of the NP+$CrCl_3$ group and this difference was highly significant (**Table 10**). The non-pregnant group kidney weight was significantly lower than the pregnant/lactating groups. The spleen weight among the groups was not significantly different. It could be speculated that the increased organ weights were related to the increased insulin sensitivity in these tissues that accompanies the decreased insulin tolerance seen in the peripheral tissues. As the insulin sensitivity in the liver increases more glucose in absorbed and the hepatocytes convert this surplus to fatty tissue, which is stored there in the liver.

***Percent Body Fat.*** The non-pregnant body fat percentage was significantly greater from the lactating groups (Table 10). Among these three groups there was no significant difference. Although problems with the DEXA lessened some numbers of samples for this analysis, it was readily seen during the autopsy that the NP+$CrCl_3$ group contained more adipose tissue than any of the pregnant groups. Among the pregnant/lactating groups, the form and concentration of chromium appeared to not make a significant difference.

# CHAPTER V

# DISCUSSION

***Pregnancy.*** All the female rats in this study were given a diet high in simple carbohydrates since it has been shown that diets high in simple sugar increase urinary chromium loss (Wright, et al., 1983, Kozlovsky, et al., 1986). The loss of chromium may impair the rats' glucose tolerance (Anderson, et al., 1990, Striffler, et al., 1995). This glucose intolerance would then be physiologically similar to gestational diabetes mellitus (GDM).

Glucose tolerance tests were administered to four male rats prior to applying this test to any of the females. This was done for three reasons. First, to provide practical experience in obtaining blood samples from the tail. Second, to provide experience in administering an oral dose of glucose to the rat. Third, to establish a likely time-frame for the return of serum glucose concentrations to "normal" after being given a glucose load. Taking repeated blood samples from the tail of a rat can result in increased hemolysis in subsequent samples. Getting a quantity of blood from the tail of a rat is a procedure of potentially limited utility after the first sample is taken. One sample was done per OGTT at two hours. These male rats received a proportional glucose dose to that administered to females (g glucose/ kg body weight) and two hours proved to be the approximate time for their blood glucose to return to normal. Gender differences and pregnancy are reasons to make using males suspect for determining how rapidly a pregnant female rat's blood glucose takes to reach baseline values.

On day 18 of pregnancy, at baseline, all three groups of pregnant rats appeared to be normoglycemic ( serum glucose < 127 mg/dL). The NP+$CrCl_3$ group were hyperglycemic (>126 mg/dL). At the end of the 2 hour oral glucose tolerance test (OGTT) the pregnant rats continued to exhibit normal glycemia while the NP+$CrCl_3$ were even more hyperglycemic. In human pregnancy, one expects to see increasing glucose intolerance and we thought it to be the case in the rat model. These normal glucose concentrations in the rat can, in part, be explained by looking at the corresponding insulin levels. The three pregnant rat groups were normal glycemic but their insulin levels were elevated above the normal range (0.5 – 2.0 ng/mL) (Morgan, et al., 1963; Linco Research, St. Charles, MO), except the P+CrPic group. The amount of insulin in these pregnant groups had increased at the end of the OGTT in all but the P+$CrCl_3$ group. The increased levels were maintaining a normal glycemic level.

The baseline insulin concentration of the P+$CrCl_3$ and the P-Cr groups were elevated above the normal range and in both cases were significantly greater than the concentration found in the NP+$CrCl_3$ group (Table 3). It would seem that chromium supplementation had no effect on the measured serum values. The insulin levels in the P-Cr and P+CrPic rats rose over the 2 hour period of the OGTT but this change was not significant. And, there were no significant differences among any of the pregnant groups at 2 hours.

Although not significant, the P+CrPic rats demonstrated the greatest glucose tolerance based on the mean measurements of serum glucose. This same group required less insulin to accomplish glucose clearance. It may be speculated that chromium picolinate made insulin utilization more efficient.

In the 1991 study by Jen, rats were fed a high sucrose diet (50% by weight). The OGTT on the 19$^{th}$ day of pregnancy, after an overnight fast, showed serum glucose values of: baseline, 4 mmol/L (about 72 mg/dL); in 2 hours, back to baseline, 4 mmol/L (Jen et al., 1991). In the Jen study, the baseline glucose concentrations were below normoglycemic levels (4.7 – 5.7 mmol/L). The glucose levels in this study, at pregnancy, are much higher than seen in the Jen study.

At day 19 of pregnancy the serum triglyceride levels of the Jen study were 150 mg/dL compared with this study's pregnant groups between 181 and 278 mg/dL. In the Jen study, the cholesterol level was considerably less than that seen in this study: 65.7 vs. group means ranging from 115 to 129 mg/dL. Normal cholesterol is in the 127 – 171 mg/dL range. The triglyceride concentration is elevated, at least in part, as a result of the increased lipolysis that accompanies late pregnancy (Herrera, et al., 1991). Cholesterol concentrations found in this study are near normal values. This might indicate that the reduction in cholesterol that might be expected from experiments with chromium (Schroeder et al., 1971) is offset by the increased cholesterol resulting from the increased lipolysis (Herrera et al., 1991).

In Koiter's study at day 18 of pregnancy, serum glucose concentrations were near 5.8 mmol (104.5 mg/dL), a value very similar to those found in this study: 5.7 – 5.8 mmol/L (103 – 104 mg/dL). The Koiter study used a 2-hour fast before conducting the OGTT as opposed to the four hours in this study.

Serum proteins undergo a non-enzymatic reaction with fructose, a ketone sugar, and one of the two components that make up sucrose. This reaction occurs in such a way that the concentration of fructosamines (glycated proteins) reflects the faster removal of

glycosylated proteins than red blood cells. This gives the glycosylated proteins a shorter half life than other blood proteins, a period of 2 to 3 weeks (Day, et al., 1979; Johnson, et al., 1982; Mosca, et al., 1987; Smart, et al., 1988; Hughes, et al., 1995). Comparing the apparent glucose tolerance among the experimental groups, one expects the group with the lowest glucose clearance to be the group with the highest fructosamine levels. Baseline glucose concentrations were normoglycemic in all three pregnant groups.

The abundance of free fatty acids found in the serum at the $18^{th}$ day of pregnancy is apparently those fatty acids released as a result of lipolysis, which accompanies late pregnancy (Herrera, et al., 1991). As the mother is transitioning from nurturing the fetuses to nurturing the litter, lipogenesis decreases and lipolysis increases. This process makes fatty acids available to the fetuses during their greatest growth period. During this time the dam also reserves glucose for the fetuses by beginning to utilize ketones for her own cellular food source. The liver, which usually exports triglycerides, begins to take up triglycerides and makes ketonic substrates (Herrera, et al., 1991).

The baseline non-esterified fatty acids (NEFA) found in the non-pregnant group are in the normal range, 0.68 – 1.02 mmol/L (Young, 1998) (Table 5). The NEFA in the pregnant groups are elevated as compared to that found in the non-pregnant group. Only the P+CrPic, whose serum concentrations are intermediate between the non-pregnant and the other pregnant groups, is not significantly different. The P-Cr group was significantly greater than the P+CrPic group but not significantly different from the P+$CrCl_3$ group. Chromium seems to be involved in NEFA metabolism. The P+$CrCl_3$ group is significantly different from the non-pregnant group. What this could mean is that the P-Cr group is less able to clear NEFA's either through conversion to glucose or across the

placenta while the P+CrPic group is the most able to clear the NEFA's, assuming that both groups are releasing NEFA's at an equal rate. This points to the need for more research that could examine this point.

The triglyceride concentrations are significantly lower in the NP+$CrCl_3$ than in any of the pregnant groups. The NP+$CrCl_3$ group exhibited near normal concentration of triglycerides (1.09 – 1.10 mmol/L) where the normal range is less than 1.8 mmol/L. The pregnant groups demonstrate a wide range of concentrations that might be significantly different from each other if it were not for their large variability.

***Lactation.*** During lactation, the dams experience increasing physiological stress as the progeny increase in size, consuming larger amounts of milk. On the 19$^{th}$ day of lactation the P+$CrCl_3$ group, alone, was not significantly different from the NP+$CrCl_3$ group in terms of serum glucose. The remaining two pregnant groups are significantly lower than the non-pregnant group. At baseline, the corresponding serum insulin concentrations among the four groups demonstrated no significant differences. Of note is the P+CrPic group. This group's glucose concentration is significantly less than the NP+$CrCl_3$ group. Among the pregnant groups, this group's mean glucose concentration is the least and the insulin concentration is the highest. Surprisingly, the P-Cr group measured a similar glucose level to the other lactating groups and yet had the lowest insulin concentration. One might have thought that the decreased chromium in the diet would result in less glucose tolerance as seen in a greater concentration of insulin to control the glucose intolerance. There seems to be little difference between the different forms of chromium. These last two groups point up the need for further study of the interaction of lactation and chromium.

At the end of the OGTT the glucose concentrations are nearly double the baseline levels. This may be at least partly explained as a response to the anesthetic (Aynsley-Green, et al., 1973, Reyes Toso, et al., 1995). In the Aynsley-Green study ketamine was found to have "no effect on blood glucose and plasma insulin" although some increase was measured. This particular study looked at the effect of ketamine and other anesthetics on male Wistar rats which had been starved for 48 hours and these differences from Sprague-Dawley female rats, most of which were pregnant, hardly are comparable. But these researchers go on to quote a study by Benke in 1971 looking at ketamine in which the blood glucose showed a marked increase in alloxan diabetic rats.

In a further study that looked at the effect of anesthetics on blood glucose, Reyes Toso used male Wistar rats that were given an intravenous glucose tolerance test after an overnight fast. The findings were an increase in blood glucose and an inhibition of insulin production (Reyes Toso, et al., 1994).

Fructosamine concentrations have been measured in several studies. In 1979 Day established that rats would make a good model for "glucosylation of rat albumins". Since then, nearly all the studies dealing with fructosamine values have been conducted with human subjects. It is for humans that the use of fructosamine as a marker is beginning to be developed.

The 1991 study of Jen, using Sprague-Dawley rats, near the end of lactation (day 21), a glucose level of 104 mg/dL. These rats had been fasted overnight and then subjected to the surgical implantation of silastic cannula into their external jugular. The cannula allowed blood to be drawn from the rat at baseline and during an OGTT. The rats were allowed to recover from this surgery for 3 to 4 hours before blood samples were

taken. The serum glucose in the present study at day 19 and baseline during lactation was about 24% higher (128 – 135 mg/dL) then in the Jen study. The diet in the Jen study was 50% sucrose by weight as in this study.

In the 1983 study by Burnol (Burnol, et al., 1983) the serum glucose at day 19 of lactation was 83 mg/dL as compared with this study measurement of 128 – 135 mg/dL. In the present study the rats exhibited about a 35% higher concentration than seen in the Burnol study. The reason for this difference is unclear. In the Burnol study, rats were fed a rat chow containing 65% carbohydrates in unspecified form but presumably complex carbohydrate. All three diets in this study for the three pregnant groups contained sucrose at 50% by weight.

The 1989 study by Koiter demonstrated insulin levels of about 1.4 ng/dL (about 30 mU/L) at day 19 of lactation after a 2 hour fast. The rats in the present study had an OGTT performed on day 19 of lactation and the range from 1.5 – 2.3 ng/dL. These values are quite similar.

The abundant triglycerides are a ready source of fats and glycerol to make milk in the mother's mammary glands. During late pregnancy and into lactation the mammary glands and liver become insulin sensitive tissues while the peripheral tissues become insulin resistant (Herrera, et al., 1991). As the pups grow and consume larger quantities of milk, the demand on the dam to supply this resource increases. The demand during the period of lactation is greater than during pregnancy. Lipolysis and diet supply the dam with these resources until such time as the pups become weaned.

No significant differences were seen in the serum cholesterol levels although some studies have shown that chromium supplementation can help lower serum

cholesterol (Schroeder, 1968). At the 19$^{th}$ day of lactation, the NEFA concentration was not significantly different among groups and again the large variation probably prevents significance among the pregnant groups.

The mean liver weight of Dr. Jen's rats was less than that found in the groups that had been pregnant in this study: [(9.7 vs 12.5 - 15.0 g ) Table 10]. Some of the differences may be due to this study's manipulation of chromium. During pregnancy and lactation, the liver becomes increasingly insulin sensitive (Herrera, et al., 1991). Since chromium has been shown to facilitate the function of insulin, the liver takes in increasing amounts of glucose that is stored or converted to glucagons. Increased stores and processing cause this organ to become larger. Kidney weight like liver weight was significantly lower in the NP+$CrCl_3$ group compared to all three pregnant groups. During this period of increased resource utilization, there is a concomitant increase in the waste products produced. This is reflected in the increased size of the kidney. There was no significant difference among the groups regarding spleen weight. Percent body fat was significantly greater in the NP+$CrCl_3$ compared to all three pregnant groups. The utilization of body fat to maintain sufficient levels of milk production during lactation is seen in the much lower body fat of the lactating rats.

# CHAPTER VI

## SUMMARY, IMPLICATIONS, AND SUGGESTIONS

In summary, whether pregnant rats consumed adequate concentrations of chromium as chromium chloride ($CrCl_3$) or chromium picolinate (CrPic) or a chromium deficient diet, they all appeared to maintain glucose tolerance better than non-pregnant rats. This seems to be made possible by increased insulin production.

Serum non-esterified fatty acid (NEFA) levels were significantly lower in the CrPic group compared with the deficient group during pregnancy. While insignificant, the CrPic group had concentrations of NEFA that were intermediate between the lower values of the non-pregnant group and the higher values of the $CrCl_3$ group. The small number of samples makes this impossible to interpret.

The triglyceride concentration was significantly higher in all three pregnant groups as compared to the non-pregnant group. The CrPic group was not significantly different from the other pregnant groups but mean levels were generally lower. This could lead one to speculate that CrPic may have a place in lowering serum triglycerides.

During lactation, the glucose concentration of the CrPic and the P-Cr groups was significantly lower than the non-pregnant group. While not significantly different, the mean numerical values in the CrPic group measured the lowest at baseline of the OGTT among the lactating groups. The insulin values on the $19^{th}$ day of lactation showed no significant differences among groups at baseline of the OGTT. It may be inferred that during lactation (day 19) dietary form and concentration of chromium played little active

part in controlling glucose tolerance or that anesthesia effects overwhelmed the physiological response to lactation by raising the glucose concentration and lowering the insulin concentration. Serum lipids were not significantly different among any of the four groups for any parameters measured: NEFA, triglycerides, or cholesterol. Whether glucose clearance is enhanced by the presence of chromium in either form cannot be determined from this research.

The liver weight was significantly greater in all three lactating groups than in the non-pregnant group. The mean liver weight of the CrPic group was the least of the lactating groups, which may indicate greater glucose sensitivity. It may be that chromium in the form of chromium picolinate can maximize glucose clearance as compared to other forms of chromium. Further investigation of the uptake of glucose in the peripheral tissue during pregnancy and lactation should be investigated. The kidney weight among the lactating groups was significantly greater than seen in the non-pregnant group. Significance due to form and concentration of chromium is not determinable from the data. There was no significant difference among the spleen weight data.

The percent body fat differences between the non-pregnant group and the lactating groups were significant. While not significant among the pregnant groups, the percent body fat was lowest in the CrPic group.

The data from this research makes any conclusive statement regarding chromium in form or concentration impossible to make. What can be said is that there seemed to be general trend, though insignificant, for 1 mg Cr as CrPic/kg diet as a supplement to aid glucose clearance and improve glucose metabolism.

Judging by the glucose levels and contrary to the expected results seen in humans, pregnancy in rats seems to be a time of greater glucose utilization and tolerance. This may be due to the proportionally greater amount of biomass represented by the developing litter and its needs and the physiological structures needed to grow and support that litter.

***Hypothesis testing results.***

H01: This hypothesis is rejected since significant differences in the serum glucose and insulin concentrations between non-pregnant and pregnant groups is seen. However, there were not significant differences among the chromium deficient diet, chromium chloride diet, and the chromium picolinate diet groups.

H02: This hypothesis is rejected. Since significant differences were seen in the serum glucose concentration but not in the insulin concentrations between the non-pregnant and pregnant groups. There were not significant differences among the chromium deficient diet, chromium chloride diet, and the chromium picolinate diet groups.

H03: This hypothesis is accepted.

H04: This hypothesis is accepted.

H05: This hypothesis is partially since significant differences were seen in both the serum non-esterified fatty acid and triglyceride concentrations between non-pregnant and

pregnant groups. However, no significant differences were seen in total cholesterol and total proteins among the chromium deficient diet, chromium chloride diet, and the chromium picolinate diet groups.

H06: This hypothesis is accepted.

**TABLE 2**

Research diet components

| Ingredient | Maintenance Diet | Basal Research Diet |
|---|---|---|
| | g/kg | g/kg |
| Sucrose | 500.0 | 500.0 |
| Casein | 140.0 | 200.0 |
| Soybean Oil | 40.0 | 100.0 |
| Fiber | 50.0 | 50.0 |
| Mineral Mix[1] | 35.0 | 35.0 |
| Vitamin Mix | 10.0 | 10.0 |
| L-Cystine | 1.8 | 3.0 |
| Choline bitartrate | 2.5 | 2.0 |
| Cornstarch | 220.7 | 100.0 |

[1] No chromium was added to the mineral mix for the basal research diet. Either chromium chloride or chromium picolinate were later added to be a level of 1 mg Cr/ kg diet for the remaining diets

## TABLE 3

Pregnancy and form and concentration of chromium effects on glucose and insulin parameters as determined by an Oral Glucose Tolerance Test (OGTT).[1]

| Measures | NP+$CrCl_3$[2] | P+$CrCl_3$[3] | P-Cr[4] | P+CrPic[5] | *P* |
|---|---|---|---|---|---|
| Glucose (mg/dL) | | | | | |
| Baseline | 143 ± 6[a] | 109 ± 5[b] | 105 ± 2[b] | 103± 3[b] | <0.0001 |
| (n) | (11) | (6) | (10) | (7) | |
| 2 hour | 158 ± 10[a] | 116 ± 4[b] | 117 ± 5[b] | 107 ± 6[b] | <0.0001 |
| (n) | (10) | (7) | (10) | (8) | |
| Insulin (ng/ml) | | | | | |
| Baseline | 1.067 ± 0.133[b] | 2.168 ± 0.583[a] | 2.276 ± 0.304[a] | 1.452 ± 0.263[ab] | 0.0361 |
| (n) | (9) | (7) | (10) | (9) | |
| 2 hour | 1.323 ± 0.232 | 1.949 ± 0.246 | 2.428 ± 0.357 | 1.866 ± 0.270 | 0.0610 |
| (n) | (10) | (7) | (10) | (9) | |

[1] Mean ± SE. Blood sample was taken from the tail after a four-hour fast on the 18th day of pregnancy. OGTT used 2 g/kg body weight glucose load. Significant differences are indicated by differing letters in each row.
[2] NP+$CrCl_3$ = Non-pregnant plus Chromium Chloride. [3] P+$CrCl_3$ = Pregnant plus Chromium Chloride.
[4] P-Cr = Pregnant plus Chromium deficient diet. [5] P+CrPic = Pregnant plus Chromium Picolinate.

**TABLE 4**

Pregnancy and form and concentration of chromium effects on serum fructosamine and total protein.[1]

| | NP+$CrCl_3$[2] | P+$CrCl_3$[3] | P-Cr[4] | P+CrPic[5] | |
|---|---|---|---|---|---|
| Measures | | | | | *P* |
| Fructosamine (umol/L) | | | | | |
| Baseline | 152.3 ± 8.7 | 144.0 ± 29.0 | 119.0[6] | 117.5 ± 12.5 | 0.3474 |
| (n) | (6) | (2) | (1) | (2) | |
| Serum Protein (g/L) | | | | | |
| Baseline | 84 ± 2 | 79[6] | 75[6] | 69 ± 4 | 0.0903 |
| (n) | (6) | (1) | (1) | (2) | |

[1] Mean ± SE. Blood sample is taken from the tail after a four-hour fast on the 18th day of pregnancy.
[2] NP+$CrCl_3$ = Non-pregnant plus Chromium Chloride. [3] P+$CrCl_3$ = Pregnant plus Chromium Chloride.
[4] P-Cr = Pregnant plus Chromium deficient diet. [5] P+CrPic = Pregnant plus Chromium Picolinate.
[6] These single entries are raw data and do not have standard errors.

## TABLE 5

Pregnancy and form and concentration of chromium effects on lipid serum parameters. [1]

| Measures | NP+$CrCl_3$ [2] | P+$CrCl_3$ [3] | P-Cr [4] | P+CrPic [5] | *P* |
|---|---|---|---|---|---|
| Non-esterifed Fatty Acids | | | | | |
| (mmol/L) | $0.705 \pm 0.077^{c}$ | $1.140 \pm 0.125^{ab}$ | $1.274 \pm 0.0970^{a}$ | $0.907 \pm 0.115^{bc}$ | 0.0013 |
| (n) | (11) | (4) | (3) | (3) | |
| Triglyceride | | | | | |
| (mmol/L) | $1.06 \pm 0.26^{b}$ | $3.13 \pm 0.65^{a}$ | $2.45 \pm 0.30^{a}$ | $2.04 \pm 0.28^{a}$ | 0.0027 |
| (n) | (9) | (3) | (10) | (7) | |
| Total Cholesterol | | | | | |
| (mmol/L) | $3.08 \pm 0.10$ | $3.33 \pm 0.34$ | $3.15 \pm 0.13$ | $2.98 \pm 0.14$ | 0.6386 |
| (n) | (9) | (6) | (10) | (6) | |

[1] Mean ± SE. Blood sample was taken from the tail after a four-hour fast on the 18th day of pregnancy. Significant differences are indicated by differing letters in each column.

[2] NP+$CrCl_3$ = Non-pregnant plus Chromium Chloride. [3] P+$CrCl_3$ = Pregnant plus Chromium Chloride.

[4] P-Cr = Pregnant plus Chromium deficient diet. [5] P+CrPic = Pregnant plus Chromium Picolinate.

## TABLE 6

Lactation and form and concentration of chromium effects on glucose and insulin serum parameters as determined by OGTT[1]

| Measures | NP+$CrCl_3$[2] | P+$CrCl_3$[3] | P-Cr[4] | P+CrPic[5] | *P* |
|---|---|---|---|---|---|
| Glucose | | | | | |
| (mg/dL) | | | | | |
| Baseline | 145 ± 4[a] | 135 ± 4[ab] | 132 ± 3[b] | 128 ± 4[b] | 0.0220 |
| (n) | (9) | (8) | (10) | (10) | |
| 2 hour | 318 ± 27 | 322 ± 36 | 259 ± 18 | 277 ± 35 | 0.3664 |
| (n) | (9) | (8) | (10) | (10) | |
| Insulin | | | | | |
| (ng/ml) | | | | | |
| Baseline | 1.350 ± 0.205 | 1.135 ± 0.255 | 0.951 ± 0.107 | 1.185 ± 0.184 | 0.5002 |
| (n) | (9) | (8) | (10) | (9) | |
| 2 hour | 0.723 ± 0.207 | 0.619 ± 0.115 | 0.621 ± 0.085 | 0.593 ± 0.175 | 0.9360 |
| (n) | (9) | (8) | (10) | (10) | |

[1] Mean ± SE. Blood sample was taken from the tail after a four-hour fast on the 19th day of lactation. OGTT used 2 g/kg body weight glucose load. Significant differences are indicated by differing letters in each column.
[2] NP+$CrCl_3$ = Non-pregnant plus Chromium Chloride. [3] P+$CrCl_3$ = Pregnant plus Chromium Chloride.
[4] P-Cr = Pregnant plus Chromium deficient diet. [5] P+CrPic = Pregnant plus Chromium Picolinate

## TABLE 7

Lactation and form and concentration of chromium effects on fructosamine and protein serum.[1]

| Measures | NP+$CrCl_3$[2] | P+$CrCl_3$[3] | P-Cr[4] | P+CrPic[5] | *P* |
|---|---|---|---|---|---|
| Fructosamine (μmol/L) | | | | | |
| 2 hour | 193.3 ± 48.1 | 128.5 ± 10.4 | 119.2 ± 6.1 | 124.1 ± 14.5 | 0.1510 |
| (n) | (9) | (8) | (10) | (10) | |
| Serum Protein (g/L) | | | | | |
| 2 hour | 73 ± 5 | 65 ± 6 | 59 ± 2 | 62 ± 5 | 0.1590 |
| (n) | (9) | (8) | (10) | (10) | |
| Fructosamine/ Serum Protein | 2.76 ± 8.0 | 2.00 ± 0.06 | 2.01 ± 0.06 | 1.97 ± 0.06 | 0.4307 |
| (n) | (9) | (8) | (10) | (10) | |

[1] Mean ± SE. Blood sample is taken from the tail after a four-hour fast on the 19th day of lactation.
[2] NP+$CrCl_3$ = Non-pregnant plus Chromium Chloride. [3] P+$CrCl_3$ = Pregnant plus Chromium Chloride.
[4] P-Cr = Pregnant plus Chromium deficient diet. [5] P+CrPic = Pregnant plus Chromium Picolinate.

## TABLE 8

Lactation and form and concentration of chromium effects on lipid serum parameters.[1]

| Measures | NP+$CrCl_3$[2] | P+$CrCl_3$[3] | P-Cr[4] | P+CrPic[5] | *P* |
|---|---|---|---|---|---|
| Non-esterifed Fatty Acids (mmol/L) | | | | | |
| 2 hour | 0.528 ± 0.062 | 0.810 ± 0.147 | 1.072 ± 0.338 | 0.695 ± 0.084 | 0.2795 |
| (n) | (9) | (8) | (10) | (10) | |
| Triglyceride (mmol/L) | | | | | |
| 2 hour | 0.96 ± 0.13 | 0.58 ± 0.07 | 0.77 ± 0.09 | 0.90 ± 0.18 | 0.2270 |
| (n) | (9) | (6) | (10) | (10) | |
| Total Cholesterol mmol/L) | | | | | |
| 2 hour | 2.74 ± 0.16 | 3.00 ± 0.23 | 2.41 ± 0.28 | 2.57 ± 0.31 | 0.4595 |
| (n) | (9) | (8) | (10) | (10) | |

[1] Mean ± SE. Blood sample was taken from the tail after a four-hour fast on the 19th day of lactation. Only the 2hr blood sample was analyzed.
[2] NP+$CrCl_3$ = Non-pregnant plus Chromium Chloride. [3] P+$CrCl_3$ = Pregnant plus Chromium Chloride.
[4] P-Cr = Pregnant plus Chromium deficient diet. [5] P+CrPic = Pregnant plus Chromium Picolinate.

## TABLE 9

Mean number of pups in each litter

| | P+$CrCl_3$[1] | P-Cr[2] | P+CrPic[3] | *P* |
|---|---|---|---|---|
| Mean number of pups | 8.5 ± 1.2 | 8.0 ± 1.0 | 8.9 ± 1.0 | 0.8183 |

[1] P+$CrCl_3$ = Pregnant plus Chromium Chloride.
[2] P-Cr = Pregnant plus Chromium deficient diet.
[3] P+CrPic = Pregnant plus Chromium Picolinate.

**TABLE 10**

Pregnancy, lactation, and the form and concentration of chromium effects on body and organ weights and body fat[1]

| Measures | NP+$CrCl_3$[2] | P+$CrCl_3$[3] | P-Cr[4] | P+CrPic[5] | *P* |
|---|---|---|---|---|---|
| Liver weight (g) | $7.36 \pm 0.30^b$ | $14.97 \pm 1.18^a$ | $14.64 \pm 0.29^a$ | $12.61 \pm 0.72^a$ | <0.0001 |
| g/100 g bw | $2.72 \pm 0.13$ | $5.42 \pm 0.44$ | $5.39 \pm 0.20$ | $4.61 \pm 0.30$ | <0.0001 |
| (n) | (9) | (8) | (10) | (10) | |
| Kidney weight (g) | $1.48 \pm 0.05^b$ | $1.79 \pm 0.06^a$ | $1.78 \pm 0.04^a$ | $1.82 \pm 0.05^a$ | <0.0001 |
| g/100 g bw | $0.54 \pm 0.02$ | $0.65 \pm 0.02$ | $0.64 \pm 0.01$ | $0.66 \pm 0.02$ | <0.0001 |
| (n) | (9) | (8) | (10) | (10) | |
| Spleen weight (g) | $0.70 \pm 0.03$ | $0.83 \pm 0.05$ | $0.79 \pm 0.02$ | $0.79 \pm 0.05$ | 0.1066 |
| g/100 g bw | $0.26 \pm 0.01$ | $0.30 \pm 0.02$ | $0.29 \pm 0.01$ | $0.29 \pm 0.02$ | 0.2027 |
| (n) | (9) | (8) | (10) | (10) | |
| Percent Body Fat | $10.43 \pm 1.20^a$ | $3.30 \pm 0.43^b$ | $3.48 \pm 0.63^b$ | $2.64 \pm 0.46^b$ | <0.0001 |
| (n) | (6) | (7) | (5) | (7) | |

[1] Mean ± SE. Organs taken of the 19th day of lactation. Significant differences are indicated by differing letters in each column.
[2] NP+$CrCl_3$ = Non-pregnant plus Chromium Chloride. [3] P+$CrCl_3$ = Pregnant plus Chromium Chloride.
[4] P-Cr = Pregnant plus Chromium deficient diet. [5] P+CrPic = Pregnant plus Chromium Picolinate.

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# VITA

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