Iron Depletion Therapy and Chromium Supplementation for Improving Insulin

by

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ABSTRACT

The effects of iron and chromium blood concentrations have been linked to blood glucose control in diabetics. It is suggested that iron causes oxidative stress in the beta cells of the pancreas and adipocytes creating insulin insufficiency and resistance. Chromium is believed to increase the action of insulin through its biologically active molecule chromodulin. Both of these mechanisms are not clear. This 20 week case study tests the feasibility of combining iron depletion therapy followed by chromium supplementation to improve insulin sensitivity. This single case study followed a protocol of two blood donations separated by eight weeks followed by chromium supplementation of 250 μg of chromium picolinate once a day four weeks after the second blood donation. Fasting blood draws were taken at baseline, post blood draws and pre and post chromium supplementation. Results were not promising for the first hypothesis of lowering HbA1c, but the results were promising for the second hypothesis of improving insulin sensitivity by lowering the HOMA score.

DEDICATION

This is dedicated to my family, my husband Damian and my children Adara, Marcus, Jason, and Chanel, who fill my life with joy and the power to succeed. I am grateful that God has blessed me with the Love and Support of you all.

I am also grateful to my brothers William and Robert who showed strength, courage, and support as we sent out loving mother and grandmother to heaven. We Love and Remember you Debra E. Johnson and Carmen A. Henry.

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I would like to thank God for being a constant source in my life and aligning me with Arizona State University and its outstanding faculty and staff. I would like to especially thank Dr. Carol Johnston for guiding me and supporting me in my endeavors at ASU and my future endeavors as a health care provider.

Dr. Sandra Mayol-Kreiser and Dr. Christy Lespron have been a tremendous support in my learning experience at ASU and a valued part of my committee, thank you. Their commitment to excellence and dedication to help students succeed is unsurpassed. Dr. Mayol-Kreiser takes students to the next level with her simulation labs and vast knowledge while Dr. Lespron takes complex biochemical reactions and simplifies them while elevating the level of enjoyment with her kindness. Thank you to all of my friends and classmates that have shared laughs and other expressions during this journey. I am grateful and honored to share this experience together.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER 1

INTRODUCTION

Overview

Type 2 diabetes produces significant health impacts on a primary and secondary level with an economic burden of approximately $245 billion for diagnosed cases of diabetes; which does not account for the burden of the undiagnosed (American Diabetes Association, April 2013). The Primary condition of uncontrolled blood glucose control leads to secondary illnesses such as neuropathy, blindness, and amputations which significantly decreases quality of life (McCormack & Grant, 2013) (American Diabetes Association,). Understanding the ability to control blood glucose concentrations through improving insulin secretion and sensitivity is critical for improving health outcomes in individuals with type 2 diabetes. Current literature recommends increasing physical activity and reducing body weight which is important in improving blood glucose concentrations (McCormack & Grant, 2013) (American Diabetes Association,2013). This study explored the impact of initial iron depletion therapy through blood donation combined with subsequent chromium supplementation on insulin secretion, insulin sensitivity, and daily blood glucose control. Blood donation has been shown to reduce iron concentrations and improve insulin production through reducing pancreatic inflammation (Hansen, Moen, & Mandrup-Poulsen, 2014). Chromium is associated with improved glucose uptake into cells through chromodulin (Quarles Jr, Marcus, & Brumaghim, 2011). Chromodulin is an intercellular protein that binds chromium III in cells and increases tyrosine kinase activity to amplify the strength of insulin receptors to

bind insulin (Quarles Jr et al., 2011). Increasing the strength of insulin binding improves the expression of GLUT 4 to the cells plasma membrane. GLUT 4 is an insulin dependent transporter that brings glucose into muscle and adipose cells for energy production (Quarles Jr et al., 2011).

Improving the amount of chromium crossing the plasma membrane is significant in improving the activity of chromodulin. Chromium is a dietary essential found in Brewer's yeast, lobster tail, mushrooms, chicken, shrimp, and black pepper (R. A. Anderson, 1981). The American diet typically contains about 5 to 125 μg of chromium (R. A. Anderson, 1981). Chromium interacts with other dietary components affecting its bioavailability. Iron in particular impacts chromium status. High dietary iron can reduce chromium absorption (Quarles Jr et al., 2011). Moreover, high iron status is believed to reduce chromium transport to cells (Quarles Jr et al., 2011). Transferrin is the protein that transports chromium into the cell which is also the primary carrier of iron (III). Reducing iron status through blood donations will lower ferritin concentrations and increase affinity for chromium to bind the C-lobe of transferrin (Quarles Jr et al., 2011) (Peffer et al., 2013). Hence, theoretically, reducing iron status via blood donations in individuals with type 2 diabetes and high iron status would enhance chromium delivery to cells, an effect that would be amplified by chromium supplementation.

This research sought to expand upon the understanding of treatments to improve glucose control in individuals with diabetes using blood donation with chromium supplementation. Current research correlates increased ferritin concentrations with elevated HbA1c concentrations (Batchuluun et al., 2014)and a reduction in HbA1c and

ferritin with whole blood donation (Hansen et al., 2014) (Peffer et al., 2013). Chromodulin, the biologically active from of chromium, research related to treatment improvements for type 2 diabetics necessitates further exploration of the benefit in human supplementation due to the lack of knowledge depth (Chen, 2011) . Additionally, to our knowledge there is no research in the literature examining these combined methods of chromium supplementation and iron depletion through blood donation. From our review many chromium studies fail to explore the role of iron in relation to chromium. Men tend to have higher transferrin saturation and higher iron stores versus women and will compose the study sample (McLaren, Li, Gordeuk, Hasselblad, & McLaren, 2001). Successful outcomes will include an increase in serum chromium concentrations, lower HbA1c, improved HOMA, and daily fasting blood glucose management within healthy limits for type 2 diabetes. Increased chromium concentrations will indicate blood donations improved chromium (III) loading on transferrin for purposes of this study. The need to further understand the implications of iron status and diabetes is imperative to further understanding of diabetes management (Hansen et al., 2014).

The Study Purpose

This case study explored the relationship of blood donation and chromium supplementation with improved diabetes management. This 20 week study included 2 blood donations separated by 8 weeks to decrease ferritin concentrations to improve HbA1c levels and increase affinity for chromium III to bind transferrin. Furthermore, supplementation of chromium as 250 µg /d of chromium picolinate was investigated to measure the effects on insulin sensitivity via chromodulin. The Participant tracked his

daily waking blood glucose and self-report taking chromium picolonate orally on a compliance calendar to confirm program consistency.

It is our belief that the combination of iron depletion through blood donations followed by chromium supplementation will show improvement in blood glucose markers. This case study was intended to be a feasibility exploration to support larger studies.

Research Aim and Hypothesis

H1: Phlebotomy and chromium treatments will improve glucose management as indicated by daily fasting glucose measurement and HbA1c levels in individuals with T2D.

H2: Phlebotomy and chromium treatments will improve insulin sensitivity as measured by HOMA in individuals with T2D.

Definition of Terms

Chromium Picolinate: A form of biologically active chromium salt that is used as an oral supplement.

Chromodulin: An amino acid residue made of carboxylate, cysteine, glycine, aspartate and glutamate which bind up to four chromium ions. It is also known as a low molecular weight substance capable of binding chromium (Chen, 2011) (Vincent, 2001).

HbA1c: Measures the amount of glucose attached to red blood cells. This gives a measure of how well blood glucose has been managed over an eight to twelve week period. This is also called glycated hemoglobin (Ezenwaka, Seales, Surujlal, & Mathura, 2009).

HOMA: Homeostasis model assessment-estimated insulin resistance (HOMA-IR), this is used to measure insulin resistance in diabetes research (Qu, Li, Rentfro, Fisher-Hoch, & McCormick, 2011).

Delimitations

The participants that met the inclusion criterion had been diagnosed by a Medical Doctor (by self-report) and displayed an elevated ferritin concentration and transferrin saturation based on sex as men tend towards higher levels (McLaren et al., 2001). Blood values set were acceptable values that indicated satisfactory management of type 2 diabetes mellitus which allowed for reasonable deductions that treatment protocol would produce results (American Diabetes Association,). Excluded participants included those with low iron status, current chromium supplementation, and elevated inflammation markers. This population is comfortable with daily finger pricks which elevates compliance and participation for study length. This study lasted for 20 weeks.

Limitations

The participant is a Type 2 diabetics and his illness is subject to change in severity which presented the need for adjustment in medications not to include

administering of insulin. Change of lifestyle had potential to affect blood glucose concentrations and HbA1c (McCormack & Grant, 2013) (American Diabetes Association,). Oral ingestion of supplements was self-reported and relied upon accuracy and consistency of participant.

CHAPTER 2

REVIEW OF LITERATURE

Overview

Type 2 diabetes mellitus is a complex disease that has shown to be improved with alternative treatments and lifestyle changes (American Diabetes Association, 2014). Understanding multiple approaches to minimize cost of treatment and management, and progression of this disease is critical due to the many other illnesses and injuries that type 2 diabetes mellitus causes (McCormack & Grant, 2013) (Pscherer, Dippel, Lauterbach, & Kostev, 2012) (Viitasalo et al., 2012) (Kannan, 2012). Iron overload and chromium deficiency has shown to be associated with type 2 diabetes mellitus (Bao, Rong, Rong, & Liu, 2012; Basaki, Saeb, Nazifi, & Shamsaei, 2012). The question this research sought to answer is how iron depletion combined with chromium supplementation affects blood glucose control. The effects of combining these therapies to improve blood glucose concentrations in type 2 diabetics are unknown. These treatments offer a cost effective method that may be added to lifestyle changes with the possibility of high compliance.

The goal of this literature review was to understand current knowledge relating to the impact of type 2 diabetes mellitus, the effects of iron overload on the organ systems in type 2 diabetes, specifically impairment in the endocrine function of the pancreas, the effects of chromium in the diabetic condition and, finally, the benefits of iron depletion with chromium supplementation on improving healthy blood glucose concentrations.

Type 2 Diabetes Mellitus Overview

The pathogenesis of type 2 diabetes mellitus is different than other forms of diabetes in that most affected have healthy functioning of blood glucose control until something goes wrong (American Diabetes Association, April 2013). The progression of this disease is a combination of decreased insulin secretion from the beta cells of the pancreas and insulin resistance which decreases the cells ability to bring glucose in for energy production. The beta cells of the pancreas begin to die or fail; the insulin receptors on the cells begin to lose sensitivity and control, and if the patient is obese controls of hunger are out of balance; which results in difficulty in managing compliance to diet regulations to control glucose (American Diabetes Association, April 2013). This disease is devastating on a personal, community and global level. This discussion will cover the impact this disease has on the health of individuals and the financial burden of society. This illness is a worldwide problem that is expanding rapidly (Weber, 2010). A continual development of methods to manage healthy blood glucose concentration in type 2 diabetics in a cost effective manner is vital (American Diabetes Association, 2014).

The American Diabetes Association recognizes diabetes as an HbA1c level greater than 6.5%. This measure gives a look at blood glucose levels over a 60-90 day period according to the ADA (American Diabetes Association, 2014). Individuals with HbA1c of 5.7-6.4% are considered levels that are a high risk for developing diabetes and considered a pre diabetic level (American Diabetes Association, 2014).

Fasting plasma glucose is another diagnostic measure that is taken after fasting for eight hours. When the plasma glucose level is greater than 126 mg/dl an individual is considered to have diabetes (American Diabetes Association, 2014). Normal fasting plasma glucose levels are less than 100 mg/dl with a pre diabetic state with levels between 100-125 mg/dl. An oral glucose tolerance test is a diagnostic test that looks at how an individual processes glucose (American Diabetes Association, 2014). Glucose levels are tested before and two hours after drinking a glucose rich beverage of approximately 75 grams of glucose, this amount can be less for shorter testing time frames and more for longer time frames (American Diabetes Association, 2014). Blood glucose levels higher than 200 mg/dl after 2 hours meet diagnostic criteria for a diagnosis of diabetes (American Diabetes Association, 2014). Normal levels are less than 140 mg/dl after 2 hours and pre diabetic levels are 140-199 mg/dl after 2 hours (American Diabetes Association, 2014). A random test of blood glucose known as a causal test resulting in a reading greater than 200 mg/dl are also considered a diagnostic criterion for diabetes (American Diabetes Association, 2014). The ADA provides a simple graphic to help with making understanding and comparing these values easy (Figure 1).

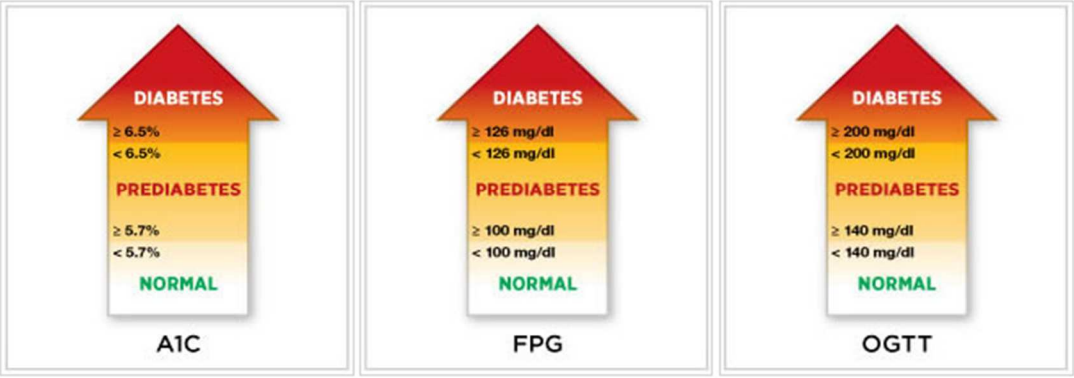


Figure 1. Diagnostic criteria for diabetes mellitus from the American Diabetes Association (American Diabetes Association, 2014).

The measure of HbA1c can be confusing as it estimates the amount of glucose on a hemoglobin molecule based on the levels of glucose in the blood for the previous two to three months (American Diabetes Association, 2014). It can sometimes be confusing to understand what the changes mean. When the percentage of glycation on a hemoglobin molecule increases by 1% there is an approximate increase in blood glucose concentrations in a rough range of 14-16 mg/dl. This range is estimated from a chart provided in an article on the ADA's website by Erika Gebel PhD (Figure 2) (Gebel,2011). The ADA highlighted the benefit of this measure as being able to diagnose diabetes without needing to complete and oral glucose tolerance test (American Diabetes Association, 2014).

A1C (%)	eAG (mg/dl)
5	97
5.5	111
6	126
6.5	140
7	154
7.5	169
8	183
8.5	197
9	212
9.5	226
10	240
10.5	255
11	269
11.5	283
12	298

Figure 2. This chart provides an estimate of the average blood glucose concentrations in relation to a HbA1c percentage (American Diabetes Association, 2014 & Gebel 2011).

Impact

Type 2 diabetes mellitus has a devastating impact of the health of individuals due to the additional disorders that arise. A 2014 article by The American Diabetes Association "Standards of Medical Care in Diabetes-2014" discusses cardiovascular disease as the number one cause of morbidity and mortality in diabetics (American Diabetes Association, 2014). The devastation to individual health is not limited to cardiovascular disease; it encompasses damage throughout the body to include amputations, renal, hepatic, and vision dysfunction. This list is not exhaustive but addresses the most devastating complications associated with type 2 diabetes mellitus. Lower limb amputation is a key insult that can be reduce with improved blood glucose concentrations which minimize lesions of the feet and lower legs (Gregg et al., 2014; Mundet et al., 2012; Pscherer et al., 2012). The impacts on hearing and oral health are additional areas of health comorbidities that are receiving attention in the literature (American Diabetes Association, 2014; Gregg et al., 2014; Kannan, 2012). These complications also lead to psychological complications due to lifestyle changes, financial burden, and reduction in quality of life from health disparities (American Diabetes Association, April 2013).

The financial burden of caring for type 2 diabetics is a global problem that is mounting (Weber, 2010). In the U.S. alone the direct medical costs reached $176 billion with an additional estimated $69 billion in lost productivity (American Diabetes Association, April 2013). One of the most eye opening revelations in the literature is the disparity in mortality rates across economic levels. Lower income individuals have a

higher mortality rate from diabetes; but, this disparity decreased in individuals as they age once their incomes is subsidized by social security, Medicare benefits, and assistance with medicine when patients are over 65 based on a study by Lipscombe and colleagues that looked at the mortality rate in Canadian diabetics over an eleven year time frame (Lipscombe et al., 2010). This helps significantly mediate complication and illness due to the development of comorbidities when medication compliance is hindered due to affordability. When Lipscombe and colleagues looked at the mortality rate in Canadian diabetics over an eleven year time frame it was shown a higher mortality in the younger diabetic population even though their medical care cost is covered, yet medications may not be (Lipscombe et al., 2010). It is speculated that this problem is elevated in the US for people who do not have health care insurance complicated by extensive medications and associated cost of other health complications (American Diabetes Association, April 2013).

Pathogenesis

Type 2 DM results when the pancreas is impaired and no longer produces adequate insulin from the beta cells and disturbances in metal concomitant has been attributed to assisting in the development and progression of this disease (Khan & Awan, 2014). In addition to the beta cells producing lower levels of insulin the insulin receptors become weaker and create an insulin resistance (American Diabetes Association, April 2013). Disturbances in metal concentrations due to polyuria and renal dysfunction are

believed to contribute to the reduced insulin sensitivity in type 2 DM (Gabrielsen et al., 2012). A study by J. Scott Gabrielsen and colleagues found a dysfunction in adipocytes insulin regulation based on iron overload. The sensitive balance of all body metals are highlighted in an article published by the Journal of Diabetic & Metabolic disorders; this article discussed the relationship of elevated iron and insufficient chromium levels in exacerbating the pathogenesis of type 2 DM(Khan & Awan, 2014).

Type 2 Diabetes and Iron

Iron and Diabetes

Research has shown that excess iron affects the beta cells of the pancreas thus reducing insulin production and contributing to impaired blood glucose concentrations(Bao et al., 2012; Batchuluun et al., 2014; Creighton Mitchell & McClain, 2014; Gupta, Palta, Singh, & Lehl, 2014; Hansen et al., 2014; Kalra, Chawla, & Madhu, 2013). It is mentioned in the literature that this association has been realized since 1865 (Hansen et al., 2014). There are various treatments for type 2 diabetes mellitus to include traditional and alternative approaches in the quest for control of healthy blood glucose concentration and minimization of complications (American Diabetes Association, April 2013). Several studies have explored iron reduction through chelation and or blood donations; this review discusses blood donations to reduce iron concentration (Ikeda, 2014; Peffer et al., 2013). Understanding the effectiveness of iron depletion therapy is helpful as the association of high iron stores and heme-iron intake have been positively associated with Type 2 Diabetes Mellitus (Bao et al., 2012).

Iron is an essential and most prevalent trace mineral in the body; responsible for assisting with storing, regulating and transporting oxygen throughout the body, immune function, energy production, metabolic processes, and DNA synthesis which is responsible for significant bodily processes. Iron is highly safeguarded when transported through the body (Figure 1) due to its ability to cause reactive oxygen species and the use of iron by any pathogens present (Creighton Mitchell & McClain, 2014)

Sources of dietary iron are found in meats, poultry, and fish and provide mostly heme forms of iron, which are absorbed easier than non heme forms from plants and dairy (Hansen et al., 2014). Iron enters the system through the cells of the intestine based on the form of iron ingested. Heme iron enters on the haem carrier protein 1 and the oxidized iron is transported on the divalent metal transporter into the intestinal cell (Hansen et al., 2014). Once inside the cell it is stored in the cell as ferritin or transported through ferroportin to the bloodstream and delivered to cells on transferrin (Hansen et al., 2014). Hepcidin is a regulation protein that is made by the liver and the pancreas, it regulates iron uptake by binding to ferroportin and stopping iron from coming in and out of the cell (Hansen et al., 2014). Iron is highly regulated in the body as it is critical in oxygen transport, protein synthesis, and other functions. It is also used by other organisms and during times of infection the body works to store iron to minimize use from pathogens (Sia, Allred, & Raymond, 2013). This protective mechanism is believed to be the mechanism that creates iron overload in diabetic patients as the cells absorbs iron during times of inflammation, which is a common condition in diabetics (Hansen et al., 2014).

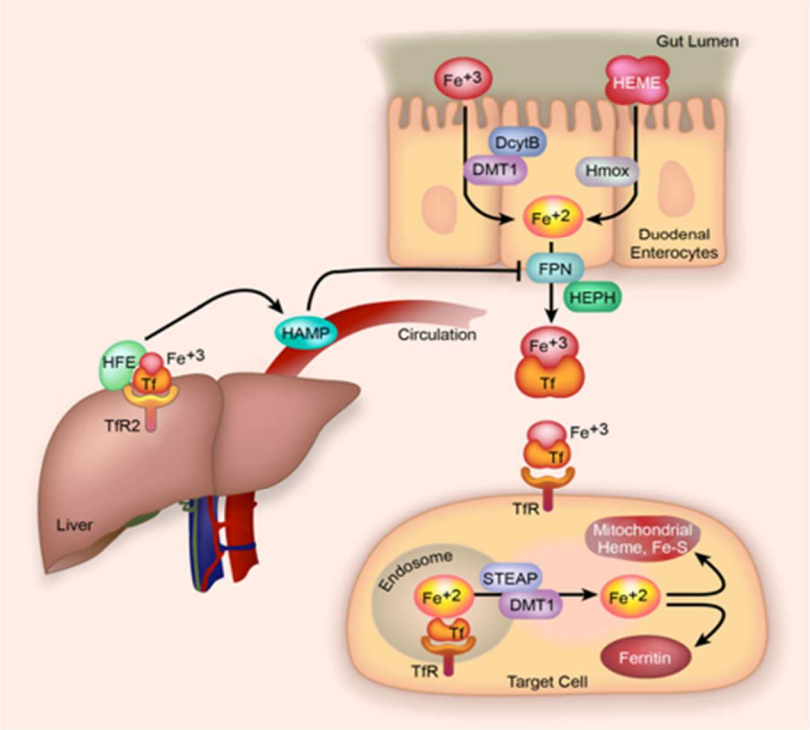


Figure 3. The transportation of iron (Fe+3 and Fe+2) is highly controlled as shown in this diagram (Creighton Mitchell & McClain, 2014).

Iron is highly regulated in the body to maintain healthy concentrations, support various biochemical reactions, and to ensure it is delivered to the cells that need it for proper functioning. The first regulation point is the absorption of iron in the intestines. This is where the form of dietary iron is significant. Heme-iron is absorbed at a significantly higher percentage than nonheme-iron and has been noted in studies as a contributing factor to type 2 diabetes mellitus as it relates to excess accumulation (Bao et al., 2012). This higher absorption is an issue because once iron is in the body there are few ways the body excretes the iron. This may be one reason why iron overload increases with age and as people age the risk for type 2 diabetes increases (Hansen et al., 2014). In a cross sectional observational study by Gupta and colleagues, they showed no correlation in elevated serum ferritin concentrations of an Indian population with type 2

diabetics noting the predominance of a vegetarian diet (Gupta et al., 2014). This study reported a lack of evidence to support the correlation of high serum ferritin and type 2 diabetes mellitus in their population which incidentally supports the previous study discussed that connected high heme intake with high iron stores. It is important to note that because their population is primarily vegetarian the predominant source of dietary iron is non-heme form thus decreasing bioavailability. In addition, a plant based diet may have other benefits that obscure the effects of the decreased bioavailability of the iron. The plant based diet may mediate pancreatic inflammation due to the high level of antioxidants.

The necessity of iron is highly regarded for all living organisms. Pathogenic bacteria in a human body produce siderophores to scavenge for iron for their own use which is why a type chaperone system of iron exists in humans as another immune mechanism to minimize the proliferation of pathogens especially of a bacterial nature. The process that the human body uses intercepts the iron that the siderophores scavenge by binding the siderophore with the iron and releasing it back to a molecule called a Siderocalin (Scn) which frees the iron to be recycled to for use of the human or mammal as this is a mammalian mechanism (figure 2) (Sia et al., 2013) . There are several forms of molecules used in the process of iron sequestering and transport (figure 3) (Sia et al., 2013).

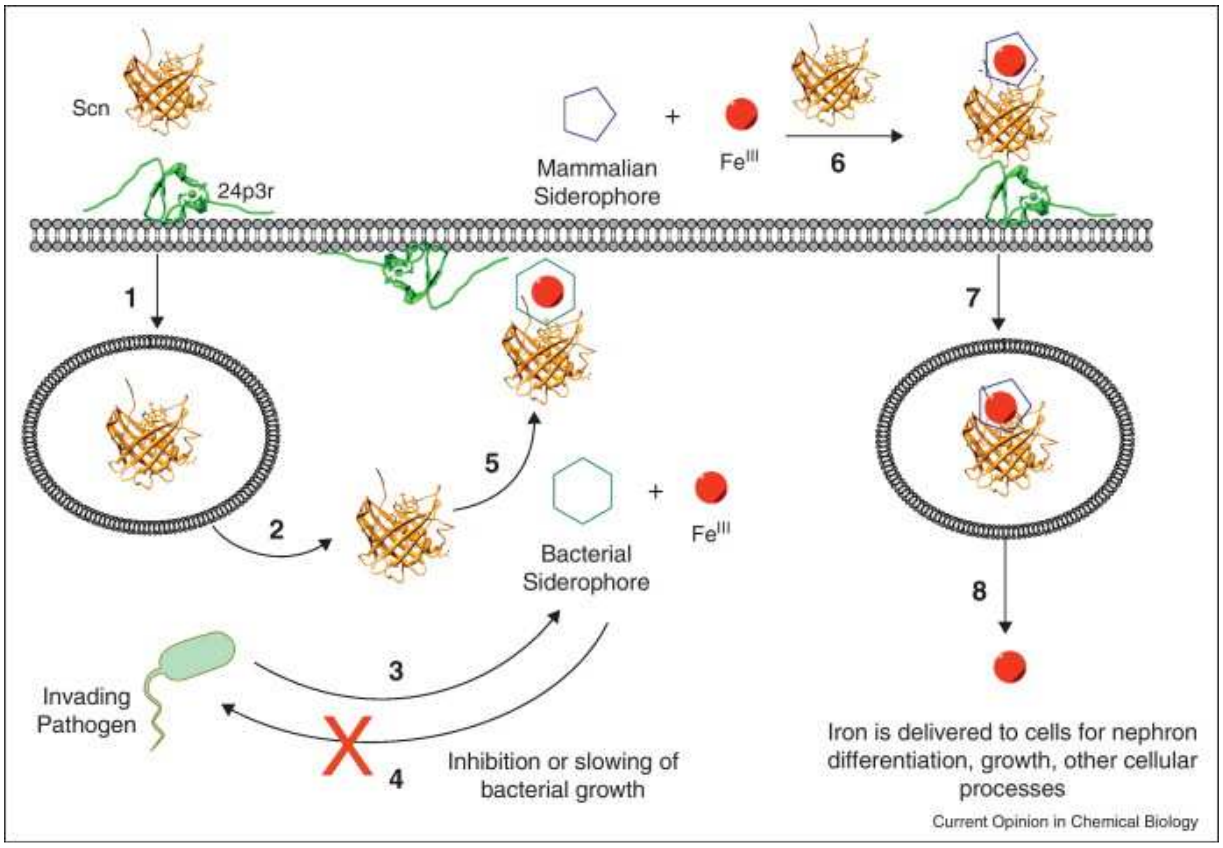


Figure 4. This shows the process that Siderocalins use to mediate bacterial growth by minimizing the use of iron by bacterial pathogens (Sia et al., 2013).

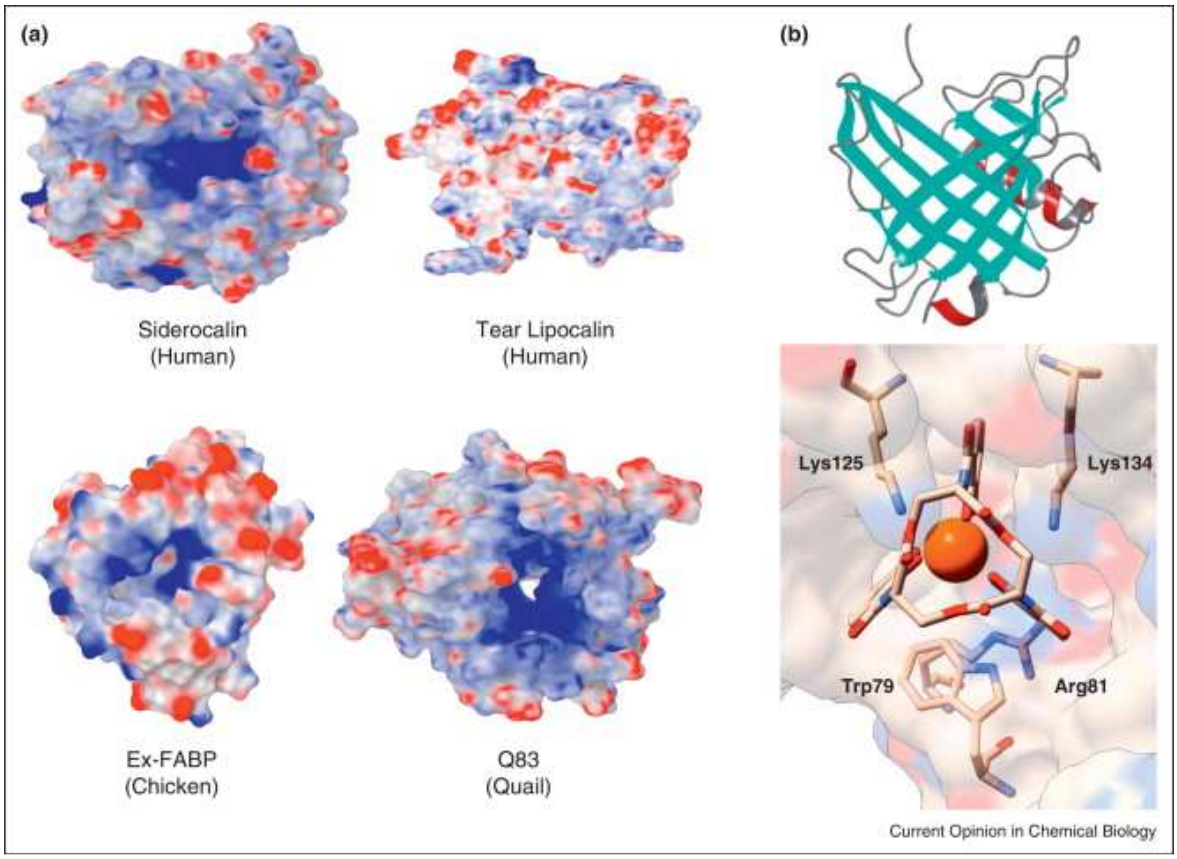


Figure 5. This shows the various types of molecules used to sequester iron in various species (Sia et al., 2013).

Once in the blood iron is carefully transported to cells where needed to assist in critical life supporting functions. In cases of diabetes mellitus type 1 and 2 there is an increase in inflammation throughout the body (American Diabetes Association, 1978). This is important because ferritin is increased to bind iron and minimize its circulation when inflammation is present. It is believed this mechanism is used to minimize pathogens from using the iron for their own life supporting functions. This is a mechanism performed by numerous cells in the body. This discussion will focus on the cells of the pancreas and the pathogenesis of type 2 diabetes mellitus which is believed to be complicated by excess systemic and cellular iron concentrations.

Iron and the Pancreas

The beta cells of the pancreas are responsible for the secretion of insulin; and iron is a key essential mineral needed for this process. However when iron is in excess the beta cells of the pancreas are destroyed by mechanisms that are speculated upon but not fully understood. In a review by Hansen et.al., two mechanisms are proposed that warrant discussion and consideration.

First, apoptosis of the beta cells of the pancreas based on the labile iron pool, free ferrous iron, is believed to be a cause (Hansen et al., 2014). When inflammation is experienced cytokine activity increases and signals cell death through the up regulation of siderophore binding proteins, Siderocalins, which sequester excess iron from pathogens (Sia et al., 2013). The uptake of extra iron in the cells increases the amount of reactive oxygen species (ROS) created from Fenton reactions with other molecules. The hydroxyl is the specific ROS that is toxic to the beta cells of the pancreas. When these toxic molecules accumulate the beta cells self-destruct, which in turn reduced the amount of insulin secreted thus diminishing blood glucose control. This mechanism is highly simplified as it is a theory and warrants further investigation.

Ferroptosis is the second mechanism of beta cell death in the pancreas that is explored. This mechanism is of great interest as it is directly related to excess cellular iron. It is believed that the ROS, hydroxyl, along with cysteine enters the cell causing an iron dependent cell death. It is not clear if this form of cell death is related to some beta cells or is just part of the process to induce cell death from iron overload.

Iron Depletion Therapy

Initial search of iron reduction and type 2 diabetes mellitus returns thousands of articles on various topics. The clear assumption is that iron and diabetes is heavily discussed and researched. The literature shows a benefit in reducing iron though iron chelation or blood donations but much more research needs to be done to support this treatment (Peffer et al., 2013).

A robust study by Peffer and colleagues showed significant reduction in ferritin and hepcidin concentrations with blood donation. Ferritin is the storage form of iron and hepcidin regulates iron balance therefore these markers are important measures for iron depletion (Peffer et al., 2013). Although this study was looking at decrease in atherosclerosis it showed a decrease of iron markers with whole blood donations. The measures of 152 men and 117 women were reviewed based on the number of lifetime whole blood donations, resulting in reduced ferritin and hepcidin concentrations. A key point was frequent blood donations improved markers more than less frequent whole blood donations. Whole blood donation was defined as any blood donation greater than 100mL.

Mensud Hatunic and colleagues studied the effects of iron depletion through blood removal on 11 subjects with hereditary hemochromatosis (HH), 7 with impaired glucose tolerance and 4 with type 2 diabetes. These subjects received regular blood removal as treatment for their HH which consists of 450 mL of blood removed to yield roughly 250 mg of iron from the blood; the goal is to reduce ferritin levels to 50 µg/L or

reduce % saturation to less than 50 %(Hatunic, Finucane, Norris, Pacini, & Nolan, 2010). Two weeks after the treatment researches concluded that small improvements in impaired glucose tolerance were achieved (Hatunic et al., 2010).

Researchers of an epidemiological study concluded that more research needs to be done to include iron depletion therapy to further enhance knowledge of the mechanism of iron related metabolic disorders to include diabetes (Basuli, Stevens, Torti, & Torti, 2014). Debargha Basulu and colleagues propose abnormal insulin sensitivity and production due to accumulation of iron which causes oxidative damage in liver, skeletal muscle, pancreas and fat cells as illustrated (figure 6) in their article.(Basuli et al., 2014).

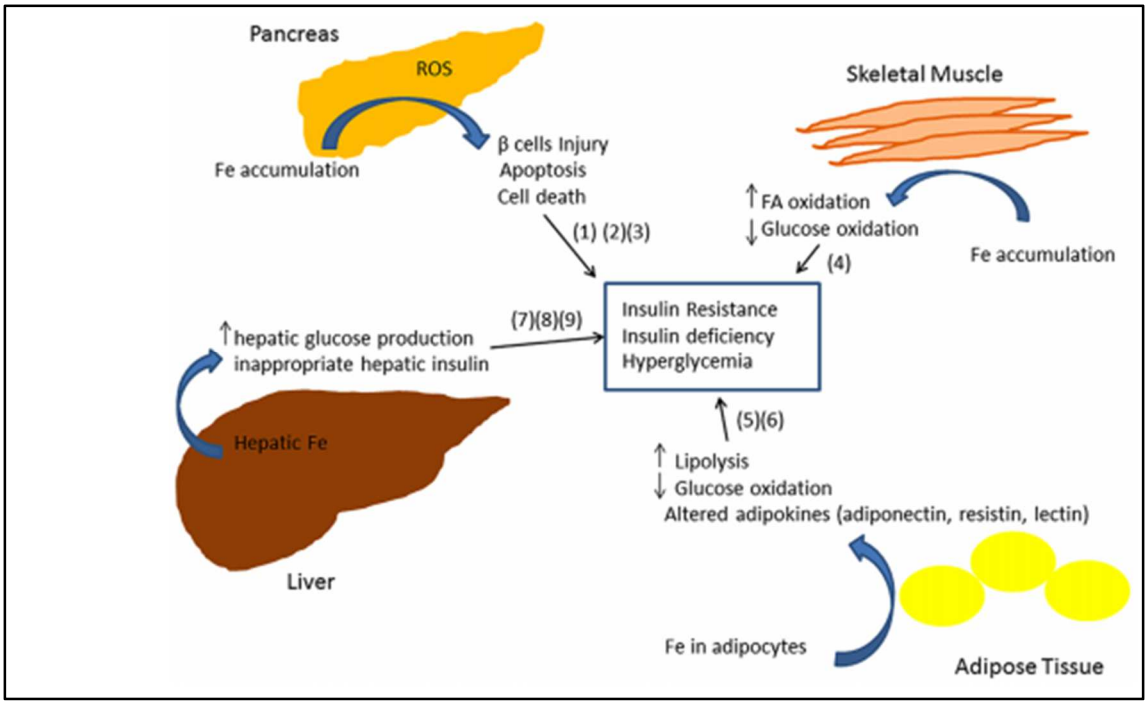


Figure 6. This illustration shows the potential mechanism of iron accumulation and effect on blood glucose control.(Basuli et al., 2014)..

It is clear that iron is a critical element in the pathology of type 2 diabetes mellitus. This mineral as well as the other molecules within the human body do not exist

alone, but react with each other and affect their efficiency. A major mineral in this research is chromium which iron has an effect on.

Type 2 Diabetes and Chromium

Chromium is a molecule that has been researched and understood to help with macronutrient metabolism and subsequently improving glucose control by improving glucose uptake into cells through chromodulin (Quarles Jr et al., 2011) . Chromodulin is an intercellular protein that binds chromium III in cells and increases tyrosine kinase activity to amplify the strength of insulin receptors to bind insulin (Quarles Jr et al., 2011) (Chen, 2011). Increasing the strength of insulin binding improves the expression of GLUT 4 to the cells plasma membrane. GLUT 4 is an insulin dependent transporter that brings glucose into muscle and adipose cells for energy production (Quarles Jr et al., 2011) (Chen, 2011).

Iron and chromium use one of the same transporters to enter the cell; human serum transferrin (Tf) (R. A. Anderson, 1981). This protein has two lobes that are comparable. These lobes are identified as a C-lobe and an N-Lobe with the C-lobe being the preferential binding site for chromium (Figure 1). This is where the exploration of iron depletion therapy arises. The C-lobe is also the preferential binding site for iron where it binds first and has a 20 times stronger bond than when it bonds to the N-lobe. In addition, as iron binds the affinity for chromium decreases which may create an inability for adequate chromium levels to enter the cell (Quarles Jr et al., 2011). Quarles Jr. and colleagues studied the competitive binding of various minerals to include chromium at

various iron concentrations. Their research suggests as suspected binding of chromium to Tf was reduced by half when iron was present in excess. This is a key point in understanding why some studies do not show an improvement with chromium supplementation due to the lack of attention given to iron concentrations. In addition, when chromium concentrations were increased iron loaded about six percent less, suggesting a potential side benefit of reducing iron uptake into cells with chromium supplementation (Quarles Jr et al., 2011). A study by Ather Ali and colleagues reject the theory that chromium supplementation improves insulin resistance. However, this study did not measure chromium concentrations and could not determine absorption of supplementation which they mentioned as a major study limitation (Ali et al., 2010). A major limitation not declared is the importance of assessing iron levels as this is a major competitor for transport into the cell. Further support for future studies to include a combination protocol of iron depletion through blood donation followed by chromium supplementation.

A meta-analysis by Christopher H. Baily rejects the use of chromium supplementation to improve fasting glucose levels. Again attention to iron levels are not addressed which would improve the overall evaluation of the effectiveness of chromium supplementation (Bailey, 2014). This analysis concludes that chromium supplementation does not benefit subjects who are not chromium deficient while noting a standard for determining deficiency has not been established. This increases the need for chromium studies to include chromium measures at baseline, throughout the study, and include iron markers.

Further research to understanding the relationship of iron and chromium binding this transporter would benefit future studies that combine the methods of iron depletion and chromium supplementation as the mechanisms are not fully understood.

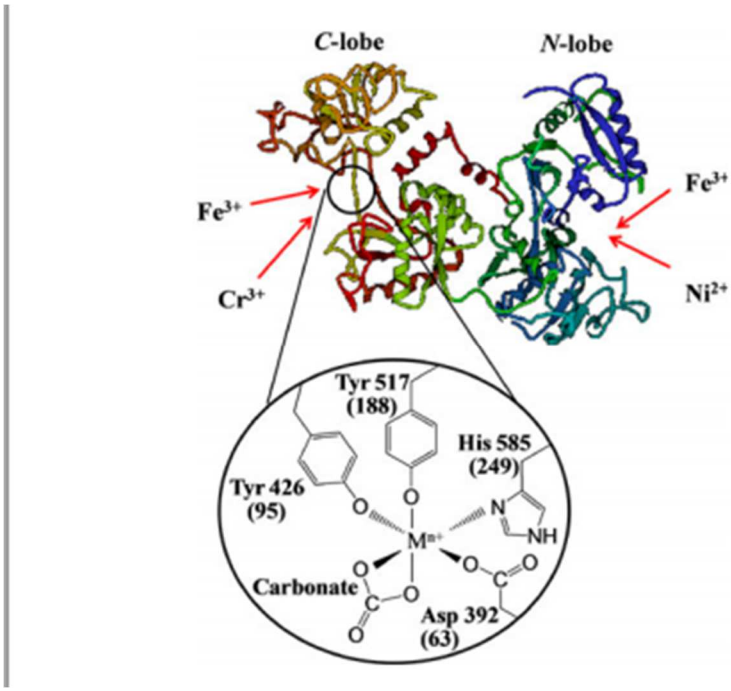


Figure 7. This shows the C-Lobe and the N-Lobe of human serum transferrin that binds minerals for uptake into the cell. The C-lobe shows the affinity for iron and chromium binding (Quarles Jr et al., 2011).

Chromium Supplementation

Research Overview

An initial search of "The effect of chromium supplementation on blood glucose control in individuals with type 2 diabetes" yielded approximately 32 articles in a PubMed search database. Several related to animal studies or did not meet point of interest. Review of several studies found a positive correlation with chromium supplementation to improve blood glucose concentrations in individuals with type 2 diabetes. A common understanding is supplementation helps to improve blood glucose concentrations when chromium status is low yet attention to iron concentrations in relation to chromium status is lacking. In addition this review of the literature found evidence of improvement with supplementation above and below the recommended intake (R. Anderson et al., 1997).

Anderson and colleagues conducted a randomized trial of 180 participants divided into three groups; group one was given a placebo, Group two was given chromium picolinate two times per day at a dosage of 1.92 mu mol (100 mu g) Cr and group 3 was treated with 9.6 mu mol (500 mu g) Cr two times per day. The participants were instructed not to modify their lifestyle in any other way. The group with the higher dose saw improvement in HbA1c after two months and the lower dose group saw improvement within four months (R. Anderson et al., 1997).

A double blind cross over trial by Suhad M.A.Bahijri and Asaad M. B. Mufti looked at seventy eight participants over twenty four weeks. This study showed that

chromium supplementation improves blood glucose concentrations in individuals with type 2 diabetes mellitus. The use of Torula yeast, CrCl3, brewer's yeast, and placebo supplements were given to all participants with a 8 week cross over method. The brewers yeast elicited the best glucose control response (Bahijri & Mufti, 2002). In addition, this study showed that urine chromium levels are a good indicator of status after a two hour glucose load test (Bahijri & Mufti, 2002).

Chromium Homeostasis was studied by Morris and colleagues, in individuals with type 2 diabetes mellitus. This controlled trial looked at chromium levels in 93 individuals with type 2 diabetes mellitus in comparison to 33 individuals without. The results showed a significant difference in the individuals with type 2 diabetes mellitus. They showed up to a thirty percent lower concentration of chromium versus the healthy participants. The levels were measured in both blood and urine. An interesting finding was in individuals that had been diagnosed over 2 years; the relationship of decreased plasma glucose levels with higher chromium serum concentrations decreased in this population. This may suggest that the benefits of chromium may be decreased in populations that have had poor chromium status with Type 2 Diabetes Mellitus long term. This indicates a possible benefit to treating individuals with chromium to prevent the onset of type 2 diabetes mellitus and for individuals who have been newly diagnosed.

A study by Jaroslav Racek and colleagues showed a significant reduction in fasting plasma glucose, and HbA1c after supplementing eleven participants with 100 micrograms of chromium enriched yeast for two weeks followed by an increased dose of 200 micrograms for six weeks (Racek et al., 2013). The participants were then taken off

the supplementation for a six week washout period which raised their previously lowered fasting plasma glucose, and HbA1c concentrations (Racek et al., 2013). This study was limited by the low number of participants as noted by the researchers.

Although no guidelines are clear for chromium deficiency a study by Giovanni Forte and colleagues showed a difference in chromium concentrations in males with and without type 2 DM. This study looked at the metal concentrations in diabetic and non-diabetic subjects. They found that there is roughly a difference of .14 ng/ml in chromium concentrations with diabetics having the deficit. Chromium is a trace mineral and it is reasonable to suggest small increases in chromium concentrations may help manage blood glucose levels. This study concluded that supplementation of chromium may be useful for blood glucose control.

The literature shows several examples of improvement of blood glucose control with chromium supplementation although the mechanism is not fully understood (Trumbo & Ellwood, 2006). Further understanding of the mechanism of chromium is not provided; however, this investigation adds to the body of knowledge and sets precedence for further testing to include evaluation of iron concentrations in relation to chromium supplementation.

CHAPTER 3

METHODS

Participants

Participants were recruited through list serve, advertisements at local places of worship and health organizations that serve diabetic populations. Participants interested in study participation were invited to complete an online survey via survey monkey. Upon identifying initial suitability, applicants were to be invited to the downtown ASU campus to further establish fit based on inclusion and exclusion criteria from blood draws and health questionnaires.

This investigation was a case study of a single participant to establish an initial understanding of this treatment. Participants recruited were men aged 30-70 years old with a Type 2 Diabetes Mellitus diagnosis from a Medical Doctor greater than 12 months with satisfactory management defined as HbA1c >6.2 or <7.5. The gender specificity was because men tend to have higher ferritin concentrations and higher transferrin saturation (McLaren et al., 2001). Additional inclusion criterion included medication stability for at least 90 days, willingness and ability to donate blood twice separated by eight weeks, no blood donations for the past 12 months, willingness to supply 4 fasting blood samples and record daily fasting glucose concentrations, and free of kidney and liver disease. Eligible participant did not regularly supplement chromium and/or iron.

Exclusion criterion included hematocrit <44%, hemoglobin <15g/100mL, ferritin < 200 µg /L because this study explored the effects of lowering iron stores participants

must have had iron markers that indicated elevated ferritin and blood measures associated with iron status. Additionally hsCRP was to be <3 and HOMA-IR score<1 as inflammation affects iron status and iron status is highly correlated with diabetes (Hansen et al., 2014).

Participant signed a written consent form upon full disclosure and informed consent. This study has been approved by the Arizona State University Institutional Review Board.

Study Design

This was a 20 week case study. Potential participants underwent an online pre-enrollment screening prior to being invited to visit one for further inclusion screening. The eligible participants visited the Downtown ASU campus on 5 separate visits to complete appropriate paperwork, receive disclosures and collect pertinent data to include anthropometric and biochemical measures. All visits varied in time between 20 -45 minutes.

Upon meeting initial screening based on survey monkey the participant came to the ABC building nutrition lab at ASU Downtown Phoenix Campus. Prior to the first visit participant had been instructed to fast for 8 hours before coming in the event he chose to proceed in study upon receiving full informed consent. Participant had the free and full authority to stop participation at this visit or any subsequent visit. This first visit included the initial informed consent after all questions have been answered, anthropometric and biochemical data collected, and delivery of instructions and supplies

for daily waking blood sample and testing for exclusion criteria. Visit 2 was scheduled at this initial visit for the following week and final determination for inclusion was conveyed to the participant.

The single participant continued in the study and was instructed to schedule his first blood donation at a convenient United Blood Services Center within one week of second visit. Daily waking blood glucose monitoring was to be continued throughout this period and duration of study. Since the participant is a diabetic he was experienced at performing this test. Eight weeks after initial blood donation participant came in for visit 3 in an 8 hour fasting state and approximately 2 tablespoons of blood was collected and the glucometer data was down loaded. This same process was repeated for visit 4 to include another blood donation separated by 8 weeks. The fourth visit was four weeks after second blood donation and at this time participants had been given chromium supplements as chromium picolinate (250 μg /d) for four weeks and a compliance sheet to track intake. Visit 5 was four weeks after visit four and the final visit which was the study conclusion, included a venous blood draw, glucometer data download, and collection of chromium compliance sheets. Monetary compensation of $10 per visit for a total of $50 was provided to participants.

Measurements

Participants submitted to venous blood draws at visits1,3,4 and 5 to include 1 finger prick at visit 1. Venous blood draws were collected by a Registered Nurse or trained phlebotomist and blood prick by trained research personnel. Prior to each venous blood draw participants was in an 8 hour fasting state. Blood analysis was performed at the nutrition laboratories at ASU. Blood concentrations of ferritin were measured by Immulite, transferrin by ELISA, Insulin by RIA, A1C by Siemens DCA, glucose and CRP by COBAS. Each sample was fully processed and plasma frozen at -80°.

CHAPTER 4

RESULTS

Recruitment for this study started in June 2014 until August 2014 through electronic list serves, flier distribution at coffee shops, places of worship, community colleges, universities in the greater Phoenix and Scottsdale area, and calls to local dietician that serve the diabetic population. Respondents (n=6) completed a questionnaire in survey monkey to determine initial qualification. Qualified respondents (n=3) received an email confirming acceptability and final inclusion pending blood tests at the ASU nutrition labs. Respondent (n=1) to follow up email met inclusion criteria and completed a 20 weeks case study commencing on September 26, 2014 and finalizing on February 13,2015.

Descriptive Characteristics

Subject

One subject was studied to explore the effects of iron depletion through blood donations combined with chromium supplementation to improve insulin sensitivity. 35 year old male diagnosed with type 2 diabetes mellitus September 2013; height 1.75m, weight 169.22 kg, BMI 55.2. Subject has a stable medication history at time of screening on September 17th 2014 of Metformin 1000 mg twice a day, Hyzaar (50mg losartan/ 12.5mg hydrochlorothiazide) once a day since September 2013. Subject was initially excluded due to a false low hematocrit reading based on milking the finger to draw

sample which resulted in an excess of serum in the blood sample. Proper retesting of subject resulted in a hematocrit within inclusion criteria of 44.5%. Subject met inclusion criteria and start date adjusted to September 26, 2014. Prior to baseline blood draws at visit 2 patient was prescribed Januvia 50 mg twice a day and discontinued this medication prior to week seven due to severe leg pain. Patient discontinued this medication but remained on Metformin and Hyzaar throughout course of study. During week 13 subject was out of all medication due to a prescription error that was corrected on week 14, and all medication was resumed with the addition of Glipizide 5 mg once a day for the remainder of study period.

Health status remained unremarkable until weeks 16-20 where patient acquired a common cold that progressed to bronchitis by week 20 when an antibiotic was prescribed just prior to final visit 5 on week 20. The second blood donation was delayed by 1 week due to a low hematocrit reading at blood donation center which extended the study to 20 weeks. Food diaries and recalls were not used due to the high burden of the study based on requirement of daily finger pricks, 6 blood withdrawals to include the 2 blood donations, and daily supplement intake and tracking, and 20 week commitment. Due to no food diaries and recalls there are no descriptive analysis of glucose reading based on diet. Compliance on daily finger pricks was 88 % (23/140) and 100% compliance on consuming chromium supplement and completing all visits to include 2 blood donations at United Blood Services. Daily finger pricks were performed upon waking which included 41% (51/123) of the reading taken after 8am due to the weekend, Thanksgiving (US), Christmas (US), and school winter break. Hypotheses outcomes based on HOMA-

IR score and HbA1c levels, HOMA-IR score calculated from online calculator and HbA1c leels from Sonoran Quest Labs (Maurer, 2015).

Lab Values and Medications

Visit 1

Subject was initially excluded on Visit 1, 9/17/14, based on Hematocrit collection error. Medication, height, and weight collected: height 1.75m, weight 169.22 kg, BMI 55.2. Blood draw conducted after error detected and baseline date adjusted to 9/26/14. Subject gave list of all medications at this visit which were Metformin 1000 mg twice a day, stable since September 2013, Hyzaar (50mg losartan/ 12.5mg hydrochlorothiazide) once a day, stable since September 2013.

Visit 2

This visit confirmed inclusion based on baseline lab data collected at visit 1. Baseline data collected 9/26/14 with reported normal levels of chromium at 0.2 mcg/L, elevated CRP levels at 10.4 mg/L, normal levels of ferritin at 212 ng/mL, elevated fasting insulin levels at 59 uIU/mL, normal levels of iron level 57µg /dL , normal TIBC at 275µg /dL , low % Saturation of 21%, elevated HBA1C of 7.5%, elevated estimated average glucose level at 169 mg/dL, and normal hematocrit at 44.5%. The range levels are based on the laboratory ranges of Sonoran Quest Laboratories.

Visit 3

Venous blood draw collected on 12/11/14 after first blood donation during week 3 on 10/16/2013. Results are relative to baseline levels. CRP levels increased to 17.2 mg/L, ferritin levels decreased to 130 ng/mL, fasting insulin levels increased to 81 uIU/mL , iron levels decreased to 51 µg /dL , TIBC levels increased to 296 µg /dL , % Saturation decreased to 17%, HBA1C decreased to 7.3%, estimate average fasting glucose levels decreased to 163 mg/dL, and hematocrit decreased to 39.5%. Chromium was excluded from this collection as we are analyzing baseline, pre, and post supplementation chromium levels. Subjects current medications at this visit were Metformin 1000 mg twice a day, stable since September 2013, Hyzaar (50mg losartan/ 12.5mg hydrochlorothiazide) once a day, stable since September 2013, and discontinuation of Januvia during study week 7 (11/13/14).

Visit 4

Venous blood draw collected 01/16/15 after second blood donation during week 13 on 12/19/2014. Results are relative to baseline levels. Chromium levels remained the same at 0.2 mcg/L, CRP levels increased to 14.7 mg/L, ferritin levels decreased to 89 ng/mL, fasting insulin levels increased to 70 uIU/mL, iron levels remained the same at 51 µg /dL, TIBC increased to 309 µg /dL, % Saturation decreased to 17%, HBA1C increased to 7.6%, estimate avg. glucose increased to 171 mg/dL, and hematocrit decreased to 42%. Subjects current medications at this visit were Metformin 1000 mg

twice a day, stable since September 2013, Hyzaar (50mg losartan/ 12.5mg hydrochlorothiazide) once a day, stable since September 2013, and started Glipizide 5 mg once a day during study week 14 (12/26/14).

Visit 5

This is the final visit and end of study. Venous blood draw collected 02/7/15 after both blood donations and 4 weeks of chromium supplementation during weeks 17-20 from 1/16/15 to 2/7/15. Results are relative to baseline levels. Chromium levels increased to 0.7 mcg/L, CRP levels increased to 19.1 mg/L, ferritin decreased to 106 ng/mL, fasting insulin levels decreased to 37 uIU/mL , iron levels decreased to 45 µg /dL , TIBC increased to 317 µg /dL , % saturation decreased to 14%, HBA1C increased to 7.7%, estimated average glucose increased to 174 mg/dL, and hematocrit decreased to 42.0%. Subject's current medications at this visit remain stable since visit 4 with Glipizide 5 mg once a day, Metformin 1000 mg twice a day, stable since September 2013, Hyzaar (50mg losartan/ 12.5mg hydrochlorothiazide) once a day, stable since September 2013.

Results Charts

Table 1. Lab Results by visit. Visit 2 did not have blood draws, this visit confirmed inclusion and scheduled first blood donation.

	Chromium	CRP	Ferritin	Fasting Insulin	Iron	TIBC	% Saturation	HbA1c	HOMA Score	Fasting Plasma Glucose	Hematocrit
Visit 1	0.2	10.4	212	59	57	275	21	7.5	20.4	140*	44.5
Visit 3	n/a	17.2	130	81	51	296	17	7.3	42.8	214	39.5
Visit 4	0.2	14.7	89	70	51	309	17	7.6	27.3	158	42.0
Visit 5	0.7	19.1	106	37	45	317	14	7.7	12.2	136	42.0

*Based on first fasting plasma glucose reading from participant.

Table 2. Daily blood glucose levels from glucometer. Missing data are days when subject did not take a measure.

	Visit 1	**Visit 2**	1st Blood Donation							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Start	9/26/14	10/3/14	10/10/14	10/17/14	10/24/14	10/31/14	11/7/14	11/14/14	11/21/14	11/28/14
Friday	140	136		154	137	129	126	180	141	151
Saturday	145	132		126		132	140	159	147	127
Sunday	169	141	142	136	146	124	157	239	149	170
Monday	163	204		123		122	146	139	145	160
Tuesday	171	114	125	162	163	128	154	123	159	214
Wednesday	163	135	141	137	148	145	167	141	157	186
Thursday	145	121		129	137	126	179	135	136	160
Avg. mg/dL	157	140	136	138	146	129	153	159	148	167
	Visit 3		2nd Blood Donation			**Visit 4**	Start Chromium			Final Visit **Visit 5**
	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16	Week 17	Week 18	Week 19	Week 20
Start	12/5/14	12/12/14	12/19/14	12/26/14	1/2/14	1/9/14	1/16/14	1/23/14	1/30/14	**2/7/14**
Friday	160	201	237	160	214		158	149	131	138
Saturday	150	168	173	176		186	158	148	134	138
Sunday	194		202	186		166	181		118	144
Monday	218	198	196	251		146	194	174	129	160
Tuesday		177	176		121	155	165	169	118	137
Wednesday	250	187			231	171	170	143	123	150
Thursday	214	187	186			183	157	162	145	134
Avg. mg/dL	198	186	195	193	189	168	169	158	128	143

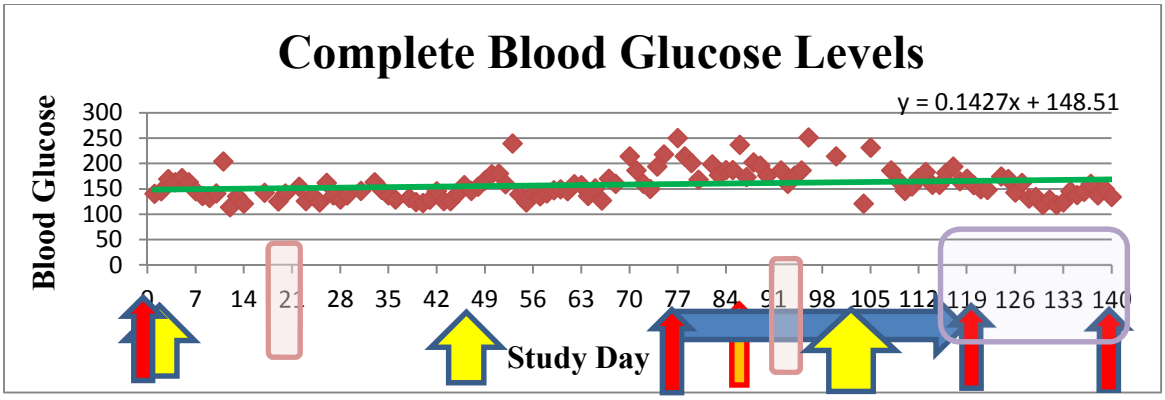
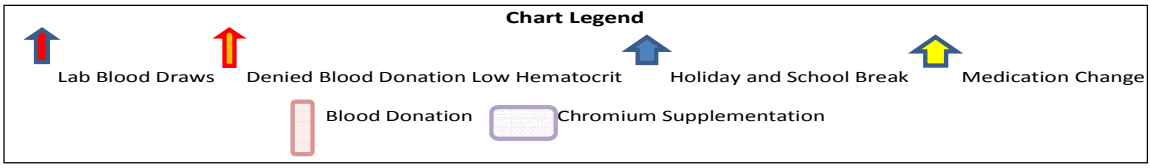


Figure 8. Graph of daily blood glucose levels from glucometer with notations of confounding factors of study.



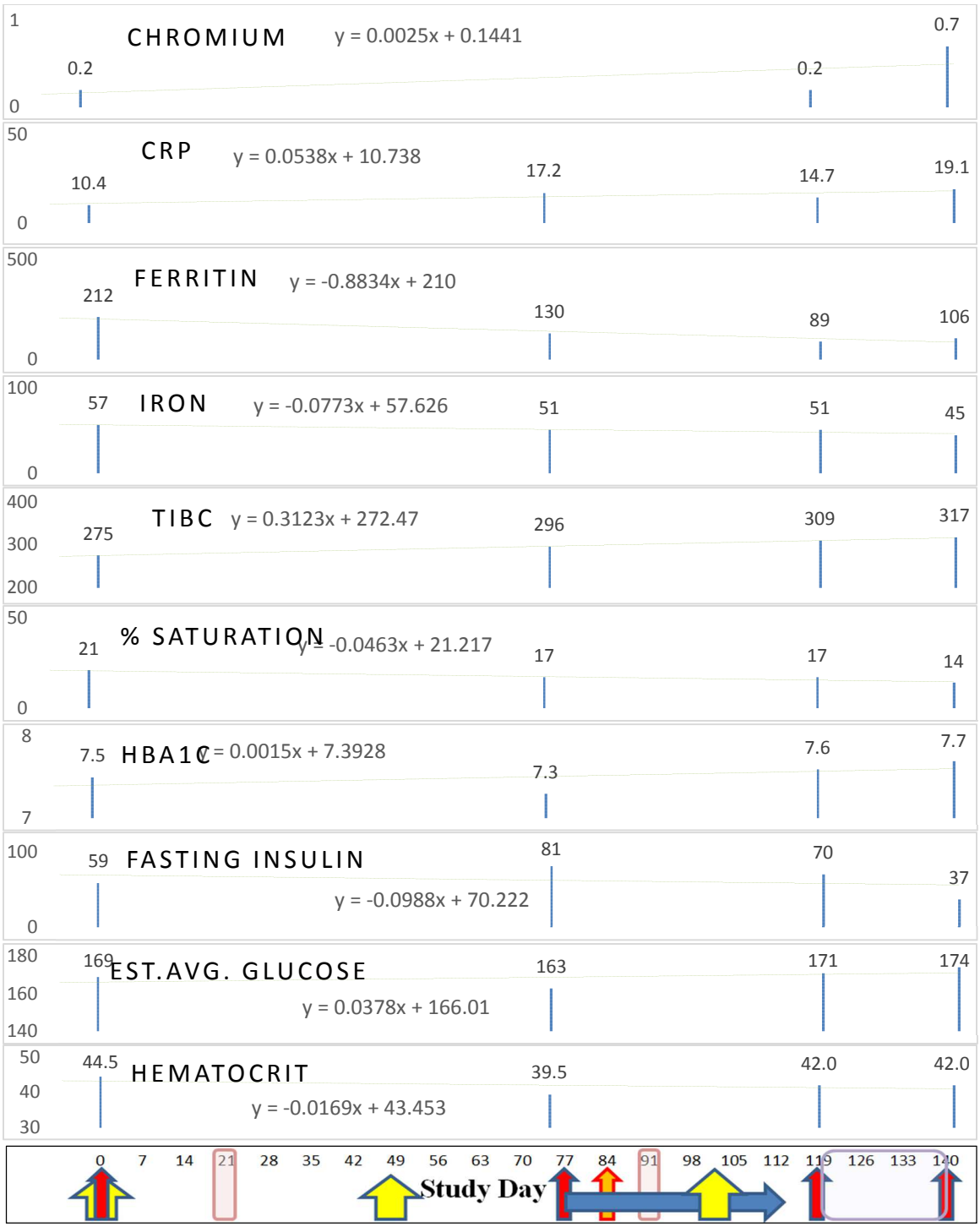


Figure 9. Line graph comparison of lab values with line equation to show change in slope and notation of confounding factors of study.

CHAPTER 5

DISCUSSION

Study results show changes in all measures that all warrant discussion. This case study investigates the effects of blood donation combined with chromium supplementation to improve blood glucose levels measured by HbA1c levels and insulin sensitivity measured by HOMA score. Complications during the study extended study time to twenty weeks, weakened interpretation of the data, and created confounding variables for the protocol.

During visit number one when colleting blood for hematocrit levels the finger was milked, squeezed to induce bleeding, which caused a false low hematocrit due to excess serum in the sample. As a result of this error our subject was initially excluded from the study which resulted in the study being extended to 20 weeks instead of 19 as proposed. The hematocrit was properly measured the following week and participant was included pending lab results from the baseline venous blood draw. Hematocrit levels were measured first to avoid unnecessary venous blood draws if this measure excluded the participant. Subject was included in study after review of all labs at visit two, and first blood donation was scheduled.

Visit 3 results shows a reduction of baseline HbA1c from 7.5% to 7.3% after the first blood donation which may be related to the decrease in iron and ferritin levels as expected. This finding is weakened due to a change in medication; however, all iron markers show a response to the blood donations which has been previously supported by

a study by Karlijn Peffer and colleagues as discussed (Peffer et al., 2013). In addition C-reactive protein levels increased which also increase ferritin levels. Despite an increase in C-reactive protein, ferritin and iron concentrations are still lower than baseline at visit 3. Additionally, ferritin levels were highest at baseline when CRP was at its lowest concentrations during the study duration. This suggests that blood donations are effective in lowering ferritin and iron despite elevated CRP concentrations. The lowering of ferritin and iron show a correlation with a lower HbA1c at visit 3. A decrease in iron levels has been associated to lower blood glucose levels which support the possibility of the blood donation contributing to the improved HbA1c score (Hansen et al., 2014). The addition of Januvia was before baseline data was collected which further supports the possibility of the blood donations contributing to a reduction in HbA1c.

The HOMA score increased from 20.4 to 42.8 based on an increased fasting insulin level of 59 uIU/mL to 81 uIU/mL and fasting blood glucose levels on the day of the blood draws of 140 mg/dL at baseline and 214 mg/dL on the morning of visit three. It is important to note that the purpose of the blood donation is to improve insulin production by decreasing ROS in the pancreas, and to reduce competitive binding to allow chromium the bind for transportation into cell. The blood donations accomplished this based on the reduction of % saturation from 21% to 17% from baseline to after the first blood donation, and the increase in TIBC. The HOMA score is a measure of insulin sensitivity as measured by the relationship to beta cell insulin production and fasting plasma glucose.

This finding is complicated by the addition of Januvia which helps the body to produce more insulin, and on the day of the blood draw the fasting glucose was not measured by subject until after 8:00 am. Subject began taking Januvia which is an oral medication that increases the body's insulin production (WHITLEY, 2007). Increase of the HOMA score supports the overall theory that iron depletion alone is not sufficient to improve insulin production and sensitivity. Results show an increase in fasting insulin with an excess of fasting plasma glucose on the day of the blood draw resulting in the highest HOMA score of the study. This may suggest poor insulin action which is hypothesized to be improved with chromium supplementation.

Visit 4 is after the second blood donation and shows an increase in HbA1c from baseline from 7.5% to 7.6% which conflicts with iron markers. Iron levels continue to decrease from baseline of 57 μg /dL to 51 μg /dL and ferritin from 212 ng/mL to 89 ng/mL which should correlate to a decrease in blood glucose levels. Although C-reactive protein is decreased form visit 3 it is still elevated from baseline which is known to increase ferritin levels. It is clear that blood donation continues to improve iron markers, however, significant confounding factors before visit 4 makes data interpretation unreasonable.

Subject is a student and his normal routine was interrupted by a winter school break and a Christmas holiday. Daily fasting blood glucose readings decreased and many of the readings were taken after 8 a.m. which suggests readings not taken upon waking and or irregular sleep patterns. The findings are further complicated due to another change in medication. First subject stopped taking Januvia and let the medication clear

his system so he could begin Glipizide, which helps the pancreas produce more insulin, so effect of decreased iron on insulin production and sensitivity cannot be interpreted (Go, Kyriakidou-Himonas, & Berelowitz, 2004). Next, due to a prescription error the subject went without all medications for week 13. Finally, once the prescription was corrected the subject resumed Metformin with the addition of a new medication, Glipizide. The HOMA score for visit 4 shows a decrease from visit 3 and an increase from baseline. This score along with the HbA1c concentrations cannot be properly interpreted due to the irregular daily fasting glucose levels and confounding variables described prior to visit 4.

At visit 5 all medication has been stable for 7 weeks; however, Januvia has been replaced with Glipizide which is different than baseline. Glipizide works primarily by increasing pancreatic beta cell production of insulin and secondarily by increasing glucose usage and decreasing the production of glucose by the liver (Go et al., 2004). This complicates the interpretation of HbA1c concentrations along with the subject presenting to visit 5 with an active case of bronchitis which may have contributed to the elevated CRP and ferritin levels and the time period of less than ninety days on the new medication may impair an accurate HbA1c reading. The combination of these factors makes interpretation of HbA1c levels unreasonable.

Chromium results show an increased concentration from baseline from .2 to .7. This is remarkable as it correlates to a decrease in HOMA score from baseline from 20.4 to 12.2 at final visit. The combination of iron depletion and chromium supplementation suggests a decrease in insulin resistance based on the HOMA score. This is further

supported by research from C. Derrick Quarles and colleagues that suggests once chromium binds Tf, it is transported into the cell and binds chromodulin which increases kinase activity and lowers insulin in the blood (Quarles Jr et al., 2011). The lowest fasting insulin levels and HOMA score of the study are seen when the chromium concentrations have been increased from baseline at .2 to .7 at visit 5 which is more than a threefold increase. The iron concentrations and percent saturation has consistently declined regardless of any confounding factors discussed throughout the study duration while the TIBC consistently increased. This further supports the theory that once iron levels are decreased the capacity for chromium to bind Tf increases and allows chromium to enter the cell and become biologically active as chromodulin (Albarracin, Fuqua, Evans, & Goldfine, 2008).

Overall, confounding factors weaken the findings based on medication changes, fluctuations in daily and seasonal lifestyle, and illnesses that arise. This is the life of a diabetic and was to be expected during the course of this twenty week study. This case study cannot be generalized to the general public and serves as an exploration for the feasibility of combining blood donations to reduce iron and chromium supplementation to improve glucose management and insulin sensitivity. Results from this study suggest that this protocol should be tested in a larger population.

Strengths of this study are the combined therapies that have not been tested and suggest feasibility of larger study. The study design is strong with a modification to extend the chromium supplementation time to six to eight weeks. This would be a better time frame as diabetics change medication dosages and types to manage glucose levels

and this time frame would give a better measure of chromium affect. Limitations of this study are recruitment and fluctuations in non-insulin medications.

CHAPTER 6

CONCLUSION

This twenty week case study showed feasibility of further testing in a larger study of this combined protocol of iron depletion through blood donation and chromium supplementation. Our results do not show promise for our first hypothesis of Phlebotomy and chromium treatments will improve glucose management as indicated by daily fasting glucose measurement and HbA1c levels in individuals with T2D based on confounding factors.

Our results show promise for the second hypothesis of Phlebotomy and chromium treatments will improve insulin sensitivity as measured by HOMA in individuals with T2D.

REFERENCES

Albarracin, C. A., Fuqua, B. C., Evans, J. L., & Goldfine, I. D. (2008,). Chromium picolinate and biotin combination improves glucose metabolism in treated, uncontrolled overweight to obese patients with type 2 diabetes. *Diabetes/metabolism Research and Reviews*, pp. 41-51. doi:10.1002/dmrr.755

Ali, A., Ma, Y., Reynolds, J., Wise, J., Inzucchi, S., & Katz, D. (2010). Chromium effects on glucose tolerance, insulin sensitivity and vascular function in people at risk for diabetes. *Diabetes, 59*, A462-A462.

American Diabetes Association.**Diagnosis and classification of diabetes mellitus** *35 no. Supplement 1 S64-S71*(January 2012), June 16, 2014. doi:10.2337/dc12-s064

American Diabetes Association. (1978). Diabetes care.

American Diabetes Association. (2014). Standards of medical care in diabtes-2014. *Diabetes Care, 37*(Supplement 1 S14-S80), 10/5/2014. doi:10.2337/dc14-S014

American Diabetes Association. (April 2013). Economic costs of diabetes in the U.S. in 2012
. *Diabetes Care, 36*(no. 4 1033-1046), 10/5/2014. doi:10.2337/dc12-2625

Anderson, R., Cheng, N., Bryden, N., Polansky, M., Cheng, N., Chi, J., & Feng, J. (1997). *Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes*. ALEXANDRIA: AMER DIABETES ASSOC.

Anderson, R. A. (1981). Nutritional role of chromium. *Science of the Total Environment, 17*(1), 13-29. doi:http://dx.doi.org.ezproxy1.lib.asu.edu/10.1016/0048-9697(81)90104-2

Bahijri, S. M. A., & Mufti, A. M. B. (2002). Beneficial effects of chromium in people with type 2 diabetes, and urinary chromium response to glucose load as a possible indicator of status. *Biological Trace Element Research, 8*5(2), 97-109. doi:10.1385/BTER:85:2:097

Bailey, C. H. (2014). Improved meta-analytic methods show no effect of chromium supplements on fasting glucose. *Biological Trace Element Research, 15*7(1), 1-8. doi:10.1007/s12011-013-9863-9

Bao, W., Rong, Y., Rong, S., & Liu, L. (2012). Dietary iron intake, body iron stores, and the risk of type 2 diabetes: A systematic review and meta-analysis. *BMC Medicine, 1*0(1), 119-119. doi:10.1186/1741-7015-10-119

Basaki, M., Saeb, M., Nazifi, S., & Shamsaei, H. A. (2012). Zinc, copper, iron, and chromium concentrations in young patients with type 2 diabetes mellitus. *Biological Trace Element Research, 14*8(2), 161-164. doi:10.1007/s12011-012-9360-6

Basuli, D., Stevens, R. G., Torti, F. M., & Torti, S. V. (2014). Epidemiological associations between iron and cardiovascular disease and diabetes. *Frontiers in Pharmacology,* 5, 117. doi:10.3389/fphar.2014.00117

Batchuluun, B., Matsumata, T., Batchuluun, B., Erdenebileg, N., Tsagaantsooj, G., Boldbaatar, K., & Khasag, A. (2014). Serum ferritin level is higher in poorly controlled patients with type 2 diabetes and people without diabetes, aged over 55 years. *Diabetic Medicine, 3*1(4), 419-424. doi:10.1111/dme.12343

Chen, Y. (2011). Characterization of the organic component of low-molecular-weight chromium-binding substance and its binding of Chromium1-3. *The Journal of Nutrition, 14*1(7), 1225.

Creighton Mitchell, T., & McClain, D. A. (2014). Diabetes and hemochromatosis. *Current Diabetes Reports, 1*4(5), 1-10. doi:10.1007/s11892-014-0488-y

Ezenwaka, C. E., Seales, D., Surujlal, R., & Mathura, R. P. (2009). Glycated haemoglobin A1c measurement in stored whole blood sample is reliable for clinical use. *The West Indian Medical Journal, 5*8(1), 17.

Gabrielsen, J. S., Hunt, S. C., Hopkins, P. N., Cefalu, W. T., McClain, D. A., Gao, Y., . . . Adams, T. D. (2012). Adipocyte iron regulates adiponectin and insulin sensitivity. *The Journal of Clinical Investigation, 12*2(10), 3529. doi:10.1172/JCI44421.

Gebel, E. (2011). Back to basics: Blood glucose. Retrieved from http://www.diabetesforecast.org/2011/apr/back-to-basics-blood-glucose.html

Go, E. H., Kyriakidou-Himonas, M., & Berelowitz, M. (2004). Effects of glipizide GITS and glibenclamide on metabolic control, hepatic glucose production, and insulin secretion in patients with type 2 diabetes. *Diabetes/metabolism Research and Reviews, 2*0(3), 225-231. doi:10.1002/dmrr.443

Gregg, E. W., Li, Y., Wang, J., Burrows, N. R., Ali, M. K., Rolka, D., . . . Geiss, L. (2014). Changes in diabetes-related complications in the united states, 1990-2010. *The New England Journal of Medicine, 37*0(16), 1514.

Gupta, M., Palta, A., Singh, R., & Lehl, S. S. (2014). Body iron stores in middle-aged north indian patients with type 2 diabetes and obesity. *Journal of Mid-Life Health,* 5(2), 72.

Hansen, J. B., Moen, I. W., & Mandrup-Poulsen, T. (2014). Iron: The hard player in diabetes pathophysiology. *Acta Physiologica, 21*0(4), 717-732. doi:10.1111/apha.12256

Hatunic, M., Finucane, F. M., Norris, S., Pacini, G., & Nolan, J. J. (2010). Glucose metabolism after normalization of markers of iron overload by venesection in subjects with hereditary hemochromatosis. *Metabolism, 5*9(12), 1811-1815. doi:10.1016/j.metabol.2010.06.005

Ikeda, Y. (2014). The ameliorating effects of iron reduction on obesity, diabetes and diabetic complications. *Journal of Pharmacological Sciences, 12*4, 49P-49P.

Kalra, S., Chawla, R., & Madhu, S. V. (2013). The dirty dozen of diabetes. *Indian Journal of Endocrinology and Metabolism, 1*7(3), 367.

Kannan, R. (2012). Diabetes mellitus and oral health. *Journal of Orofacial Sciences,* 4(1), 7.

Khan, A. R., & Awan, F. R. (2014). Metals in the pathogenesis of type 2 diabetes. *Journal of Diabetes and Metabolic Disorders, 1*3(1), 16. doi:10.1186/2251-6581-13-16

Lipscombe, L. L., Austin, P. C., Manuel, D. G., Shah, B. R., Hux, J. E., & Booth, G. L. (2010). Income-related differences in mortality among people with diabetes mellitus. *CMAJ : Canadian Medical Association Journal = Journal De l'Association Medicale Canadienne, 18*2(1), E1-E17. doi:10.1503/cmaj.090495

Maurer, R. (2015). The blood code. Retrieved from http://www.thebloodcode.com/homa-ir-calculator/

McCormack, S., & Grant, S. F. (2013). Genetics of obesity and type 2 diabetes in african americans. *Journal of Obesity, 201*3, 396416. doi:10.1155/2013/396416 [doi]

McLaren, C. E., Li, K. T., Gordeuk, V. R., Hasselblad, V., & McLaren, G. D. (2001). Relationship between transferrin saturation and iron stores in the african american and US caucasian populations: Analysis of data from the third national health and nutrition examination survey. *Blood, 9*8(8), 2345-2351. doi:10.1182/blood.V98.8.2345

Mundet, X., Cano, F., Mata-Cases, M., Roura, P., Franch, J., Birules, M., . . . Cos, X. (2012). Trends in chronic complications of type 2 diabetic patients from spanish primary health care centres (GEDAPS study): Ten year-implementation of st. vincent recommendations. *Primary Care Diabetes,* 6(1), 11-18. doi:10.1016/j.pcd.2011.06.005

Peffer, K., den Heijer, M., Holewijn, S., de Graaf, J., Swinkels, D. W., Verbeek, A. L. M., & Atsma, F. (2013). The effect of frequent whole blood donation on ferritin, hepcidin, and subclinical atherosclerosis. *Transfusion, 5*3(7), 1468-1474. doi:10.1111/j.1537-2995.2012.03916.x

Pscherer, S., Dippel, F., Lauterbach, S., & Kostev, K. (2012). Amputation rate and risk factors in type 2 patients with diabetic foot syndrome under real-life conditions in germany. *Primary Care Diabetes,* 6(3), 241.

Qu, H., Li, Q., Rentfro, A. R., Fisher-Hoch, S. P., & McCormick, J. B. (2011). The definition of insulin resistance using HOMA-IR for americans of mexican descent using machine learning. *PloS One,* 6(6), e21041. doi:10.1371/journal.pone.0021041

Quarles Jr, C. D., Marcus, R. K., & Brumaghim, J. L. (2011). Competitive binding of Fe3+, Cr3+, and Ni2+ to transferrin. *JBIC Journal of Biological Inorganic Chemistry, 1*6(6), 913-921. doi:10.1007/s00775-011-0792-9

Racek, J., Sindberg, C. D., Moesgaard, S., Mainz, J., Fabry, J., Müller, L., & Rácová, K. (2013). Effect of chromium-enriched yeast on fasting plasma glucose, glycated haemoglobin and serum lipid levels in patients with type 2 diabetes mellitus treated with insulin. *Biological Trace Element Research, 155*(1), 1-4. doi:10.1007/s12011-013-9758-9

Sia, A. K., Allred, B. E., & Raymond, K. N. (2013). Siderocalins: Siderophore binding proteins evolved for primary pathogen host defense. *Current Opinion in Chemical Biology, 1*7(2), 150-157. doi:10.1016/j.cbpa.2012.11.014 [doi]

Trumbo, P. R., & Ellwood, K. C. (2006). Chromium picolinate intake and risk of type 2 diabetes: An evidence-based review by the united states food and drug administration. *Nutrition Reviews, 6*4(8), 357-363. doi:10.1111/j.1753-4887.2006.tb00220.x

Viitasalo, K., Lindström, J., Hemiö, K., Puttonen, S., Koho, A., Härmä, M., & Peltonen, M. (2012). Occupational health care identifies risk for type 2 diabetes and cardiovascular disease. *Primary Care Diabetes,* 6(2), 95. doi:10.1016/j.pcd.2012.01.003

Vincent, J. B. (2001). The bioinorganic chemistry of chromium(III). *Polyhedron, 2*0(1), 1-26. doi:10.1016/S0277-5387(00)00624-0

Weber, C. (2010). Challenges in funding diabetes care: A health economic perspective. *Expert Review of Pharmacoeconomics & Outcomes Research, 1*0(5), 517-24. doi:http://dx.doi.org.ezproxy1.lib.asu.edu/10.1586/erp.10.48

WHITLEY, H. (2007). Sitagliptin (januvia) for the treatment of patients with type 2 diabetes. *Am Fam Physician, 7*6(6), 861-862.

APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

APPROVAL FULL BOARD

Carol Johnston
SNHP -Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 6/6/2014 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	The feasibility and tolerability of using iron depletion therapy and chromium supplementation for evaluating insulin sensitivity and hemoglobin A1c in type 2 diabetes
Investigator:	Carol Johnston
IRB ID:	STUDY00001141
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• consent, Category: Consent Form; • Protocol, Category: IRB Protocol; • calendar, Category: Other (to reflect anything not captured above); • flyer and verbal script , Category: Recruitment Materials; • health history questionnaire, Category: Screening

The IRB approved the protocol from 6/4/2014 to 6/3/2015 inclusive. Before 6/3/2015, you are to submit a completed "FORM: Continuing Review (HRP-212)" and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 6/3/2015 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc: Nia Jarrett