INVESTIGATING HOW HIGH FERRITIN LEVELS ARE ASSOCIATED WITH GESTATIONAL DIABETES

by

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As members of the Master's Committee, we certify that we have read the thesis prepared by Rodrick Antonio Millner titled Investigating How High Ferritin Levels are Associated with Gestational Diabetes and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

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Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

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Abstract

Gestational diabetes mellites (GDM) is now a widely recognized condition that affects over 10% of mothers within the United States. The CDC estimates between 2-10% of pregnancies in the US are affected by gestational diabetes every year. Gestational diabetes was discovered over a century ago, and our current understanding has enabled us to determine multiple mechanisms that lead to Gestational diabetes. It is becoming clear that placental hormones are the major contributor toward development of Gestational diabetes. Placental growth hormones have the ability to inhibit insulin receptors as a mechanism for causation of gestational diabetes. However, iron has recently emerged as a potential contributor to the development of gestational diabetes. The focus of my thesis will be how high iron levels are associated with the increased risk of gestational diabetes development in mothers. I will base my model on Fu, *et al*, 2016 whose research aimed to summarize the available proof for correlation of body iron status, dietary total iron, and risk of gestational diabetes and Ganz *et al*. who introduces the idea that hepcidin is a potential factor contributing to gestational diabetes. Finally, I will conclude with the role specific cytokines have on insulin resistance, based on the work of Nieto-Vazquez , *et al*. and how this may integrate with iron-mediated effects. My interpretations are based on quantitative data, prospective cohort studies[1]and case control studies[2]. I will discuss how consuming large amounts of red meat is correlated with risk of developing gestational

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[1] A cohort study or panel study is a quasi-experiment in the form of a longitudinal study

[2] A case-control study is a type of observational study in which two existing groups differing in outcome are identified and compared on the basis of some supposed causal attribute.

diabetes. Fu, *et al*, mentioned several studies (Sharifi,2010; Chan,2009; Kinnunen,2014; and Gungor,2007) with conflicting arguments on whether or not heme iron affected blood glucose. Iron is an important factor in gestational diabetes development because too much iron can lead to hemochromatosis in the pancreas which could develop to Type-1 diabetes from tissue destruction. Tissue damage from hemochromatosis attracts immune cells (Monocytes) that release Interleukin-6 cytokines, which are known to interfere with blood glucose homeostasis. The liver is an important organ involved in gestational diabetes because of its role in releasing hepcidin[3], which is a hormone involved in the inhibition of heme-iron's release into circulation. The goal of my thesis is to contribute to the growing knowledge pertaining to gestational diabetes by focusing on the relationship that iron has in increasing a mother's risk of developing gestational diabetes. Given the recent literature regarding iron and gestational diabetes, the discovery of iron as a potential risk factor may contribute to development of effective new protocols for pregnant women to prevent gestational diabetes.

Keywords: Gestational Diabetes, Ferritin, Hepcidin, Interleukin-6

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[3]A protein that in humans is encoded by the *HAMP* gene. Hepcidin is a key regulator of the entry of iron into the circulation in mammals (Ganz, 2003).

Investigating How High Ferritin Levels are Associated with Gestational Diabetes

Introduction

Diabetes Mellitus is a disease that has been recognized as early as the 15th century B.C. The first recorded person to be diagnosed with diabetes dwelled in Egypt in the year 1552 B.C. The patient presented with excessive urination; later in the start of the early 11th century A.D., patients who presented with excessive urination were asked to give urine samples to their physician, who would drink the urine sample to determine if it tasted sweet. Sweet-tasting urine was a positive indicator for diabetes. Medical personal has come a long way from ingesting bodily fluids of patients suspected to have diabetes. We now depend on the modern, evidence-based diagnostic procedures, seen today in most primary care offices, hospitals, and clinics. One such test is the Oral Glucose Tolerance Test (OGTT). The patient is ordered to consume a drink that is high in sugar. Then two hours later, the medical provider will administer a test to determine the patient's blood glucose level. This test is objectively used to determine how fast glucose is cleared from the blood but at the molecular level the test tells medical providers what is happening to either beta cells and/or insulin receptors. Another popular test is the hemoglobin A1C (HbA1c) test which measures the patient's glycated hemoglobin. Glycated hemoglobin is a form of hemoglobin that reflects the three-month average plasma glucose concentration. HbA1c is a measure of the beta-N-1- deoxy frucsoyl components of hemoglobin (Miedema, 2005). This test is limited to three months because the average life span of red blood cells (RBC) is four months (120) days.

When blood glucose levels are high, glucose molecules can be conjugated to the hemoglobin molecules within erythrocytes. The longer the duration of hyperglycemia in the vascular system, the more glucose is coupled to hemoglobin within RBCs, thus increasing the glycated hemoglobin levels. Since glycation of a hemoglobin molecule is irreversible, the buildup of glycated hemoglobin within erythrocytes reflects the average level of glucose the cell has been exposed to during its life cycle. Measuring glycated hemoglobin helps determine how effective a course of therapy is when monitoring long-term serum glucose. High levels of glycated hemoglobin will indicate an unhealthy control of blood glucose levels which results in hyperglycemia (Miedema, 2005). Glycated hemoglobin prevents oxygen from binding to the heme molecules which decrease oxygen absorption in surrounding muscle cells (Miedema, 2005).

Types of Diabetes

Type-1 Diabetes emerges as a result of an autoimmune disorder, where the host cells start attacking pancreatic beta cells[4], leading to insulin deficiency (Clark, 2016). As a result of insulin loss, patients become insulin dependent and would require synthetic insulin to control blood glucose levels. A consensus report from the American Diabetes Association (ADA) estimates 1.25 million Americans have Type-1 Diabetes. Type-2 diabetes is another form of diabetes which results from the pathological condition in which healthy insulin-sensitive receptors become resistant to insulin. The CDC reports that 90% of diagnosed cases are Type-2 Diabetes (T2D). Gestational Diabetes (GDM) is described as a condition in which a woman without prior evidence of diabetes develops

[4]insulin-producing cells in the islets of Langerhans of the pancreas.

high blood sugar levels during pregnancy (Zhang, 2017). GDM pathophysiology is similar to T2D; both cause insulin receptor insensitivity. Healthy women without a family history of diabetes can also be at risk for GDM (Mari-Sanchis, 2018) which is why it is important to study other risk factors for GDM.

GDM is impacted by environmental factors like diet and exercise, or genetic factors such as autoimmune disorders. Family history can also contribute to increasing risk of developing GDM. As we continue to study the development and causation of GDM specifically, there is more evidence suggesting that a variety of factors can contribute to the diabetes. Studies by Zhang,2017; Helin,2012; Fu, 2016; Zhao, 2016, explored how iron levels affected blood glucose levels and how it specifically relates to GDM. The daily dietary need for iron in women who are menstruating is 2-3 mg per day, but that value increases to 6-7 mg per day during pregnancy. However, the iron levels in a typical Western diet are on average 15-20 mg/day, of which 15-35% gets absorbed into specialized cells (Hurrell,2010). Therefore, these investigators considered the effects of high or low heme iron has on development of diabetes. The potential role of heme iron in GDM will be discussed in detail below.

Gestational Diabetes Mellitus

Uncontrolled GDM can have devastating effects on both the mother and the fetus. The children of women who had GDM will have an increased risk for childhood and adult obesity (Kawasaki, 2018). Kawasaki, *et al*. observed that such children are at increased risk for developing Type-1 (T1D) diabetes in early childhood and also increased risk of developing T2D in early adulthood (2018). They based their statements

on observational studies which involved 26,509 children who were offspring of mothers whom had developed GDM. As GDM manifests through pregnancy, glucose is unable to be efficiently get taken up by specialized cells (Funk, 2011). If glucose is poorly taken up by specialized cells i.e. muscle cells, the blood stream, then glycogen storage persists ultimately leading to increased adipose tissue mass in both the mother and fetus (Mack, 2017).

The mixing of maternal and fetal circulation does not occur directly, instead, the placenta acts as a filter to allow specific nutrients to reach the fetus from the mother and allows waste to reach the mother from the fetus. Glucose is a molecule that is able to cross the placental barrier into the fetus's blood stream. The blood glucose levels of the fetus increase leading to fetal hyperglycemia (Carr,1998). This becomes problematic when the fetal pancreas responds to the rise in blood sugar by activating beta cells to release fetal pancreatic insulin, the insulin production is inadequate compared to the increasing serum glucose from the mother (Mack, 2017). Macrosomia[5] it the most common consequence of GDM (Stainhart, 1997; Shepard 2017). Excessive levels of glucose stimulation on the fetal pancreatic beta cells can cause decreased insulin production and lead to degradation of the pancreatic cells themselves. Hyperglycemia in the fetus results in the stimulation of insulin, insulin like growth factors, growth hormones, and other growth factors that stimulate fetal growth and deposition of fat and glycogen, these factors lead to macrosomia which results in oversized babies (Shepard, 2017). Geraghty, *et al*. collected blood samples from 331 mother-child couples in a

[5]a condition in which babies are born much larger than normal.

prospective cohort and they found that maternal high serum glucose levels correlated positively with birth weight. These studies both demonstrate and highlight the complexity of how together maternal obesity and maternal glucose intolerance not only increase birth weight but also lead to increased neonatal adiposity, putting the child at a risk for developing macrosomia. Catalano, *et al*. found that infants born to women with GDM have increased fat mass when compared to infants born to women with normal glucose tolerance. However, GDM can be prevented and controlled through exercise, dieting and medication.

Exercise in Prevention of GDM

"The first-line of treatment" in GDM cases is the application of prevention skills. Prevention of GDM is becoming a bigger concern as the prevalence of GDM increases worldwide. Shepherd, 2017 investigated how diet and exercise could help control or prevent GDM in pregnant women. Patients who exercise regularly can lower their risk of developing GDM. Even pregnant women who have not been physically active prior to pregnancy, can gradually increase their exercise during pregnancy to help reduce potential risk of gestational diabetes (Gregg, 2017). Shephard, *et al*. used cluster-RCT (randomized control trial) and compared results from data that focused on mothers who participated in the diet and exercise program with the cohort of mothers that did not have any eating restrictions or exercising tasks. They analyzed results from 23 RCTs (which included 8918 women and 8709 infants from high-income countries) that compared combined diet and exercise interventions. The primary outcome produced a P value of 0.07, demonstrating a possible reduced risk of GDM in the diet and intervention group compared to the standard care group. Overall, Shepard *et al.* were able to provide

moderate evidence suggesting that risk of GDM can be reduced from combined diet and exercise intervention during pregnancy. A limitation of this study is that the authors failed to include the specifics of the diet and exercise regimens used in the interventional group. Another control measure for GDM is the pharmacological approach.

Pharmacologic Control of GDM

Metformin and other Glucagon-like peptide-1 (GLP-1) drugs reduce the effects of GDM. The four most commonly used antihyperglycemic medications used to help patients manage hyperglycemia: are sulfonylureas, dipeptidyl peptidase-4 (DPP-4) inhibitors, Glucagon-like peptide-1(GLP1) receptors agonists, and basal, long-acting insulin (Maryam, 2017). There are many medications on the market that are proven to assist a diabetically-ill patient control their hyperglycemia. Metformin is recommended as a first-line of therapy drug to treat T2D. The Professional Practice Committee of the American Diabetes Association recommended the use of metformin for patients with pre-diabetes mellitus (Romero, 2017). Metformin works to decrease blood glucose levels by inhibiting the production of glucose and reducing intestinal glucose absorption (Gong, 2012). Metformin also controls hepatic glucose production by suppressing gluconeogenesis and acts on the liver to increase insulin activity to make insulin receptors on muscle cells more sensitive. Januvia (Sitagliptin) it is an oral antihyperglycemic drug that inhibits dipeptidyl peptidase-4 (DPP-4), a protein that is expressed on the surface of most cell types and is associated with immune regulation. Sulfonylureas include glyburide and glipizide which works by increasing insulin release from pancreatic beta cells. Gerstein, (2000) designed a trial to determine whether insulin injections worked better then sulfonylureas in diabetic patients. This trail provided good

evidence on how beneficial Sulfonylureas are compared to synthetic insulin as a replacement therapy.

Pancreas, Primary Organs and Cells Involved in Glucose Homeostasis

In order to fully understand the devastating effects of GDM, it is first important understand the normal physiology of the pancreas in response to glucose. Glucose can be introduced into the body in multiple ways. Glucose can be introduced following consumption of food sources high in carbohydrates, where it is metabolized from more complex molecules. The metabolism of carbohydrates requires enzymes secreted from the pancreas to break carbohydrates down to glucose. This process is accomplished by the pancreas and salivatory glands interacting with the digestive, endocrine and exocrine systems. The endocrine component of the pancreas is responsible for important hormones such as insulin, glucagon and somatostatin (Marieb, 2014). These hormones are secreted into the serum and act on receptors that are throughout the body to help control glucose homeostasis. The exocrine function of the pancreas produces digestive enzymes that metabolize carbohydrates into simple sugars (Young, 2013). In addition, there are several enzymes within the mouth and stomach that assist in the metabolism of carbohydrates. The pancreas assists in digestion by releasing pancreatic juices that further break down carbohydrates into molecules of glucose (Silverman, 2002). Pancreatic amylase is an important component in pancreatic juice; it causes the digestion of carbohydrates molecules such as starch into glucose. Once the starch is broken down to its most basic form of glucose, glucose will be absorbed in the intestine and transported to the blood, where it will be taken up by cells that utilize glucose for production of ATP through glycolysis (Berg, 2019). Pancreatic amylase is secreted and acts on the remaining

polysaccharides (maltose and oligosaccharides) and breaks them down into disaccharide units of maltose. Maltase is present in the lining of the small intestine where it will breakdown maltose into units of glucose. Glucose is then absorbed and enters the blood stream and as the concentration of blood glucose increase, the pancreas will start releasing insulin into the blood stream. Insulin binds to receptors located on the surface of hepatocytes, myocytes and adipocytes, and to a lesser extent on many other tissues. Insulin is released by the pancreatic beta cells in response to high glucose serum levels. Insulin helps to regulate metabolism of carbohydrates by promoting absorption of glucose from the blood into specialized cells.

Insulin Signaling Pathway

Insulin Signaling Pathway. The insulin transduction pathway is a biochemical pathway involved in maintaining glucose homeostasis and it is initiated when insulin binds to its corresponding insulin receptor proteins located on the surface of cell membranes. GLUT-4 storage vesicles will fuse into the cellular membrane. This process is important because it results in the GLUT-4 protein channels rising to the surface of the membrane. Once the GLUT-4 proteins are exposed on the surface of the cell membrane, glucose can passively transport into the cell where metabolism will take place. Glucose is an essential component to produce ATP, however it is also a factor that could leads to GDM if not properly regulated (Berg, 2019).

Pancreatic Alpha Cell

Alpha cells contribute one of the major components involved in homeostasis, glucagon. Glucagon is secreted when blood glucose levels decrease, causing a feed-back

loop that increases the concentration of blood glucose (Kerr, 2000). Glucagon inhibits glycolysis when serum glucose is below a normal range. The process is called glycogenolysis and is the breakdown of glycogen to glucose-1-phophate (Nelson, 2008). Glycogenolysis promotes the production of glucose from glycogen stored within cells of the liver to increase blood glucose levels. Glucagon-like peptide (GLP-1) suppress glucagon secretion in human pancreatic alpha cells by inhibiting calcium channels present in the alpha and beta cells. Insulin is released from alpha cell secreted from action potentials generated, calcium influx through voltage-gated channels and changes the signaling of beta performance and increases the risk of developing diabetes. (Nelson, 2008). GLP-1 is expressed by intestinal epithelial cells under high glucose conditions which stimulates insulin and inhibits glucagon secretion to lower glucose levels (Holst, 2007).

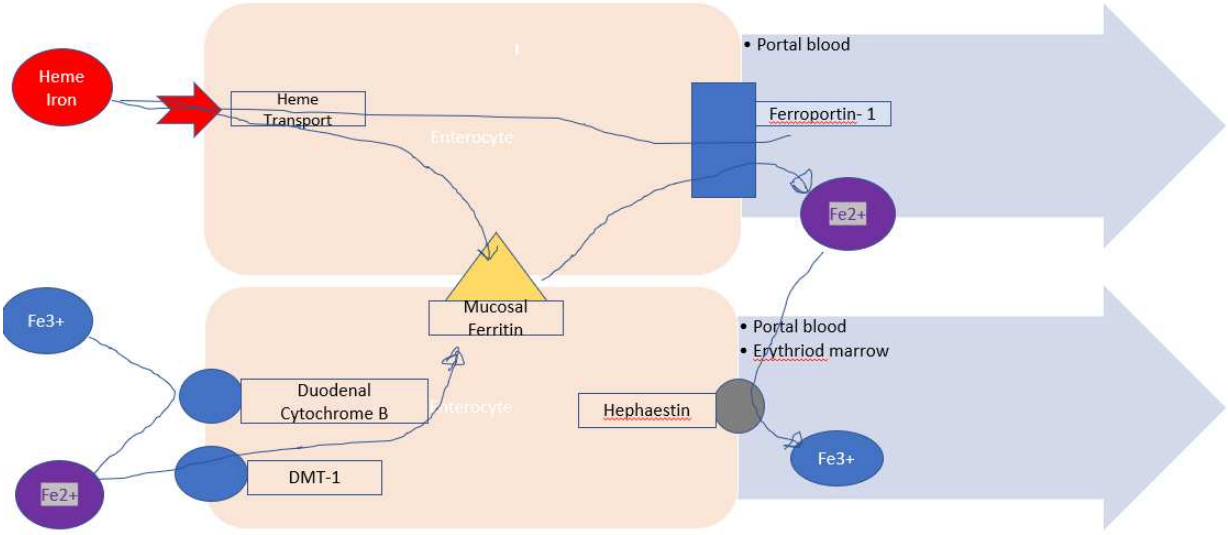
Iron Metabolism

Iron is essential for many cellular processes, but its unique chemical properties require special mechanisms to make it available for use by the cell. There are two forms of iron that the body utilizes: functional heme iron, (which is present in 80% of the hemoglobin within erythrocytes) and ferric iron (nonfunctional stored iron). Heme iron and nonheme iron come from different food sources (Iqbal, 2017). Heme iron is derived from the myoglobin and hemoglobin present in red meat, seafood, and poultry. Heme transporters are located on the apical surface of the enterocytes and they are specific to allowing heme iron to cross the membrane (Leong, 2011). Heme iron absorption ranges

from 15% to 35% while the absorption of non-heme iron ranges from 2% to 20% (Hurrell, 2010).

Ferric iron is most commonly stored in either the parenchyma of the liver, the macrophages of the spleen, or within the marrow of flat bones. Stored ferric iron gets reduced from its ferric form to the ferrous state before passing through the surface of the enterocyte. Ferritin is reduced, it is then able to cross through the DMT-1 receptors (Mangels, 2013). Pregnant women generally have an increased demand for iron because iron is needed for the growing process of the fetus (Bothwell, 2000). Because of this, patients are more likely to become anemic and would require iron supplements to restore iron levels.

Figure 1



Adapted from (Kumar, Abbas, Aster, & Perkins, 2018)

Figure 1. Iron Absorption. In order for ferritin (Fe 3+) to be absorbed, it is reduced from its ferric form to the ferrous state (Fe 2+). This is achieved once ferritin binds to the duodenal cytochrome B (DCYTB) receptors located on the surface of the enterocytes. Ferritin picks up an electron and is then able to cross through the DMT-1 receptor becoming mucosal ferritin. Stores regulator: as iron stores increase in the liver, the hepatic peptide hepcidin is released to prevent iron from exiting the enterocyte for further absorption. The enterocytes retain any absorbed iron and are sloughed off during a bowl movement; as body iron stores fall, hepcidin diminishes and the intestinal mucosa is signaled to release their absorbed iron into circulation (Kumar, Abbas, Aster, & Perkins, 2018).

Hepcidin

The body regulates how much iron enters the blood stream via a negative feedback loop. When the liver detects that the body is receiving high concentrations of iron, the liver produces a molecule, hepcidin, that will bind to the ferroportin-1 transporter and inhibit the release of iron from enterocytes (Rossi, 2005). Notably, it has been suggested that excess ferritin levels from heme iron increase the risk of developing GDM (Zhang,2017; Shrista, 2017) is most likely contributed to hepcidin levels (Ganz, 2012). Emerging evidence supports the idea that hepcidin is the master regulator of iron homeostasis, regulating iron absorption from dietary sources in the gut, recycled iron from macrophages, and iron stores in the liver (Ganz, 2012).

Links between Iron Intake and GDM

Reports by Afkhami-Ardekani, 2009,; Chan, 2009,; Kinnunen, 2014 indicated that elevated ferritin concentration in serum are positively correlated with increased risk of GDM. This prompted an evaluation by Fu, *et al*., of the results of numerous studies (Afkhami, 2009; Lao, 2001; Sharifi, 2010; Gungor, 2007; Tarim, 2004; Zein, 2015; Qiu, 2011; Soubasi, 2010; Chen, 2006; Behboudi, 2013; Bowers, 2011; Helin, 2012; Javadian, 2014; Derbent, 2013; Amiri, 2013) regarding relationships between blood iron levels and GDM. Fu, *et al*, acknowledged that heme iron intake was positively correlated to an increased risk of developing GDM, yet they questioned the reliability of the results from the relatively small number of studies. One cohort study (Helin, 2012) was able to make a direct association between dietary iron and GDM. However, when Fu, *et al*., repeated the study, they found no relationship between dietary iron and the risk of GDM (Fu, 2016).

After conducting a systematic review and meta-analysis to appraise the relationship between iron and GDM risk, Fu, *et al*., performed supplementary analyses, with subgroup analysis, and sensitivity analysis and made the conclusion that limitations exist because the available studies are based strongly on observational studies. Although, ferritin-GDM links shows positive correlation to suggest possible risk contributed to heme iron levels, it was stated that observational studies, if poorly designed, are relatively weak because of the possibly of overestimating details and results of the experiment (Fu, 2016).

The effect of iron supplementation on the risk of GDM has been examined in 2 large randomized control trials (Chan, 2009; Kinnunen, 2016). Chan, *et al*. conducted a trial comparing 300 mg of ferrous sulfate tablet containing 60 mg of elemental iron to a placebo tablet prescribed to women with hemoglobin concentrations within the usual range. No effect of iron supplementation was observed on the risk of GDM. However, the study was limited by low compliance and the exclusion of women with elevated iron status at baseline (Chan, 2009). In the more recent analysis by Kinnunen, *et al.* based on secondary analyses of randomized controlled trials of (Omuladsahebmadarek, 2011) that examined the impact of iron supplementation on multiple pregnancy outcomes, no significant differences in the GDM incidence were observed between iron supplementation and the placebo group (Kinnunen, 2016). However, the study only consisted of a few GDM cases (n=5) and may have not been adequately powered to detect a significant effect (Zhang, 2017). Taken together, data from existing clinical trials on iron supplement do not provide conclusive findings on the impact on GDM occurrence because of their inherent limitations in study design resulting mostly from observational studies (Zhang, 2017).

In a large study which included 500 GDM cases, iron supplementation lasting 2 weeks or longer during pregnancy was related to a 3-fold increased risk of GDM (Bo, 2009). However, inference from this study was limited in that iron supplementation was assessed in mid-pregnancy at the same time as GDM diagnosis (Zhang, 2017). Iron intake from diet other than the supplement was not assessed and accounted for in this study (Bo, 2009; Zhang, 2017). Studies of other indicators of iron status, such as transferrin concentration, hemoglobin, or serum iron concentration, are lacking in the context of GDM risk (Zhang, 2017). Overall, these findings suggest that high ferritin levels contribute to an association with GDM not causation. Although the findings were not consistent across all studies (Zhang, 2017). Helin *et al*. investigated the association between iron intake during pregnancy and examined 399 pregnant women at risk for developing GDM. They found that of the 399 pregnant women who participated in the GDM prevention trail, 72 women were found to have develop GDM (18.1%). Using a prospective cohort study (based on a cluster-randomized controlled trial, where the intervention and the usual care groups were combined) using patients from various maternity clinics in southwestern Finland, Helin, *et al.* concluded that iron intake during pregnancy increases the risk of GDM (Helin, 2012). However, these results were based on a food questionnaire which means (Helin, 2012) results were based on observation.

Mari-Sanchis, *et al*., examined the association that iron has on pregnant women by measuring their total iron intake from red meat, both processed and unprocessed, to understand total iron intake with risk of developing gestational diabetes (Mari-Sanchis, 2017). Mari-Sanchis, *et al.* conducted a prospective study amongst 3,322 disease-free Spanish women who reported at least one pregnancy over a 13-year timeframe. They

found that 172 (5%) women developed GDM resulting from consumption of red meat, suggesting that high heme iron intake is associated with higher risk of developing GDM. Based on these analyses a positive association of GDM with iron intake was concluded. Zhao, *et al.* concluded that dietary intake of heme iron was significantly associated with GDM risk. They also found that body iron stores (ferritin) were correlated with GDM risk. Ultimately, they concluded that both serum ferritin and serum heme iron were increased in GDM patients compared with non-GDM patients (Zhao, 2017).

How does iron impact diabetes? Zhang, *et al*. state that free iron can contribute to increased oxidative stress because iron is known for its pro-oxidant properties and its ability to generate reactive oxygen species (ROS). Pancreatic beta cells are damaged by high levels of oxygen radical intermediates caused by the high ferritin accumulation. As a result, less insulin production will occur because the oxygen radicals damage pancreatic cells leading to diabetes (Zhang, 2017). Kishimoto, *et al*., performed immunohistochemical analysis to study an autopsy case of a patient who suffered from aplastic anemia and diabetic hemochromatosis. They found extensive hemosiderin deposits [6]in the liver and pancreas, but the hemosiderin deposits were exclusively distributed in the Beta cells. Their conclusion was that selective iron-induced damage to the beta cells, which affects insulin synthesis and secretion, is what led to glucose intolerance in those patients (Kishimoto, 2010).

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[6]An iron-storage complex. It is only found within cells Iron with deposits of hemosiderin is very poorly available to supple iron when iron is needed. +

Hepcidin, a hepatic hormone that plays a key role in iron homeostasis, is linked to development of GDM. Two studies (Derbent, 2013; Rawal, 2017) support the claim that hepcidin levels demonstrate a positive association with GDM. One study was a cross-sectional study[7] that included 30 GDM cases (Derbent, 2013). It demonstrated that hepcidin concentrations during the time of GDM screening were significantly elevated in women with GDM compared to women with normal glucose tolerance. A large prospective longitudinal study (Rawal, 2017), which included 107 GDM cases from women of multiple races during the 16-24 weeks of gestation period, found that increased hepcidin concentration was associated with an increased risk of developing GDM. Because hepcidin concentrations could be influenced by inflammation this longitudinal study was adjusted for C-reactive protein (CRP[8]) concentration and it was observed that a significant and positive association between hepcidin and GDM persisted (Zhang, 2017). CRP level increase in response to inflammation, which is known to increase in response to IL-6 secretion by macrophage and monocytes (Pepsy, 2003).

Immune influences on GDM

It is known that elevated interleukin-6 (IL-6) cytokines have a strong association with insulin-resistant states as seen in T2D (Nieto-Vazquez, 2008). IL-6 is the cytokine that is most commonly present during such insulin-resistant states. Nieto-Vazquez, *et al*.

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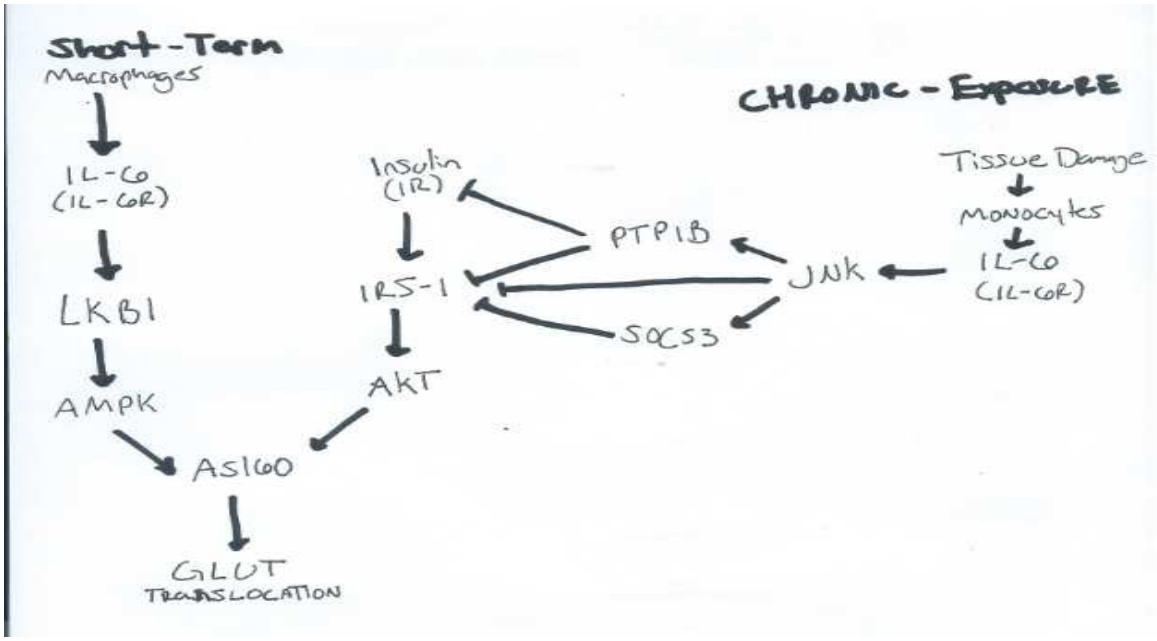
[7]In medical research and social science, a cross-sectional study is a type of observational study that analyzes data from a population, or a representative subset, at a specific point in time—that is, cross-sectional data.

[8] C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells (Pepsy, 2003).

studied the dual effect IL-6 had on the function of insulin and observed that in mice, low levels of IL-6 increase glucose uptake and systemic insulin sensitivity and chronic exposure of IL-6 promoted insulin resistance by impairing insulin signaling at the insulin receptor substrate (IRS-1) [9](Carry, 2006; Febbraio, 2004). The role of IL-6 in the etiology of insulin resistance is not fully understood and has been a matter of controversy (Kristiansen, 2005). IL-6 is a pleiotropic cytokine produced by T-cells, B-cells, monocytes, fibroblasts or endothelial cells. IL-6 is one of the main cytokines produced in response to inflammation. IL-6 has a broad range of cellular responses ranging from immune response, hemopoiesis, cellular growth regulation, gene activation and regulation, proliferation and differentiation (Carry, 2006). Data presented below illustrate the impact IL-6 has on the action of insulin.

Figure 2



[9] Insulin receptor substrate 1 (IRS-1) is a signaling adapter protein that in humans is encoded by the IRS-1 gene (Sun, 1991).

Adapted from (Nieto-Vazquez, Fernandez-Veledo, Alvaro, & Lorenzo, 2008)

Figure 2. *Dual role of IL-6 in modulating insulin sensitivity in skeletal muscle*. IL-6 per se increases GLUT4 translocation to the plasma membrane by activating the KB1/AMPK/AS160 pathway. A dual effect on insulin action is observed when myotubes are exposed to this cytokine. Short-term IL-6 treatment has an additive effect with insulin on glucose uptake, mimicking the positive effect of IL-6 on insulin sensitivity when released from muscle after exercise. However, chronic exposure (such as when secreted by obese adipose tissue) produces insulin resistance, with impaired GLUT4 translocation and defects in insulin signaling. Accordingly, IL-6 impairs insulin signaling at the level of IRS-1 by three mechanisms that involve 1) serine phosphorylation by JNK, 2) impairment on tyrosine phosphorylation by SOCS3, and 3) tyrosine dephosphorylation by PTP1B. LXR agonists and SP600125 overcome such resistance by producing downregulation of SOCS3 and PTP1B expression and inhibition of JNK, respectively (Nieto-Vazquez, 2008).

Discussion

Throughout this thesis, I have presented research from various investigators that links high levels of iron to GDM. Observational studies showed that women who consume more red meat before and during gravidity are more likely to develop GDM (Mari-Sanchis, 2018). The evidence presented suggests that dietary iron intake during and before pregnancy is associated with GDM, which remained true after removal of major dietary factors and other major documented risk factors of GDM (Zhang, 2017). This information suggests, based on evidence from meta-analysis, that high ferritin from consuming red meat or iron supplements puts healthy mothers at risk of developing

GDM. Additional observational studies (Bowers, 2011; Darling, 2016; Palma, 2008) demonstrated potential links between iron and GDM were indirect links to GDM. However, observational studies cannot demonstrate how ferritin increases the risk of developing GDM. Research (Afkhami-Ardekani, 2009; Fu, 2016; Zhang, 2017) presented evidence to indicate an association between increased iron blood serum with GDM but did not present a potential pathway to demonstrate how iron leads to high blood glucose. In my opinion, the molecular mechanisms underlying the observed associations of GDM with heme iron have not been researched in sufficient depth (Zhang, 2017). Although heme iron intake was positively related with an increased risk of GDM after adjustment for known potential confounders, considering the small number of studies, the reliability of the results can be effected (Fu, 2016). Although Helin, *et al.*, stated that dietary total iron intake has been associated with GDM in a cohort study (Helin, 2012), Fu, *et al.,* did not find any statistically significant relationship between ferritin levels and GDM risk (Fu, 2016). Ford, *et al*., and Cauza*, et al.,* hypothesized that elevated ferritin levels may represent elevated body iron stores and may reflect inflammation. Some researchers (Qui, 2004) found that C Reactive Protein (CRP) levels, a marker of the acute-phase inflammatory response, at mid-pregnancy, is correlated with GDM. CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes); it is an acute-phase protein that increases following IL-6 secretion by monocytes and T cells (Pepsy,2003; Lau, 2004). This suggests that inflammation can play an important role in the development of GDM (Li, 2007). Based on the theories, research, and studies presented above, I have proposed a model for what best explains how increased ferritin levels during gestation lead to GDM.

Figure 3

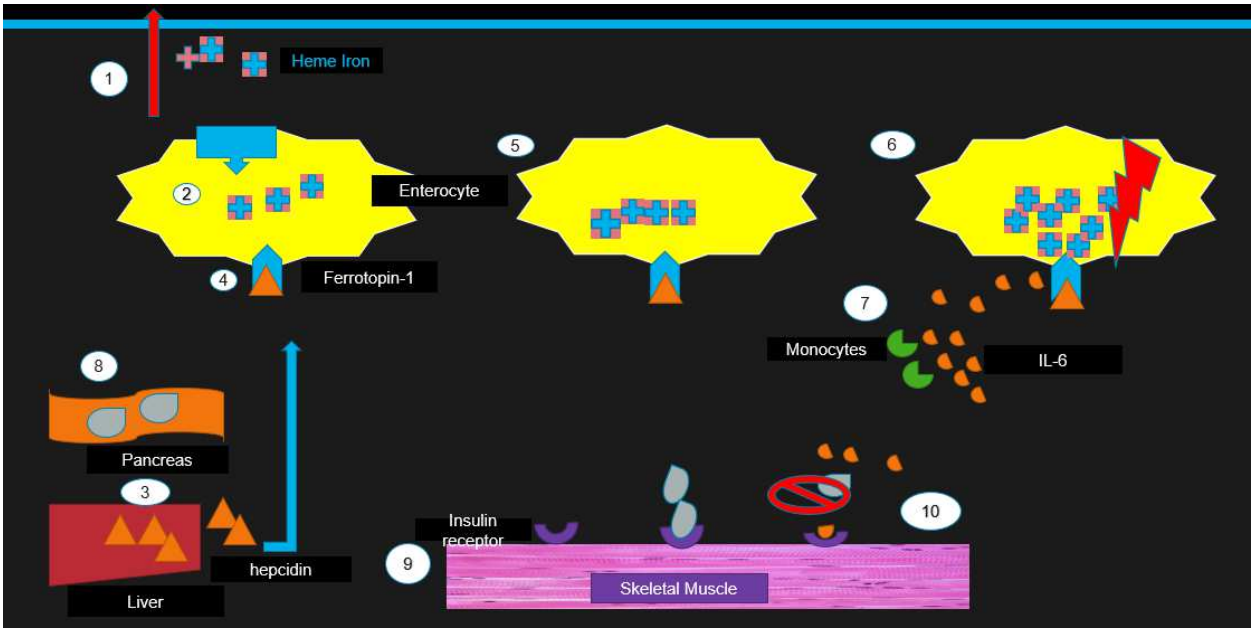


Figure 3. 1.) As the concentration of Heme iron increases, (2) heme iron enters the cell through heme transporters and is able to absorb into circulation through ferrotopin-1 transporters. 3.) The liver starts releasing hepcidin (4). The hepcidin will bind to ferrotopin-1 transporters to prevent iron from getting absorbed. 5.) heme iron will continue to cross through the heme transporters but the heme iron is unable to absorb into circulation. 6.) Oxidative stress causes damage to the cell which recruits monocytes (7) to the area, they release IL-6. During chronic exposure to IL-6 (10), it binds to its receptor and alters the signaling downstream of the insulin receptor.

My hypothesis on how iron leads to GDM in women is as follows: 1) Starts with high consumption of iron, either through red meat or through ferrous sulfate, increasing the iron concentration in the serum. 2) As the iron levels increase, hepcidin is released from the liver to inhibit iron from being absorbed into the body. This would be due to

hepcidin's ability to block ferroprotin-1, an iron transporter, which prevents iron absorption. However, when excess iron is being consumed, in the presence of hepcidin, iron builds up within the gut. Prolonged exposure to iron leads to hemochromatosis resulting in tissue damage from the oxidation of iron. This will allow for iron to bypass the ferrotopin-1 transporters to leak iron into the blood stream through the damaged tissues. I hypothesize that when serum hepcidin levels are elevated, iron builds up in the gut leading to hemochromatosis. The excess iron build-up causes tissue destruction which will lead to inflammation of enterocytes within the gut. During the inflammatory process, the damaged tissues releases histamines to increase the blood flow to the damaged area. The histamine causes the capillaries to open to release phagocytes to the damaged tissue. The phagocytes upon detection of damage tissues will secrete pro-inflammatory cytokine IL-6 to act on the muscle cells to stimulate energy mobilization to increase the temperature of the surrounding cells. However, it is the presences of IL-6 produced by monocytes, that interferes with glucose absorption by interfering with insulin receptor signaling.

Based on the available data reviewed throughout my thesis, it seems there is not enough evidence to conclude that elevated ferritin leads to GDM. The current research regarding high ferritin levels as a risk factor for GDM should be examined more closely. To better understand how iron is linked to GDM, more research should be steered to determine molecularly the influence iron has on blood glucose levels. A logical interpretation of presented data suggests that tissue damage from hemochromatosis in the gut generates tissue damage to recruit enough immune cells, to release IL-6, a known cytokine that interferes with signaling that happens after insulin binds to it receptor,

indirectly effecting blood glucose. In addition, more research should be done to determine if high concentrations of iron increase systemic or local IL-6 levels and to determine which cell types contribute to chronic levels of IL-6. There is abundant evidence that certain pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are involved in response to tissue damage. Perhaps these cytokines are also involved as factors that increase the risk of GDM and more research on these specific cytokines and their relationship to blood glucose should be examined. Other challenges include the complication of genetic variation between individuals. Patients who have sickle cell anemia could produce different results from the typical population because their erythrocytes handle iron differently. In conclusion, I believe that iron indirectly increases blood glucose levels. The major cause of hyperglycemia is due to cytokines released from monocytes in response to tissue damage. In particular, IL-6 has been linked to altered insulin receptor signaling. Therefore, this cytokine (and perhaps others) should receive additional attention for future research on potential links with GDM.

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