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THE ROLE OF INFLAMMTION IN TYPE II DIABETES

A Major Qualifying Project Report

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ABSTRACT

Insulin resistance is one of the main causes of Type II diabetes. Recent studies have shown that inflammation plays a key role in the onset of insulin resistance. A high fat diet and elevated levels of inflammatory cytokines have been found to interfere with insulin signaling. In this study, we explored the effect of a high fat diet on insulin resistance in immunodeficient mice. Following a high fat diet, these NOD-*scidIL2rγ*^{null} mice remained leaner and significantly more insulin-sensitive compared to their wild type counterparts. These data will aid our understanding of how inflammation promotes insulin resistance.

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ACKNOWLEDGEMENTS

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BACKGROUND

Diabetes is a metabolic disorder affecting millions of individuals worldwide. Diabetes is a disease in which the body becomes unable to use glucose as a source of energy, and this failure to utilize the body's main source of energy has many severe ramifications. Because the patient is unable to produce or respond to insulin (depending on the type of diabetes), this disease prevents glucose from being delivered inside cells in the body either for immediate use, or storage for later. According to the American Diabetes Association (2010), 25.8 million people currently suffer from this disorder in the United States. There does not seem to be any gender discrimination with diabetes, affecting about 11.8% of men under the age of 20, and 10.8% of women in the same age group (American Diabetes Association, 2010).

Diabetes: Types I and II

The two main types of diabetes are type I and type II. Type I diabetes is an autoimmune disease that destroys the pancreatic islet beta cells and keeps them from creating and secreting insulin. In this type, the body's own T-lymphocytes target the pancreatic beta cells leading to their destruction. The lack of insulin prevents the body from maintaining homeostatic levels of carbohydrates and fats circulating throughout the body. Type I diabetes commonly affects children (and is sometimes termed juvenile onset diabetes), but it also occasionally presents in the adult population. Recent statistics show that nearly 1 in every 400 children and adolescents has type-1 diabetes (American Diabetes Association, 2010). The more common type II diabetes, which mostly affects adult patients, is frequently associated with poor diet and obesity, and causes the body to become unresponsive to the insulin that is produced.

Diabetes Physiology: Mechanism and Role of Insulin Receptors

Delivering the necessary energy to and from cells is performed by the body's metabolism. Two hormones essential for this process are insulin and glucagon. The homeostasis of the blood levels of glucose, fatty acids, triglycerols, and amino acids is maintained by these two hormones working in unison to take up and delivery energy to the body (Herrera, 2000). These two hormones are secreted by pancreatic cells known as the islets of Langerhans. The islets are composed of alpha, beta, and gamma cells. The alpha and beta cells are responsible for secreting glucagon and insulin, respectively, and the gamma cells secrete somatostatin, which helps regulates the secretion of growth hormone. The body is extremely sensitive to the amounts of glucose in the circulatory system. The body responds when glucose levels fluctuate from the normal range, between 5 to 8 mmol/l. When glucose levels dip below 5 mmol/l, the body increases the amount of glucose in the blood by secreting glucagon, which signals the body to break down glycogen reserves into glucose for use, in a process known as gluconeogenesis. The body frequently experiences low glucose levels in the morning hours after fasting overnight. Alternatively, when blood glucose levels rise above 8 mmol/l, such as after the ingestion of a meal, beta cells release insulin to signal the uptake of glucose into cells. The glucose taken up by the cell is then used as an immediate energy source, or is converted to glycogen through a process known as glycogenesis. This glucose homeostasis balancing act is shown in **Figure 1**.

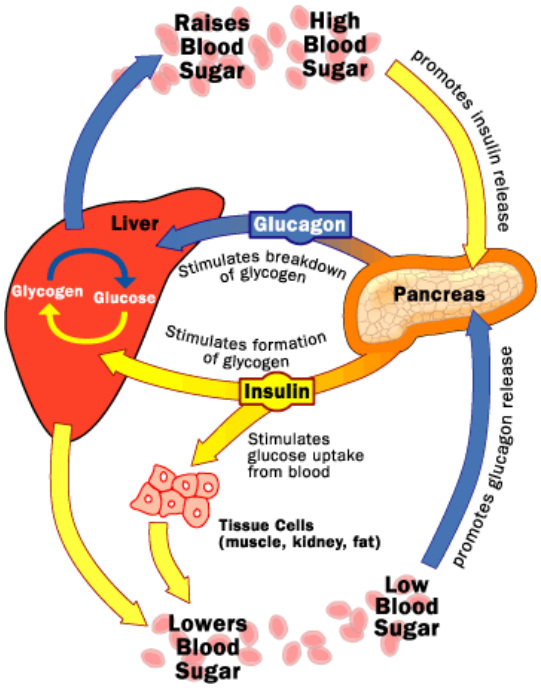


Figure 1: Glucagon and Insulin Secretion. The figure shows the two scenarios that occur during an imbalance in plasma glucose levels. When the body is no longer in homeostatic levels of glucose in the blood, the secretion of insulin (the bottom half of figure 1) or glucagon (the top half of figure 1) is performed to re-establish homeostasis. (Freudenrich, 2010)

The pancreatic hormones are produced through a series of pathways in the endocrine system. When plasma glucose levels are above or below the normal levels, signals are sent to the brain. In normal insulin-sensitive individuals, the body will sense the increase in blood glucose levels and signal to the pancreas to secrete more insulin. This newly secreted insulin travels through the blood stream and binds insulin receptor sites located on tissues such as liver and muscle. The binding of insulin to its receptor triggers a cascade of intracellular signaling that allows the cells to take up more glucose via glucose transporters (**Figure-2**). When insulin binds its receptor, the receptor is auto-phosphorylated which leads to a series of signaling events that culminate with the up-regulation of glucose transporters on the cell surface that bind glucose and transport it inside the cell (White, 1997).

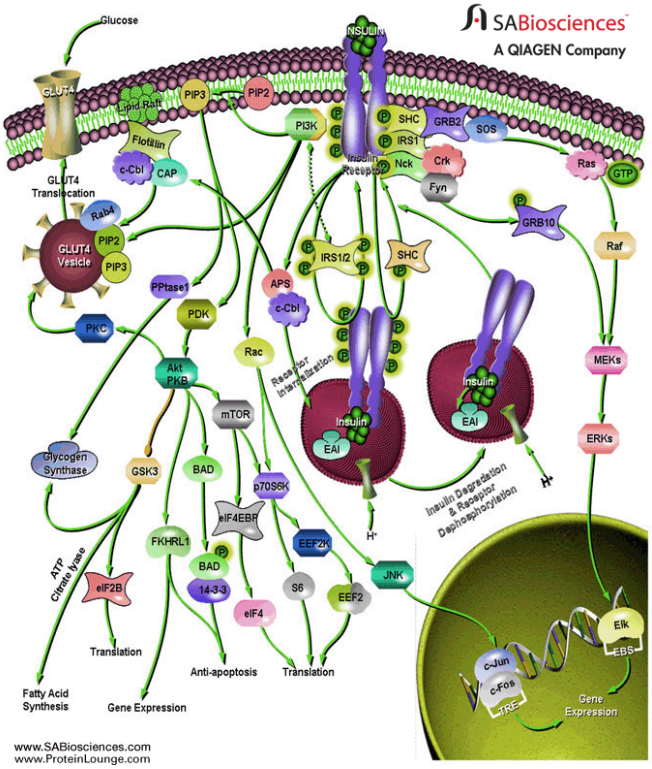


Figure 2: Diagram of Insulin Signaling Pathway. Figure illustrates the cascade of signaling events that occur when insulin molecules bind the insulin receptor (diagram upper center) resulting in auto-phosphorylation. The end result is the migration of the glucose transporter GLUT storage vesicle to the cell surface to bind glucose and transport it into the cell. (SABiosciences, 2010)

Type II Diabetes: Insulin Resistance and its Various Mechanisms

Although Type II diabetes is the more common type, less is known about its various causes. All type II patients show one consistent phenotype, insulin resistance, a lack of response to secreted insulin. This resistance is a notable characteristic of diseases such as high blood pressure and heart disease, and is primarily associated with type II diabetes. Insulin resistance can be observed 10 to 20 years before the onset of Type II diabetes (Shulman, 2000). In response to decreased insulin sensitivity, the pancreas secretes more insulin to compensate. Diabetes results when the increased levels of insulin are insufficient to compensate for the insulin resistance. Insulin resistance can manifest in three types: 1) increased amounts of insulin

are required to elicit normal physiological responses; 2) the increased levels of insulin are insufficient to maintain glucose homeostasis; 3) a combination of the first two (Proietto et al., 1982).

Fatty Acid-Induced Insulin Resistance

One mechanism that can induce insulin resistance is an increased level of plasma free fatty acids. Since free fatty acids compete with glucose for substrate oxidation, one model proposes that increased fat oxidation associated with obesity causes insulin resistance (Shulman, 2000). The increased fatty acid levels directly affect insulin signaling and diminish glucose uptake (**Figure-3**). Other factors such as adipokines secreted by adipose tissue may also lead to diminished glucose uptake (Mlinar et al., 2006).

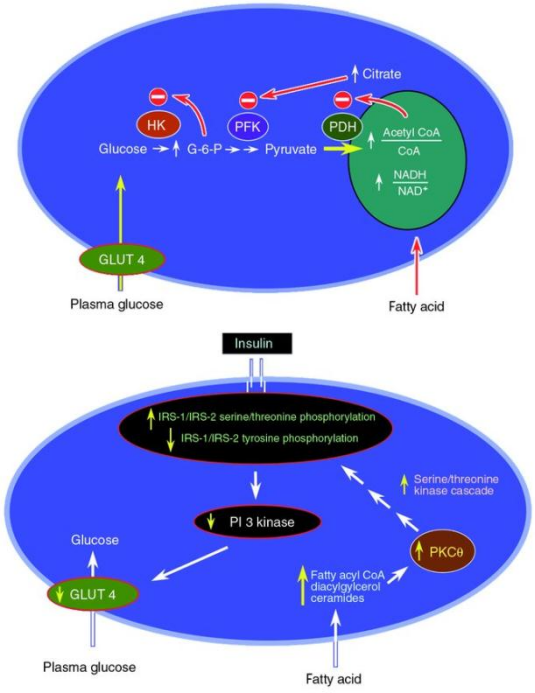


Figure 3: Two Proposed Mechanisms for Fatty Acid Induced Insulin Resistance. The top diagram shows fatty acid-induced insulin resistance occurring in skeletal muscle, and focuses on the role of mitochondria and an inhibition of phosphofructokinase which would increase intra-cellular levels of

glucose-6-phosphate and inhibit the activity of hexokinase II, increasing intracellular glucose and decreasing the amount of glucose taken up by the muscle. The bottom diagram is an alternative mechanism in which increased levels of fatty acid in the muscle leads to the activation of a serine/threonine kinase cascade, which initiates phosphorylation of serine/threonine sites on the insulin receptor substrates, causing insulin resistance. (Shulman, 2000)

ER Stress in Type II Diabetes

The endoplasmic reticulum (ER) is a highly specialized organelle that functions with intracellular trafficking, and lipid and protein synthesis. The ER synthesizes transmembrane proteins and lipids for most cells, and is responsible for the synthesis of almost all secreted proteins. The ER also has an important role in Ca^{2+} storage and signaling. Thus, the ER is a multi-functional organelle that functions in organogenesis, transcriptional activity, stress responses, and apoptosis (Berridge, 2002). With respect to diabetes, ER stress plays a role in β-cell loss and insulin resistance. In the presence of high glucose levels, insulin production in the ER as a secreted protein can exceed 10-fold normal levels which can stress the ER, increasing the chances of incorrect protein folding. When the ER is stressed, it utilizes an unfolded protein response (UPR) (Berridge, 2002), which aims to halt protein translation to restore normal folding. The response normally includes the production of molecular chaperones, which aid in the protein folding process. However, prolonged exposure to ER stress can be detrimental to β-cell function (Eizirik et al., 2008). Therefore, chronic levels of glucose and fatty acids which exceed the normal range inflict a great deal of stress on the ER, affect insulin folding and production, and ultimately lead Type II diabetes.

Mitochondrial Oxidative Stress in Type II Diabetes

Another organelle whose impairment is related to diabetes is the mitochondrion. Studies have shown that subjects with a history of diabetes in their family experience reduced rates of mitochondrial ATP synthesis in the mitochondria, before experiencing any diminished tolerance for glucose, providing evidence that mitochondrial dysfunction plays a significant role in the onset of diabetes (Petersen et al., 2004). Within β-cells, an ATP/ADP ratio determines the opening and closing of the K_{ATP} channel that allows the secretion of insulin, thus mitochondrial dysfunction affects glucose-induced insulin secretion (Lowell and Shulman, 2005). As glucose is normally processed into pyruvate in the cytoplasm, the pyruvate enters the mitochondria, and the ratio of ATP/ADP increases. The increase in the ATP/ADP ratio eventually triggers the exocytosis of insulin secretory vesicles by the β-cells via a cascade of events including the closing of ATP-sensitive K+ channels, and the depolarization of voltage-sensitive Ca2+ channels (Rolo and Palmeira, 2006). However, when this ATP/ADP ratio increases too high, hyper-polarization of the mitochondrial membrane occurs. The proton gradient's high electrochemical potential difference, which is indicative of a high glucose state, eventually causes partial inhibition of the electron transport in complex III (Rolo and Palmeira, 2006). Electron transport complex III is one of the four complexes responsible for transporting electrons along the inner mitochondrial membrane, and helps transfer H^+ ions across the membrane to establish the proton gradient. As a result of complex III's inhibition, electrons accumulate at a molecule known as coenzyme Q, another member of the electron transport chain. Free radical anions are then formed from the partial reduction of O_2. This accelerated reduction of coenzyme Q appears to be the fundamental cause of mitochondrial dysfunction leading to diabetes-related metabolic disorders (Rolo and Palmeira, 2006).

Type II Diabetes Mouse Models

To better understand diabetes, scientists have developed several types of mouse models that mimic the disease. The **Akita mouse** contains a C96Y mutation in the insulin-2 gene, which prevents disulfide bonds from connecting insulin A and B chains together. This results in the accumulation of misfolded pro-insulin molecules in the ER, and eventually causes diabetes from β-cell loss induced by ER stress (Eizirik et al., 2008).

Another model, known as the **Munich mouse**, has a C95S mutation in the insulin gene, resulting in a loss of the disulfide bond of the intra-A chain. The loss of this bond leads to insulinopenic glucose tolerance in mice that are heterozygous, and causes severe diabetes in mice that are homozygous (Eizirik et al., 2008).

Tfam-mutant mice develop diabetes from having a knockout of the nuclear gene Tfam in pancreatic β-cells, which causes impaired insulin secretion and β-cell loss. Tfam, a transcriptional activator imported into mitochondria, is essential for mtDNA expression and maintenance. These mice can be used to investigate how mitochondrial dysfunction affects diabetes since they also show severe mtDNA depletion, deficient oxidative phosphorylation, and abnormal-appearing mitochondria (Rolo and Palmeira, 2006).

The **non-obese diabetic-severe combined immunodeficient (NOD-SCID)** mouse was reported in 1995 (Shultz et al., 1995) and was generated by crossing a NOD mouse with a SCID mouse. In contrast to the NOD mouse, which is our best model to date for Type I diabetes, the NOD-SCID mouse lacks an immune system so lacks the autoreactive T cells which destroy the islet tissue in Type I diabetes. This mouse does not have the phenotype of type 1 diabetes which is a major characteristic of NOD mouse. Type-1 diabetes is an autoimmune disease in which insulin-producing β-cells are destroyed by T cells (the T and B lymphocytes in this mouse fail to

properly rearrange their antigen-specific receptors and fail to generate functional T or B cells). The NOD-*scidIL2rγ*^{null} mouse has the targeted mutation in the IL2 receptor common γ chain gene (*IL2rγ*)**.** The *IL2rγ* chain is required for high-affinity signaling through the IL2, 4, 7, 9, 15 and 21 receptors. The receptor also directs the growth and maturation of lymphocyte subtypes: T cells, B cells, and natural killer cells so mutation of this gene in mice exhibits severe impairment in innate and adaptive immunity. Recent studies have shown that insulin resistance is related to inflammation and our previous study found the increase of various inflammatory markers in skeletal muscle characterized by insulin resistance. Based on these results, we selected this NOD-*scidIL2rγ*^{null} mouse model in order to identify the role of inflammation in type-2 diabetes.

Kim Lab Interests in Immune System Involvement in Type II Diabetes

Dr. Jason Kim, a Professor of Molecular Medicine at the University of Massachusetts Medical School, is involved in a variety of research projects involving Type II diabetes and metabolism in general. One project is currently investigating the relationship between Type II diabetes and the immune system. Although Type II is not considered an autoimmune disease like Type I, there is evidence of a relationship between type II and the immune system. One line of evidence is the link between inflammation and insulin resistance. Although their role is complex, it is clear that immune molecules such as macrophages, lymphocytes, etc. are involved in atherosclerosis and obesity, which are associated with insulin resistance syndrome (Tracy and Lewis, 2002). In 1993, the discovery of the overexpression of TNF-α in adipose tissue of obese individuals allowed Hotamisligil et al. to conclude there is a relationship between inflammatory cytokines and insulin activity in type II diabetes (Hotamisligil and Spiegelman, 1993). Cytokines are hormones involved with cellular signaling that are produced by the immune

system and nervous system. When an individual becomes obese, macrophages penetrate adipose tissue and cause the adipose cells to secrete inflammatory cytokines such as TNF-α (Kim, 2010). TNFα is released from macrophages and lymphocytes after inflammatory stimulation, trauma, or as previously mentioned by obesity. Among its multiple types of signal transductions, TNFα signals the activation of c-Jun NH2-terminal kinase-1 (JNK-1) which helps regulate energy balance, and glucose and lipid homeostasis in a variety of tissues (Aguirre et al., 2000) (**Figure-4**). When bound to insulin receptor substrate-1 (IRS-1), JNK-1 is phosphorylated at a key serine residue (not tyrosine) which prevents insulin signaling, leading to insulin resistance (Aguirre et al., 2000).

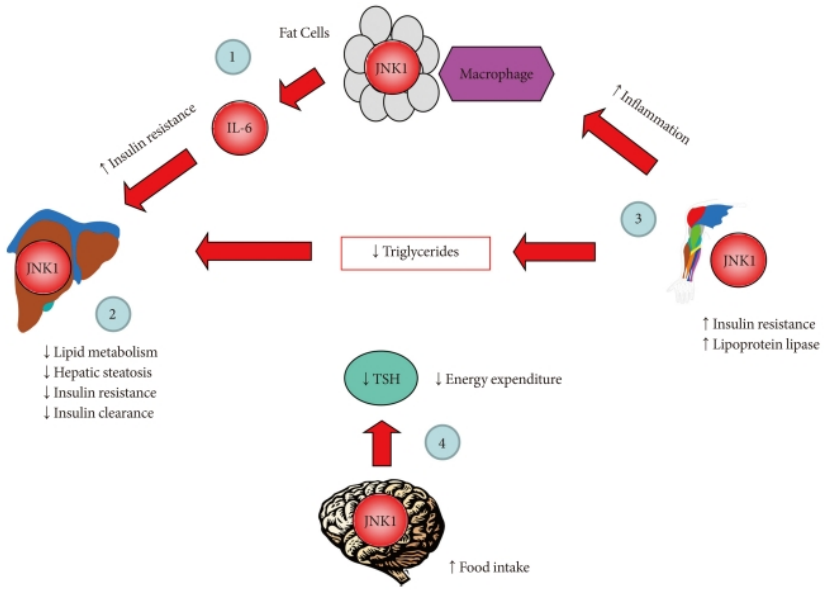


Figure 4: The Role of JNK in Insulin Resistance. Each labeled number indicates observations made with mice deficient in JNK1 in adipose tissue, liver, skeletal muscle, or nervous system: 1) In adipose tissue, JNK1 promotes the secretion of interleukin-6 (IL-6) which causes hepatic insulin resistance in obesity, 2) In liver, JNK1 reduces lipid metabolism and insulin clearance, thereby preventing hepatic steatosis and decreasing insulin resistance, 3) In skeletal muscle, JNK1 mediates insulin resistance, adipose tissue inflammation, and suppresses muscle lipoprotein lipase thereby altering circulating triglyceride levels, and 4) In the nervous system, JNK1 mediates the negative feedback regulation of hypothalamic pituitary-thyroid axis and promotes negative energy balance by increasing food intake and reducing energy expenditure. (Kim, 2010)

PROJECT PURPOSE

Various factors induce insulin resistance in type-2 diabetes. Recently, many investigators found that insulin resistance is related to inflammation. In our lab's previous data we established that inflammation was induced in skeletal muscle with insulin resistance after a short-term high-fat diet. To further investigate this relationship between insulin resistance and inflammation, we assessed glucose metabolism and insulin resistance in diabetic immune-deficient mice.

METHODS

Body Composition

The ^{1}H-MRS instrument was used to measure the changes in the mouse's total body weight, fat mass, and lean mass. Body composition of the mice was measured weekly. The ^{1}H-MRS machine allowed the measurements to be taken while the mice were fully awake.

Metabolic Cage

Metabolic cages were used in conscious mice to measure energy expenditure, physical activity, and food/water intake. Energy expenditure was calculated by measuring O_2 consumption and CO_2 production in individual mice. Physical activity was calculated by quantitative measurement of horizontal and vertical movement (XYZ-axis) for 3 days. Twelve mice were measured at a time (6 NOD-*scidIL2rγ*^{null} and 6 wild-type). The metabolic cages were performed on both normal chow and 4 weeks of high-fat diet (55% fat).

Hyperinsulinemic-Euglycemic Clamp

A hyperinsulinemic-euglycemic clamp (**Figure-5**) was used to measure glucose metabolism in conscious mice. The clamp was performed on normal chow and 4 weeks of high-fat diet with the NOD-*scidIL2rγ*^{null} mice and wild type mice. Following the basal period, a 2-hr hyperinsulinemic-euglycemic clamp was conducted with a primed (150 mU/kg body weight) and continuous infusion of human insulin at a rate of 15 pmol/kg/min to raise plasma insulin within a physiological range. Blood samples were collected at 10~20 min intervals for the immediate measurement of plasma glucose, and 20% glucose was infused at variable rates to maintain basal glucose levels. Insulin-stimulated whole body glucose metabolism was estimated with a

continuous infusion of [^{3}H]glucose throughout the clamps (0.1 μCi/min). To estimate insulin-stimulated glucose uptake in individual organs, 2-[1-^{14}C]deoxy-D-glucose (2-[^{14}C]DG), which is a non-metabolizable glucose analogue, was administered as a bolus (10 μCi) at 75 min after the start of clamp. Blood samples were taken at 80, 85, 90, 100, 110, and 120 min of clamp for the measurement of plasma [^{3}H]glucose, $^{3}H_2O$, and 2-[^{14}C]DG concentrations. An additional blood sample was taken at 120 min to measure plasma insulin concentrations (clamp parameters). At the end of clamp, mice were anesthetized, and tissue samples were taken for biochemical and molecular analyses. Both the 3H-glucose and 2-[1-^{14}C]deoxy-D-glucose were purchased from PerkinElmer.

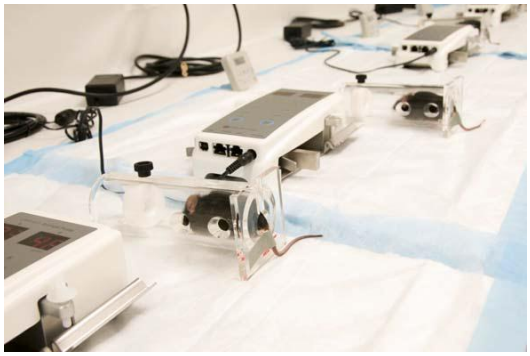


Figure 5: The Hyperinsulinemic-Euglycemic Clamp. This figure shows conscious mice inside oversized restrainers. The mouse's tail is exposed from the back of the restrainer in order to take blood samples.

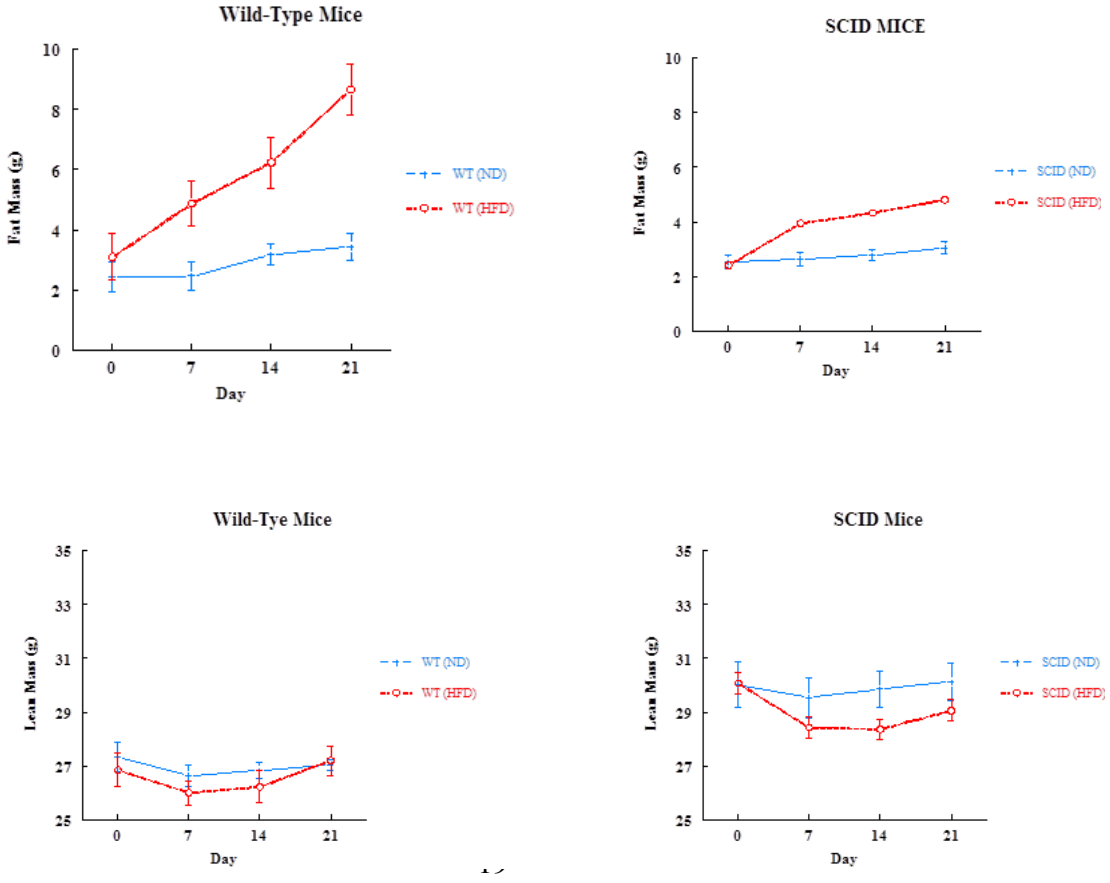
Whole Body Assay

The Whole body assay was performed following each clamp to measure the radioactivity in each of the plasma samples taken during the clamp. Barium hydroxide monohydrate (0.3 N) as well as Zinc sulfate heptahydrate (0.3 N), which were purchased from Sigma-Aldrich, were used to deproteinize plasma samples to measure whole body glucose metabolism. A scintillation

cocktail and liquid scintillation counter, with dual channels for the separation of ^{3}H and ^{14}C, were used to measure radioactivity in plasma samples. Basal whole body glucose turnover was determined as the ratio of the [^{3}H]glucose infusion rate to the specific activity of plasma glucose at the end of basal period. Insulin-stimulated whole body glucose uptake was determined as the ratio of the [^{3}H]glucose infusion rate to the specific activity of plasma glucose during the final 30 min of clamps. Hepatic glucose production during insulin-stimulated state (clamp) was determined by subtracting the glucose infusion rate from the whole body glucose uptake. Whole body glycolysis was calculated from the rate of increase in plasma $^{3}H_2O$ concentration from 90~120 min of clamps. Whole body glycogen plus lipid synthesis was estimated by subtracting whole body glycolysis from whole body glucose uptake.

RESULTS

Four groups of mice were studied during the project: 1) C57BL/6J wild-type mice on a normal diet, 2) NOD-*scidIL2rγ*^{null} mice on a normal diet, 3) C57BL/6J mice on a high-fat diet for 4 weeks, and 4) NOD-*scidIL2rγ*^{null} mice on a high-fat diet for 4 weeks. Body composition was measured weekly to observe the changes in fat mass and lean mass of both mice on both diet conditions (**Figure-6**). The fat mass of wild-type mice increased threefold on the high-fat diet compared to standard chow, but there was no significant change in fat masses of NOD-*scidIL2rγ*^{null} mice between chow and the high-fat diet condition. The body weight of wild-type mice increased significantly after high-fat diet compared to chow diet in contrast to that of NOD-*scidIL2rγ*^{null} mice.



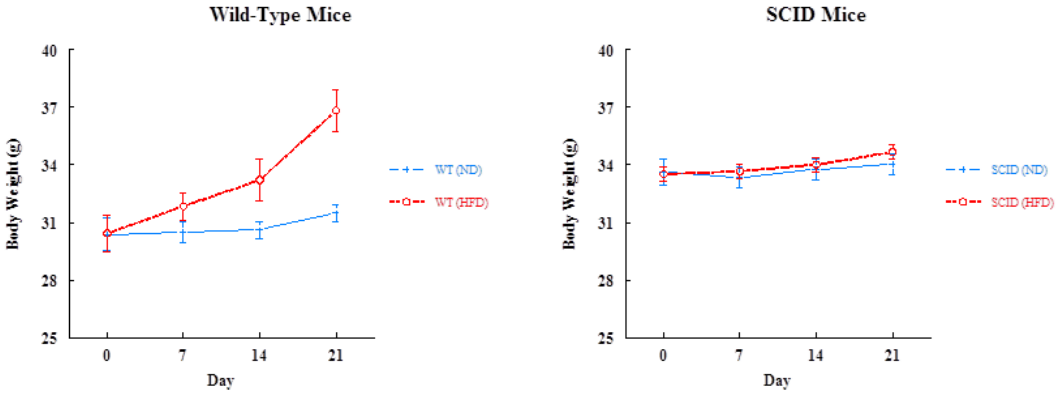
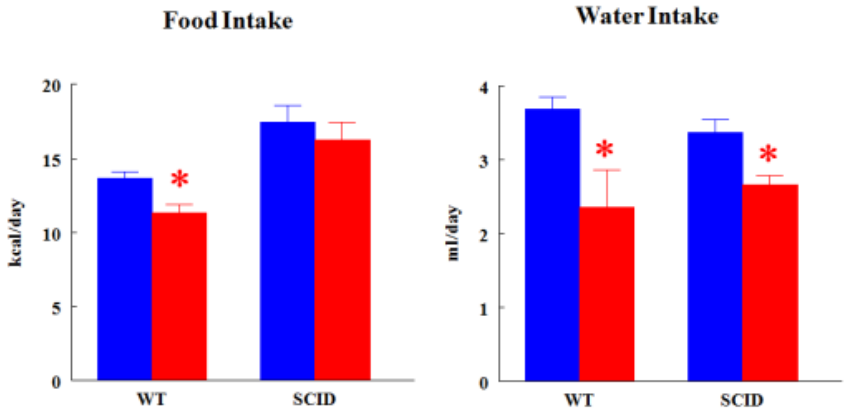


Figure 6: Body composition data of wild-type and NOD-*scidIL2rγ*^{null} mice (SCID) on normal chow diet and a high-fat diet**.**

After 4 weeks of chow and high-fat diet, wild-type mice and NOD-*scidIL2rγ*^{null} mice were put in the metabolic cages and their food intake, physical activity, and energy expenditures were measured for 3 days (**Figure-7**). Food intake and physical activity were significantly reduced in wild-type mice after high-fat diet, but there was no significant change in food intake and physical activity in NOD-*scidIL2rγ*^{null} mice. Interestingly, food intake was higher in NOD-*scidIL2rγ*^{null} mice than wild-type mice on both chow and high-fat diet conditions. VO_2 consumption, VCO_2 production, and energy expenditure significantly decreased in wild type mice after high-fat diet compared to NOD-*scidIL2rγ*^{null} mice.



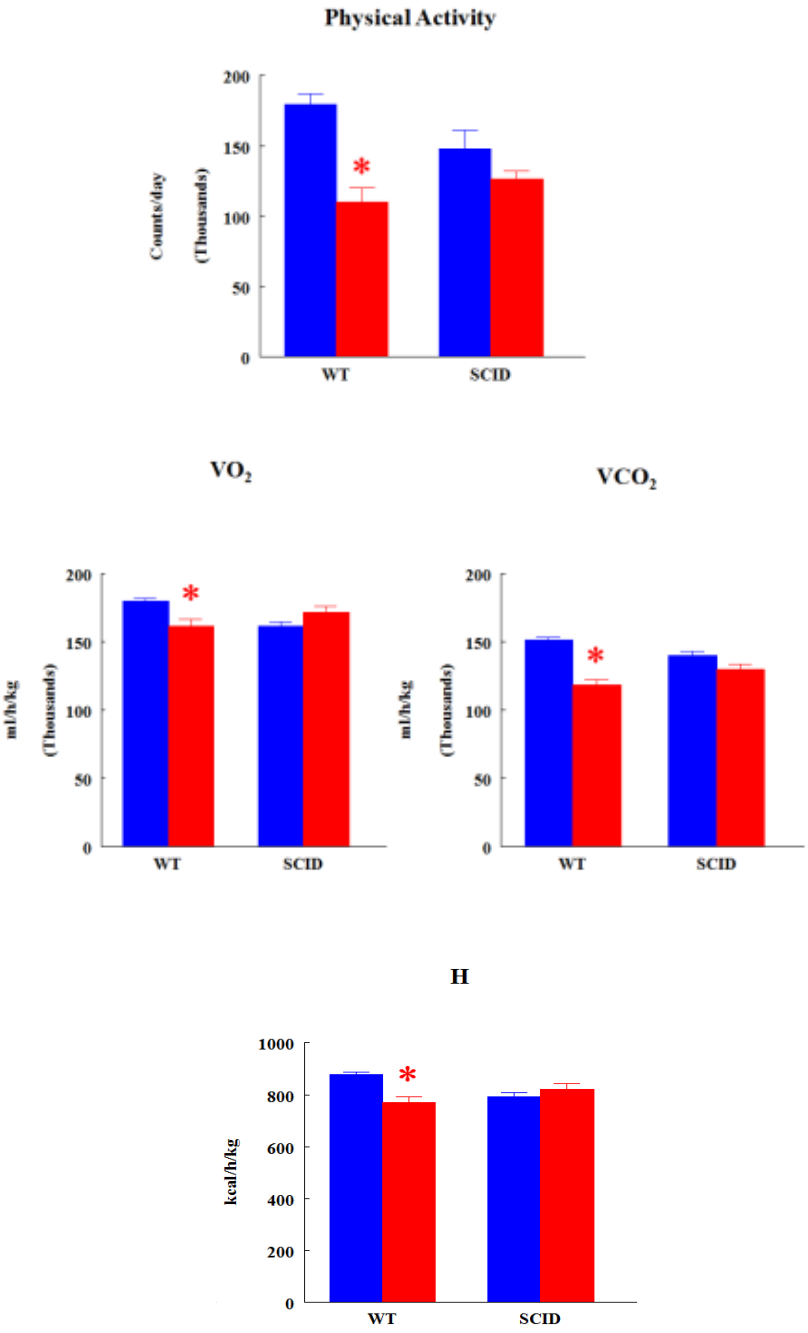
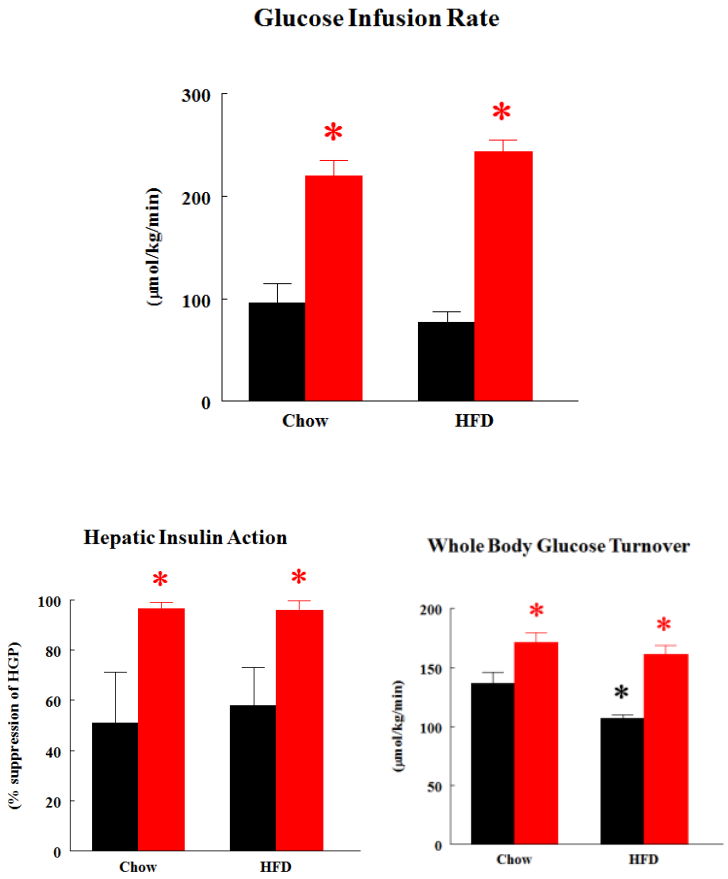


Figure 7: Metabolic Cage Data. The graphs display comparisons of food intake, water intake, physical activity, VO_2 consumption, VCO_2 production, respiratory exchange ratio, and energy expenditure in mice on a high fat diet versus mice on a normal diet. The blue columns represent the normal chow diet and the red columns represent the high fat diet. NOD-*scidIL2rγ*^{null} (SCID) and wild-type (WT) columns are labeled. The asterisk indicates a significant difference in the compared groups.

Following a 4 weeks of normal chow and a high-fat diet, a hyperinsulinemic-euglycemic clamp was performed on each group of mice to assess glucose metabolism (**Figure-8**). NOD-*scidIL2rγ*^{null} mice fed a chow diet were more insulin-sensitive than wild-type mice, as indicated by significant increases in steady-state glucose infusion rates and whole-body glucose turnover during clamp. Following high-fat diet, NOD-*scidIL2rγ*^{null} mice remained more insulin-sensitive and showed a ~ 40% increase in insulin-stimulated whole-body glucose turnover, and more than a twofold increase in while-body glycogen plus lipid synthesis compared with the high-fat diet wild-type mice. Hepatic insulin action in the NOD-*scidIL2rγ*^{null} mice was much higher than that in wild-type mice on both chow and high-fat diet conditions.



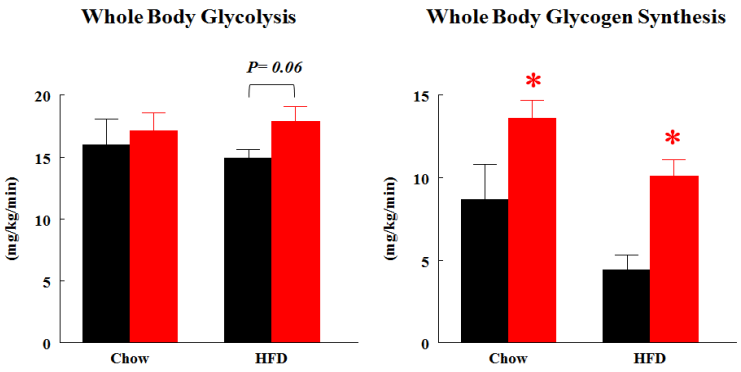


Figure 8: Hyperinsulinemic-Euglycemic Clamp Data. The graphs show the changes in glucose infusion rate, hepatic insulin action, whole body glycolysis, whole body glycogen synthesis and whole body glucose turnover in mice on a high fat diet versus on a normal diet. The black columns represent the normal wild-type (WT) mice and the red columns represent the NOD-*scidIL2rγ*^{null} mice. The asterisk indicates a significant difference in the compared groups.

DISCUSSION

The focus of this project was to further assess the inflammation that is observed during diabetic insulin resistance. During the project, wild-type and NOD-*scidIL2rγ*^{null} mice were placed on a high fat diet or a normal chow diet for 4 weeks. A difference in adiposity was observed between the wild type mice and the immunodeficient mice. Our initial hypothesis was that mice of the NOD-*scidIL2rγ*^{null} background, which lack an antigen specific immune system, and would be less prone to inflammation, would become less insulin-resistant than the normal wild type mice. Following the analysis of the body composition data and the hyperinsulinemic-euglycemic clamp data, it was clear that the NOD-*scidIL2rγ*^{null} mice were less obese and remained extremely insulin sensitive. As expected, the wild type mice became obese and significantly insulin resistant. VO_2 consumption and VCO_2 production of wild-type mice were reduced after high-fat diet but not in the NOD-*scidIL2rγ*^{null} mice. The NOD-*scidIL2rγ*^{null} mice ate more than the wild-type, performed the same amount of physical activity after the high-fat diet, and yet still remained leaner than the wild type mice. Often, when a mouse gets older or is feeding on a high fat diet, their energy expenditure decreases significantly. In the case of the NOD-*scidIL2rγ*^{null} mice, it was observed that even following a high fat diet for four weeks, their energy expenditure remained unchanged.

During a 2h hyperinsulinemic-euglycemic clamp, the glucose infusion rates of the NOD-*scidIL2rγ*^{null} mice were much higher than that of wild-type mice, a clear indication of insulin sensitivity. A high glucose infusion rate indicates that insulin-stimulated glucose uptake is high in various tissues. In order to keep the mice at a euglycemic level of between 100 and 150 mg/dl the glucose infusion rate was adjusted accordingly. In normal insulin sensitive subjects, insulin

causes the inhibition of glucose production from the liver. Hepatic insulin action in NOD-*scidIL2rγ*^{null} mice was shown to be much higher than that in wild-type mice on both chow and high-fat diet conditions. This result indicates that hepatic glucose production was more reduced in NOD-*scidIL2rγ*^{null} mice compared to wild-type mice during the clamp, which illustrates that the liver is insulin resistant in wild-type mice. As indicated by whole-body glucose turnover, glycolysis and glycogen synthesis, NOD-*scidIL2rγ*^{null} mice used and stored more glucose even though they were fed high-fat diet. These results indicate that NOD-*scidIL2rγ*^{null} mice remain more insulin sensitive following high-fat diet, which may have come from the lack of inflammatory responses.

There is a proven relationship between the immune system, inflammation and insulin resistance. A previous study performed by Kim et al. (2010) which collaborated with Dr. Roger Davis, demonstrated the relationship between inflammation and insulin resistance by observing the activity of JNK-1 in mice following high-fat diet. Following the high fat diet in WT mice, JNK-1's activation by the cytokine TNF-α caused phosphorylation of serine residues as opposed to the normal tyrosine residues, thus inhibiting the normal phosphorylation cascade performed during normal insulin signaling. Mice, which contained a selective deletion of JNK-1 in white and brown adipose tissue, remained insulin sensitive, whereas normal mice became insulin resistant following high-fat diet. The removal of JNK-1 prevented the inflammatory cytokine from causing an abnormal signaling pathway and rescued the mice from insulin resistance. Similarly in this project, the removal of an even larger component of the immune system in the NOD-SCID mice caused the same event. Taken together, based on results of the previous JNK-1 study and our data, inflammation is one of the major causes to induce insulin resistance.

Further studies will measure the inflammation-related mediators and targets (for example macrophages, TLR4, SOCS3, MCP-1, etc.), and will use immunoblots to measure the levels of insulin signaling proteins from muscle, white adipose tissue, brown adipose tissue, and liver. Future studies will also address whether the tissue-specific delivery of inflammation related proteins using an AAV expression system can re-induce insulin-resistance in NOD-*scidIL2rγ*^{null} mice following a high-fat diet. This study will further characterize the role of inflammation on insulin resistance in type 2 diabetes.

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