THE ROLE OF LIPOXYGENASE AND INTERLEUKIN-6 ON ISLET β-CELL OXIDATIVE STRESS AND DYSFUNCTION

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DEDICATION

To my family, thank you for all your support and encouragement as I pursue my dreams.

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β-CELL OXIDATIVE STRESS AND DYSFUNCTION

Type 1 and Type 2 diabetes (T1D/T2D) share a common etiology that involves an increase in oxidative stress that leads to dysfunction and subsequent β cell death. Lipoxygenases are enzymes that catalyze the oxygenation of polyunsaturated fatty acids to form lipid metabolites involved in a variety of biological functions including cellular oxidative stress response. On the other hand, Interleukin 6 (IL-6) signaling has been demonstrated to be protective in islets. In this study, we explored the effect of lipoxygenase enzymes 12-Lipoxygenase, 12/15 Lipoxygenase and IL-6 on β cell function and survival in mice using both STZ and high-fat diet (HFD) models of diabetes. *Alox12*$^{-/-}$ mice showed greater impairment in glucose tolerance following STZ and HFD compared to wild-type mice (WT), whereas *Alox15*$^{-/-}$ were protected against dysglycemia. These findings were accompanied by evidence of islet oxidative stress in *Alox12*$^{-/-}$ mice and reduced oxidative stress in *Alox15*$^{-/-}$ mice, consistent with alterations in the expression of antioxidant response enzymes in islets from these mice. Additionally, islets from *Alox12*$^{-/-}$ mice showed a compensatory increase in *Alox15* gene expression and treatment of these mice with the 12/15-lipoxygenase inhibitor ML-351 rescued the dysglycemic phenotype. IL-6 was able to significantly attenuate the generation of reactive oxygen species by proinflammatory cytokines in human pancreatic islets. Furthermore, we find that IL-6 regulates the master antioxidant response protein NRF2. Collectively these results show that loss of *Alox12* activates a compensatory increase in *Alox15* that sensitizes β cells to oxidative stress and signaling by IL-6 is required for maximal antioxidant response under conditions of increased ROS formation, such as obesity.

Raghavendra G. Mirmira, MD, Ph.D., Chair

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1 Introduction

The pancreas was first described by Herophilus, a physician who practiced in the ancient city of Alexandria, who was dubbed as the father of Human Anatomy (1,2). Since the pancreas was first identified, our knowledge of its structure and function has dramatically improved. The pancreas anatomy can be subdivided into two categories, the exocrine pancreas and the endocrine pancreas, that encompass the functions of the cells within each group. The exocrine pancreas is composed of cells whose primary function involves aiding in digestion. This portion of the pancreas is comprised of small acinar cells that together form small glandular structures termed acini. These acini secrete enzymes and proenzymes into their lumen which then drains into the duodenum via the pancreatic ductal system. Spread out among the vast sea of exocrine tissues, making up only 1-2% of the total pancreatic mass, are the functional units of the endocrine pancreas termed islets of Langerhans. The islets of Langerhans are a richly vascularized cluster of 5 different types of endocrine cells. The dominant cell type in the islets is the β cell which comprises 28-75% of cells in the human islet and 61-88% of cells in the rodent islet (3). β cells secrete insulin as their principal product but also produce and co-secrete amylin with the insulin. Following β cells, the next major component of islets is the α cell. α cells account for 10-65% of endocrine cells in human islets and 9-31% in murine islets (3). The primary function of the α cell is to produce and secrete glucagon. Delta cells make up about 1.2-22% in human islet population and 1-13% of murine islet population and produce and secrete somatostatin(3). Pancreatic polypeptide and ghrelin-producing cells make up the remaining architecture of the islet in very small percentages (<2% each) (4,5).

1.1 Islet Structure and Function in the Regulation of Blood Glucose

Interestingly, studies have identified interspecies variability in the endocrine cell arrangement of pancreatic islets (3,6). The classic islet architecture of β cells forming an inner core with α and other endocrine cells in the periphery stems from studies of normal

rodent islet histology. Studies of human islets show an islet architecture composed of an intermixture of endocrine cells (5). Additionally, the architecture of islets has been shown to be dynamic as it changes with age and disease states. In normal islets, the interspecies differences are attributed to the different metabolic needs of each species (6). For example, the structural differences between human and rodent islets lead to specific functional differences. Endocrine cells in both human and islets form micro-organs composed of interconnected gap channel networks that allows cell to cell communication. The unique heterogeneous architecture of the human islet would promote more β cell to α cell contact compared to the larger-ordered architecture of the murine islet (6).

The primary function of β cells is to produce the hormone insulin in response to changes in blood glucose levels (7). Insulin is a polypeptide hormone encoded by the INS gene in humans, which produces a 110-amino acid hormone precursor known as preproinsulin (8–10). The N-terminal signal peptide of preproinsulin interacts with cytosolic signal recognition particles that facilitate the translocation of preproinsulin into the lumen of the rough endoplasmic reticulum. Here, the N-terminal signal peptide is cleaved by signal peptidases to produce proinsulin (11). Proinsulin is then transported from the ER to the Golgi and packaged into immature secretory vesicles where it is further cleaved to yield mature insulin and C-peptide. These secretory vesicles store the mature insulin that is ready to be secreted immediately upon stimulation of the β cell (12).

Insulin biosynthesis is primarily regulated by the metabolism of glucose in the β cell. Glucose regulates both the transcription, stability, and translation of the preproinsulin mRNA. This is not surprising as insulin secretion requires precise regulation in order to meet the metabolic demands of the organism. The dense vasculature that is found in the islets gives the β cells access to monitor the precise level of glucose in the circulation by uptake via glucose transporters and release the appropriate amount of insulin in response to changes. β cells constitutively express glucose transporter 2 (GLUT2) localized to their

plasma membrane (13). GLUT2 is a low-affinity glucose transporter that enables a high rate of glucose influx into β cells (14). Immediately upon entry into the cytoplasm, glucose is phosphorylated by glucokinase (15,16). This phosphorylation of glucose by glucokinase effectively traps it in the cell and is the rate-limiting step in β cell glucose processing (16). Glucose is then converted to pyruvate through glycolysis.

Unlike other cell types, β cells normally do not convert pyruvate to lactate due to the negligible expression of the catalytic enzyme pyruvate dehydrogenase (17). Therefore, all glucose that enters β cells is converted to pyruvate which is then oxidized through the tricarboxylic acid (TCA) cycle in the mitochondria to produce ATP (18). This leads to a change in the ATP/ADP ratio in the β cell that is sensed by ATP-sensitive potassium channels (KATP) (19). KATP channels switch to a more closed state increasing the extracellular to cytoplasmic potassium gradient and inducing depolarization of the plasma membrane (20). Depolarization of the β cell plasma membrane then leads to the opening of voltage-dependent Ca^{2+} channels and the influx of Ca^{2+} (21–23). Ca^{2+} induces fusion of insulin-containing secretory granules to the plasma membrane and secretion of insulin (24–26).

Although glucose is the primary regulator of insulin secretion, insulin secretion is also modulated both positively and negatively in response to diverse types of stimuli. Metabolism of specific combinations of amino acids such as glutamine and leucine have been demonstrated to enhance glucose-stimulated insulin secretion (GSIS). Metabolic processing of amino acids can also lead to an increase in the ATP/ADP ratio of the β cell and stimulate insulin secretion (27,28). Hormones such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) function similarly to enhance insulin secretion. These hormones are released by K- and L-cells in the intestine in response to ingestion of glucose and amino acids (29), and can directly enhance GSIS through binding to their specific cell surface receptors on β cells in a process known as the incretin effect (30,31).

Free fatty acids have also been demonstrated to increase the secretion of insulin (32–34), whereas the actions of catecholamines and somatostatin inhibit insulin secretion (35). With these various inputs, a normal β cell can integrate all these signals and modulate the amount of insulin needed to fit the metabolic needs of the organism (36).

Once insulin is secreted, it is quickly dispersed into the bloodstream via the extensive vascular network present throughout the islet. Insulin has a wide variety of both acute and chronic effects on the function of peripheral organs. Acute effects of insulin include the rapid induction of glucose transport into cells and regulation of metabolic enzyme activity. Insulin-stimulated glucose uptake regulates 40% of glucose disposal in mammals with the majority occurring in the skeletal muscle (37,38). Insulin-stimulated glucose uptake is mediated primarily through glucose transporter 4 (GLUT4) (39). GLUT4 is normally sequestered intracellularly in vesicles. Upon binding of insulin to its receptor, multiple signaling cascades lead to targeted translocation of GLUT4 to the plasma membrane allowing for the influx of glucose. The insulin receptor is a heterotetrameric glycoprotein membrane receptor (40). It is composed of 2α and 2β subunits that are linked by disulfide bonds (41). Insulin binds to the extracellular α chains of the receptor and induces a conformation change that activates the intrinsic tyrosine kinase activity of the cytosolic β chain (42). Several tyrosine residues on the β chain are autophosphorylated and further catalyzes the phosphorylation of proteins known as insulin receptor substrates (IRS) (43). The IRS proteins then couple activation of insulin receptor to multiple pathways such as the phosphatidylinositol 3 kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (44). Activation of PI3K is the critical pathway linking insulin signaling to its metabolic effects. Binding of the regulatory subunits of PI3K to phosphorylated IRS proteins induces activation of the catalytic subunit of PI3K (45). The catalytic subunit then phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2), generating the lipid second messenger phosphatidylinositol (3,4,5)- triphosphate (PIP3) (46,47). PIP3 acts on

downstream targets, such as AKT, by recruiting them to the plasma membrane and enabling their activation. PDK-1 (3-phosphoinositide-dependent kinase 1) is a kinase that is activated upon binding to membrane-bound PIP3 allowing it to phosphorylate its targets at serine or threonine residues. AKT is a primary target of PDK-1 and is also recruited to the plasma membrane by PIP3. PDK-1 phosphorylates AKT at Thr-308 inducing the start of its activation sequence (48). AKT is then fully activated when it is further phosphorylated at Ser 473 by mammalian target of rapamycin complex 2 (mTORC2) (49). This fully activated AKT then activates many downstream targets to induce most of the downstream actions of insulin. AKT induces activation of mTORC1, regulating many metabolic pathways, cell growth, and protein synthesis (50,51). Most importantly, AKT also activates translocation of GLUT4 to the plasma membrane, thus increasing glucose uptake into cells (52).

The MAPK pathway is the other major downstream signaling pathway activated by insulin signaling. Once activated, the insulin receptor acts as a scaffold for the adaptor proteins Growth factor receptor-bound protein 2 (Grb2) and Src homology 2 domain containing (SHC) (53). Grb2 binds and actives the Ras guanine nucleotide exchange factor Son of Sevenless (SOS). SOS catalyzes the GDP to GTP exchange required to activate Ras which then activates signaling cascade that eventually leads to activation of ERK 1/2 (54). Activated ERK 1/2 can then act to regulate cellular proliferation and differentiation (55). Besides its role in glucose control, insulin also regulates other important metabolic processes. For example, in adipose tissue, insulin signaling leads to inhibition of lipolysis and ketogenesis as well as activation of lipogenesis (56). In hepatocytes, insulin increases the expression of enzymes such as pyruvate kinase and glucokinase leading to enhanced glucose utilization (57,58). Glucose storage is also increased by activation of glycogen synthesis. Thus, the primary action of the pancreatic β cell is to produce and secrete insulin in response to glucose (Figure 1). Furthermore, as

described above, the actions of insulin are diverse and permissive across cell types. Hence in pathological states, such as diabetes or pre-diabetes, dysregulation of this crucial signaling hormone can lead to devastating consequences.

1.2 Consequences of Islet β Cell Dysfunction

Diabetes is a broad description of several pathologies that are characterized by anomalous carbohydrate metabolism and hyperglycemia (59). Development of the disease is ultimately due to a relative or complete impairment of insulin secretion and variable levels of peripheral insulin resistance. The global prevalence of diabetes and glucose intolerance has reached epidemic proportions swiftly in recent decades(62). This dramatic pace of change in diabetes has been attributed to rapid changes in lifestyle in many countries and regions towards a more sedentary lifestyle. A recent report by the international diabetes foundation highlights the exponential increase in diabetes prevalence observed over decades and predicted a dismal future outcome if the trends continue. Worldwide there were 425 million cases of diabetes reported in adults aged 20-79 years in 2017 and 451 million cases in adults aged 18-99 years(63). Projections estimate that by the year 2045 prevalence of diabetes in age 18-99 years will rise to 693 million worldwide(63). A country's economic status influences the prevalence of diabetes. High-income countries have the highest prevalence of diabetes at 22% followed by middle-income countries at 19% and low-income countries at 8%(63). The influence of economic growth on diabetes prevalence makes it difficult to estimate the future prevalence of diabetes accurately. The current predicted prevalence of 693 million by 2045 is a conservative prediction as it fails to consider changes in the economic status of the world. Furthermore, there is also a high prevalence of individuals with impaired glucose tolerance. Globally an estimated 374 million individuals were categorized as impaired glucose tolerance (IGT) in 2017 (60). The prevalence of this "pre-diabetic" state,

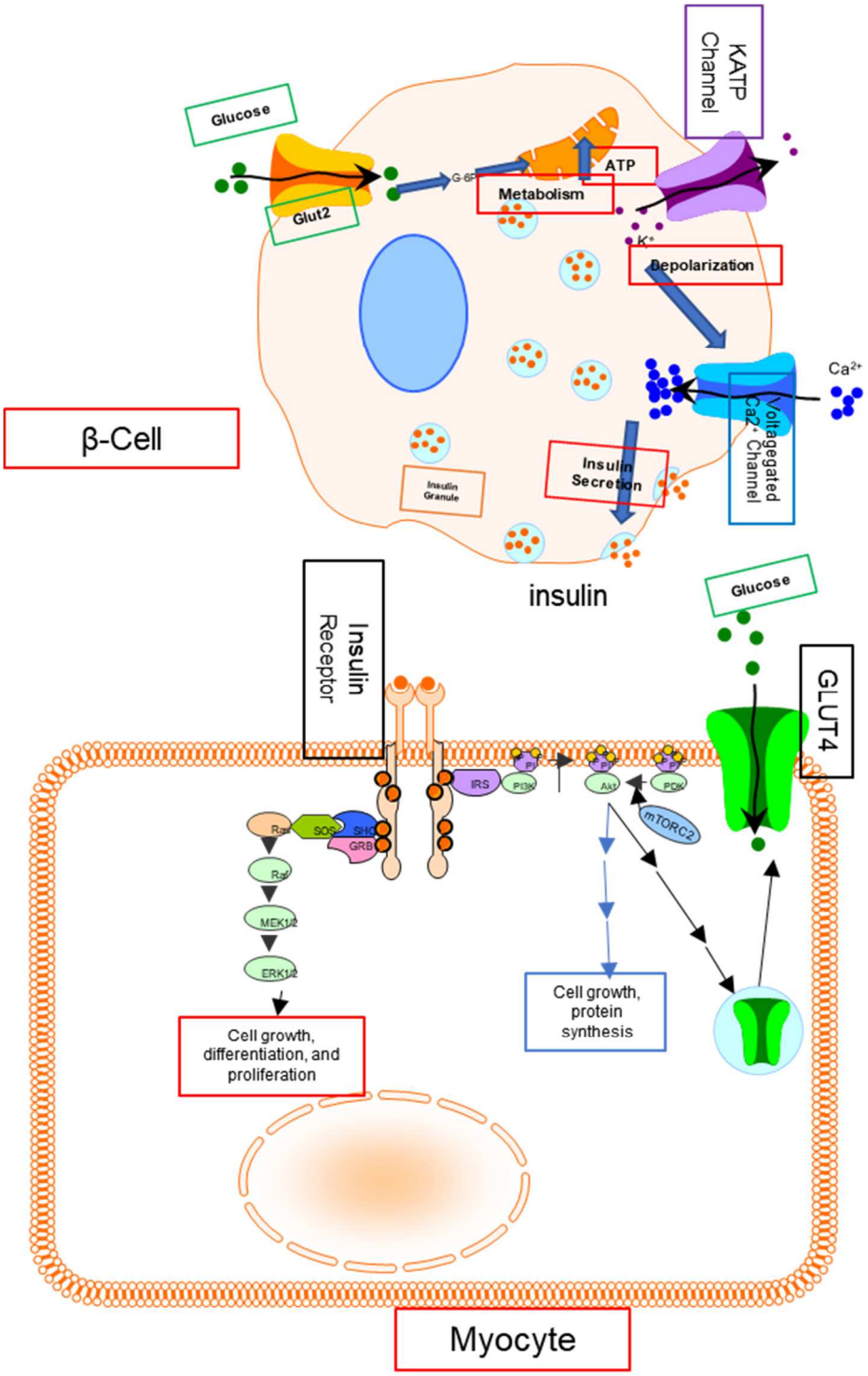

Figure 1. Glucose-stimulated insulin secretion in β cells and Insulin signaling in skeletal muscle

like that of individuals with diabetes, is also expected to increase to 587 million individuals by 2045 (60). Finally, the economic impact of diabetes is also staggering. The total estimated global expenditure in 2017 was over $850 billion (60). This number is also expected to increase in concert with the increase in prevalence to close to nearly $1 trillion annually. More importantly, while diabetes is a chronic disease, it also ushers a host of very lethal comorbidities. In 2017, approximately 5 million deaths were attributable to diabetes, accounting for nearly 10% of global all-cause mortality (60).

1.3 Type 1 Diabetes

Diabetes is classified into two major categories Type 1 (T1D) and Type 2 (T2D). T1D is classically characterized by autoimmune destruction of pancreatic β cells, leading to insulin deficiency. The incidence of T1D varies widely between countries ranging from the age-adjusted incidence of 0.1 per 100,000 per year in countries such as Venezuela and China to 40.9 per 100,000 per year in Finland (61). T1D can be diagnosed at any age, but in general, most diagnosis occurs between birth and 14 years of age.

The onset of T1D involves an intricate interplay between genetic susceptibility and environmental factors. The initiating event of T1D has not been completely elucidated, but studies have identified multiple genetic markers that contribute to T1D. The genetic contribution to T1D has been the focus of numerous studies (62). Interestingly, most cases of T1D occur in individuals with no known family history of T1D. Studies have shown that individuals with first degree relatives with T1D demonstrate an increased risk of developing diabetes with offspring and siblings demonstrating a 6% and 5% increased risk respectively (63–65). Additionally, concordance studies in identical twins show a 50% increased risk in the twin of a patient with T1D (66).

The major genes that contribute to T1D are in the human leukocyte antigen (HLA) region of chromosome 6p (67). These genes code for MHC class II molecules that are typically expressed on the cell surface of antigen presenting cells such as macrophages

(68). MHC molecules are composed of α and β chains that together form a binding pocket for antigens to be held in place during antigen presentation by antigen presenting cells to antigen receptors found on T cells (69). The antigen recognition specificity of MHC molecules is dependent on the amino acid composition of their α and β chains (70). Therefore, mutations of critical amino acids can alter the ability of MHC molecules to bind and present specific molecules as antigens to T cells. Studies have identified that over 90% of patients with Type 1 diabetes carry HLA-DR3-DQ2 or HLA-DR4-DQ8 haplotypes, and individuals that are heterozygote for both haplotypes possess the highest susceptibility of developing Type 1 diabetes (71).

Interestingly, not all polymorphisms of MHC genes lead to increase susceptibility to T1D. Individuals with HLA-DQB1*0602 have increased protection from T1D even if they are related to someone with positive autoantibodies (72). Polymorphisms in antigen presenting genes are not by themselves enough to initiate T1D, as the antigens to present are still required. Several islet cell autoantibodies have been detected in the serum of prediabetic and newly diagnosed T1D patients. One of the earliest autoantigens to appear is an anti-insulin antibody (73). This antibody is first detectable in children before the development of T1D. Mouse studies have demonstrated that $CD8^+$ and $CD4^+$ T cells can recognize different epitopes of insulin as antigens (74–76).

Furthermore, immune cells isolated from T1D individuals are also responsive to insulin. Another important antigen is Glutamic acid decarboxylase (GAD) (77,78). This enzyme is found primarily in islets and cells of the central nervous system. Antibodies for GAD are found in approximately 70% of newly diagnosed T1D (73).

Other non-MHC genes that can modulate susceptibility to T1D have been identified. Polymorphisms in the cytotoxic-T lymphocyte-associated antigen-4 (CTLA4) has been shown to increase the risk of T1D (79,80). Additionally, polymorphisms of insulin

promoter and the tyrosine phosphatase PTPN22 are also known modulators of T1D (81–83).

Genetics alone is not enough to explain the occurrence of T1D, and environmental factors have been demonstrated to contribute heavily to the development of T1D. This is evident by the increased incidence of T1D observed in multiple different populations. Additionally, twin studies have only shown a 50% concordance of T1D. The major candidates for environmental factors include enteroviral infections, sanitation, and diet (84,85). Enteroviruses are small RNA viruses that cause a variety of human diseases such as polio, hand-foot-mouth disease, and myocarditis, especially in children (86). Enteroviral infection is thought to lead to diabetes by direct infection of β cells or inducing autoimmune attack against β cells (87). Studies have shown that children with congenital rubella syndrome have a higher incidence of developing diabetes in the future (88). Enteroviruses are thought to trigger autoimmune destruction of β cells by molecular mimicry by viruses of β cell-specific molecules such as GAD that can then lead to immune recognition of β cells are foreign agents leading to their destruction. The incidence of T1D has been shown to be increasing in highly developed nations with increased sanitation. The hygiene hypothesis first introduced in the 1990s hypothesized that reduction in infections leads to malfunction in immune activity (89). This hypothesis has created a cultural misnomer of increased hygiene being detrimental. Efforts are currently focused on clarification that it might be a decrease in symbiotic, not pathogenic microbes that are detrimental to proper immune system development (90). Diet is also thought to play a substantial role in T1D development. Consumption of β-casein, a component in cow's milk has been demonstrated to have a strong correlation with the incidence of T1D (91). Additionally, consumption of nitrate-contaminated substances also increases the incidence of T1D (92).

The presence of one or more of these precipitating environmental factors in individuals that are genetically susceptible to T1D induces a series of immunological

attacks on β cells that eventually leads to their destruction. Autoreactive T cells residing in the pancreatic lymph nodes are activated by interaction with peptides presented by APCs such as dendritic cells and macrophages. These activated cells can then directly cause β cell death by direct secretion of perforins and granzyme moles on β cells. Additionally, β cell death can be mediated through cytokine production by T cells that invade pancreatic islets. Secretion of pro-inflammatory cytokines such as TNF-α and IFN-γ cause direct cytotoxicity to β cells and activate surrounding to immune cells such as macrophages leading to augmented immune activity (93) (Figure 2). β cell destruction by immune activity steadily progresses over time. During this period the surviving β cells can compensate for the loss, and no clinical symptoms are detected until β cell mass reaches a critical threshold. T1D can present in several different ways. Classically, the most common form of presentation is hyperglycemia with chronic polydipsia, polyuria, and weight loss. Patients can also present in a state of diabetic ketoacidosis (DKA) (94). These patients present with hyperglycemia (blood glucose >200 mg/dL), metabolic acidosis, and ketosis (95). For infants that have known risks of T1D, such as first degree relative with T1D, diagnosis is made before overt clinical symptoms are presented. The presence of autoantibodies before clinical symptoms allows screening to assess risk for T1D. Definitive clinical diagnosis is made upon elevated blood glucose. Initial management of T1D requires exogenous insulin administration in order to control blood glucose levels (96). Long term management of T1D involves a tenuous balance of exogenous administration of insulin and strict glycemic control in order to avoid chronic hyperglycemia and severe hypoglycemia (96). While this is enough to manage symptoms of T1D, it is not a cure for the disease. Exogenous administration of insulin and strict dietary control eventually can take a financial, physical and mental toll on patients leading to noncompliance (97).

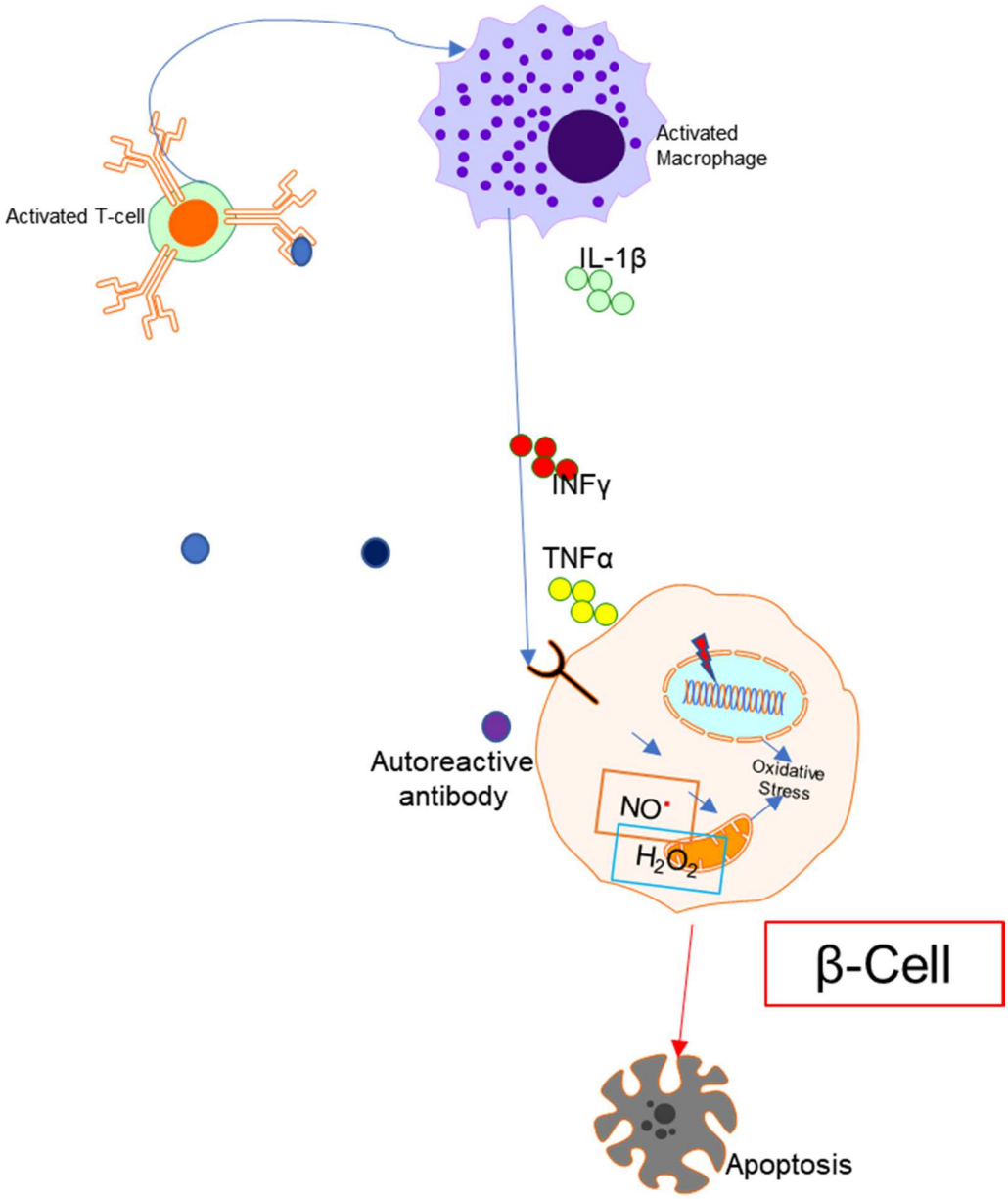

Figure 2. Autoimmune mediated attack of Pancreatic β cell in T1D

Additionally, exogenous insulin increases the chance of a fatal hypoglycemic episode. Hence, it is critical to identify alternative treatment strategies for T1D. The vital role that the immune system plays in this disease makes it a suitable target for the treatment of T1D, yet immunotherapies for T1D have proved ineffective. Other avenues being pursued involves transplantation of whole pancreas or islets to individuals with serious progressive complications (98). Furthermore, studies have identified a possible treatment window following the diagnosis of T1D and complete β cell destruction. During this so call honeymoon phase, it is hypothesized that introduction of therapies that halt destruction or restore β cell mass can lead to prolong or increase endogenous function (99). Identification of molecular targets that can halt β cell destruction and improve function is critical to the future of T1D treatment.

1.4 Type 2 Diabetes

T2D is the most common class of diabetes in adults and is characterized by chronic hyperglycemia and varying degrees of insulin deficiency and resistance. Over 90% of individuals with diabetes are diagnosed with T2D (100). There has been an overall trend of increased prevalence of T2D in every country since 1980, but the majority of T2D cases are in low- and middle-income countries (101). The rise in the prevalence of T2D is associated with rising obesity and a sedentary lifestyle. T2D is a detrimental disease that is associated with many fatal comorbidities. Individuals with T2D have an increased risk of all-cause mortality compared to individuals without T2D (102–104). In particular, the risk of vascular diseases such as coronary heart disease, and ischemic stroke is significantly higher in T2D patients (105,106).

Similar to T1D the pathophysiology of T2D is a complex interplay between genetics and environmental factors. Genetic susceptibility influenced by increase BMI, sedentary behavior, and high caloric intake modulate the onset of T2D. The underlying genetics of T2D is not as well understood as it is for T1D. Only a few cases of monogenic T2D have

been identified and inherited T2D polymorphisms have shown only small changes in risk. The lack of currently identified genetic factors that can actively contribute to disease risk does not mean that genetics plays little role in T2D (107). There numerous observations that provide strong evidence for genetic influence on T2D development (108). Individuals with first degree relative with T2D have 5 to 10 times higher lifetime risk than individuals with no family history of diabetes. Additionally, 39% of patients with T2D have at least one parent with T2D. Finally, among monozygotic twins, 96% percent of unaffected twins eventually develop T2D (109). The search for driver genes that could explain the strong genetic contribution is still underway, and of the over 100 genetic loci identified to contribute to T2D, each locus has only been shown to cause a minuscule increase in risk (108).

The role of environmental factors in T2D has been well documented. Especially the role of obesity and diet on incidence of T2D has been well demonstrated in multiple studies. One of the classical hallmarks of patients diagnosed with T2D is overt weight gain and history of a sedentary lifestyle. Interestingly, this was previously thought to be a main feature of T2D and not T1D individuals, but this viewpoint has shifted as the obesity epidemic spreads across all forms of diabetes (110). Obesity, by itself has been demonstrated to contribute to 55% of diabetes worldwide and increases hazard ratio of diabetic complications (114,115). Numerous studies have established the ability of obesity to induce insulin resistance. The exact mechanism involved is still unclear but a matter of intense investigation. Several pathways have been demonstrated to play a role in obesity-induced insulin resistance. Obesity has been demonstrated to increase c-Jun amino-terminal kinase (JNK) pathway activity leading to a decrease in insulin activity (116). Additionally, mice harboring knockout of JNK1 show enhanced insulin sensitivity and decrease weight gain (117). Furthermore, increased adipocytes in obese individuals secrete numerous factors that are inhibitory to insulin signaling such as TNF-α, resistin,

and free fatty acids (FFA)(118,119). Insulin signaling sensitizing factors such as adiponectin are further decreased in obese individuals(120).

TNF-α is a pro-inflammatory cytokine that has been shown to directly inhibit insulin signaling. Binding of TNF-α to its membrane receptor induces a cascade of MAPK signaling that leads to serine phosphorylation-mediated inhibition of IRS proteins. Additionally, neutralization of TNF-α in obese rodents significantly improved glucose uptake and TNF-α levels in humans are positively correlated with insulin resistance(121). Resistin is a relatively newly identified protein that has been implicated in the pathogenesis of obesity-mediated insulin resistance through its action on AMPK and SOCS-3 signaling (122). Circulating resistin levels are positively associated with obesity, and insulin resistance (123). Insulin signaling resistance in adipocytes leads to increased lipolysis and increased FFAs. Additionally, FFAs have been demonstrated to increase JNK signaling, serine phosphorylation of IRS proteins and cellular stress(124). Altogether, through multiple mechanisms high levels of circulating FFAs increases risk of T2D. Adiponectin is an insulin-sensitizing adipokine that is secreted by adipocytes of non-obese individuals(125). The secretion of adiponectin in impaired in obese individuals leading to a loss of its ability to improve glycemic control, reduce inflammation and improve lipid profile in obese individuals(126). The levels of adiponectin are inversely correlated with risk of diabetes and cardiovascular disease in nondiabetic populations(127).

Obesity has also been demonstrated to induce system-wide inflammation that involves components of the classic inflammatory response to pathogens such as increased circulating inflammatory cytokines, increase acute phase response proteins, and leukocyte recruitment and activation(128). However, unlike the normal pathogen-induced inflammatory response obesity-induced response is chronic, low-grade inflammation that involves multiple organs. This insidious state of inflammation also

contributes to metabolic dysfunction by induction of multiple molecular signaling pathways that affect insulin production and response(129).

High caloric intake in an individual with a maligned genetic predisposition creates an environment of high circulating glucose levels that eventually decreases the sensitivity of cells to respond to insulin-mediated glucose uptake. This decrease sensitivity to insulin increases the workload of β cells to secrete more insulin leading to a transient increase in cell mass that can reach up to 50%(130). During this period the β cells can maintain glucose homeostasis at the cost of increased circulating insulin. The ability of the β cells to maintain this state for an extended period varies between individuals but eventually β cell failure develops and insulin production drops. This leads to chronic hyperglycemia and T2D (Figure 3).

Diagnosis of T2D relies on elevated random plasma glucose test ≥200 mg per dl with signs of hyperglycemia, fasting plasma glucose ≥126 mg per dl, a 2-hour post glucose load ≥200 mg per dl following 75g oral glucose or repeated HbA1c ≥6.5%. A recent problem that is now being more readily observed is the difficulty of distinguishing T1D from T2D. Patients with T1D now exhibit classic pathophysiologic element of T2D. The rise in obesity among youths and adults has increased the frequency to patient's with T1D that also suffer from obesity and peripheral insulin intolerance. This was not an issue in the past as the poor metabolic control prevented T1D patients from gaining weight, but improvement in therapies has made this scenario more common as 20 to 30% of T1D patients present with features of T2D(131). Classic auto-antibody screening is still used to distinguish uncertain diagnosis of T1D or T2D.

Management of T2D is complicated due to its many related comorbidities. T2D treatment requires concomitant life style modifications and drug therapy. Due to the important detrimental role of obesity and physical inactivity, life style modifications that incorporate diet and exercise to promote weight loss is a standard recommendation for

T2D patients(132). However, despite successful weight loss, most patients regain weight over subsequent years and still progress to diabetes(133). Hence it is recommended to combine anti-obesity medications to promote and maintain weight loss. In cases of severe obesity (BMI > 35 kg/m^2) bariatric surgery such as Roux-en-Y bypass or sleeve gastrectomy is recommended as it has proven more effective than anti-obesity medications(134). Like T1D there is no cure for T2D, and management involves decreasing symptoms and lowering chances of fatal comorbidities. With the progressive increasing prevalence of T2D and obesity further research into more effective therapies is warranted.

1.5 Oxidative Damage in the Pathogenesis of Diabetes

A common feature of both T1D and T2D is the loss of pancreatic β cells. Although many mechanisms have been attributed to the pathogenesis of both diseases recent evidence has highlighted a common theme. It has been hypothesized and shown in multiple studies that metabolic perturbations and immune activity involved in both T1D and T2D lead to increase β cell oxidative stress(135). This increase in oxidative stress leads to cellular dysfunction and eventually apoptosis. Uncovering the mechanisms by which this occurs and methods to protect β cells from oxidative damage will be beneficial for both T1D and T2D.

The ability of eukaryotic cells to generate adenosine triphosphate (ATP) as a source of energy requires molecular pathways that involve the reduction of molecular oxygen. The consequence of pathways such as the tricarboxylic acid cycle and the electron respiratory chain is the generation of reactive oxygen species (ROS) that can lead to the synthesis of free radicals. Dioxygen (O_2) is essential for energy metabolism in most living organisms. The stable orbital structure of O_2 allows it to be chemically unreactive but act as strong oxidant (136). Conversly, ROS such as superoxide anion ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), or peroxyl radicals ($^{\bullet}OOH$)

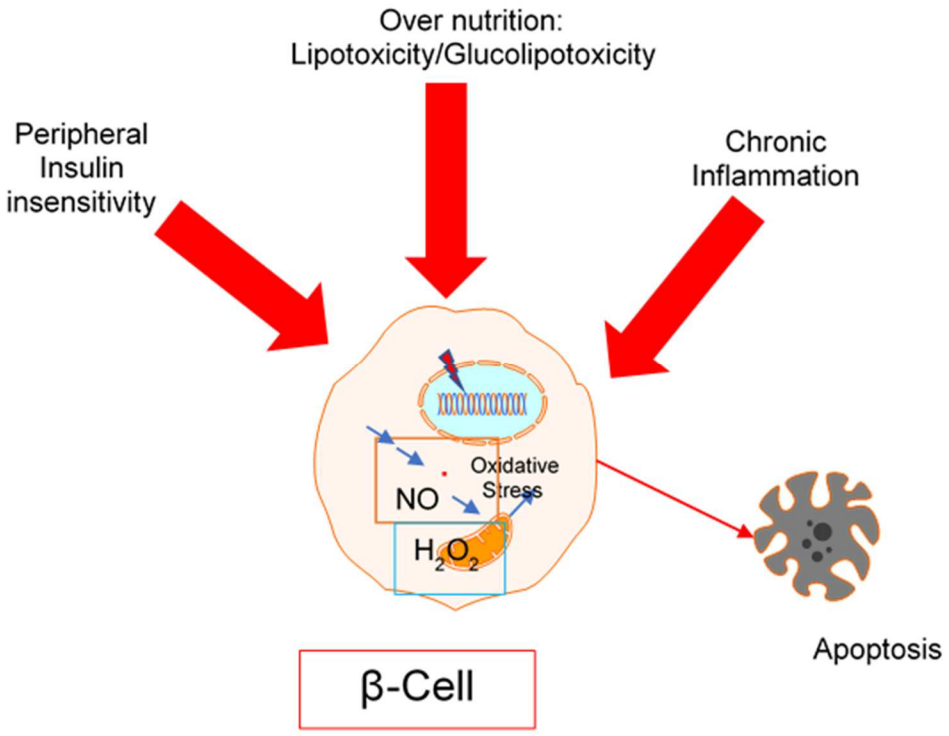

Figure 3. Mediators of Pancreatic β cell stress in the setting of obesity and T2D

are very chemically reactive and can induce cellular and tissue damage(137). Additionally, there are other biologically relevant reactive species such as reactive nitrogen species (RNS), reactive sulfur species (RSS), and reactive carbonyl species (RCS) that play an important role in redox biology and oxidative stress(138,139).

Reactive species are a natural biproduct of many biological reactions and are generated by various cellular organelles (Figure 4). The mitochondria are the major source of cellular generation of ROS. Electron transfer at complex I and III of the electron transport chain leads to production of $O2^{\bullet-}$(140). Additionally, many flavin-dependent enzymes in the mitochondria also contribute to production of ROS(141). Under normal conditions the generated $O2^{\bullet-}$ is rapidly converted to H_2O_2 by the superoxide dismutase enzyme family(142). Peroxisomes are another major source of ROS production in mammalian cells. A major function of peroxisomes is the breakdown of fatty acid molecules through β oxidation to acetyl CoA to be used in other metabolic reactions. Peroxisomes contain many enzymes such as Acyl-CoA oxidases, Urate oxidase, and Xanthine oxidase that utilize oxygen in their reactions and produce H_2O_2 as a secondary product(143,144). Furthermore, besides intracellular generated ROS, there are also extracellular derived ROS that can increase the oxidative burden of a cell(145). H_2O_2 is an uncharged molecule that can freely diffuse through plasma membranes(146). Therefore, H_2O_2 is not confined to its site of generation but can spread between cellular compartments and adjacent cells(147). Immune cells such as macrophages, and neutrophils also produce ROS in order to fight invading microorganisms(148,149).

1.6 The Role of Antioxidant Response in Reducing Oxidative Damage

In order to maintain redox homeostasis biological systems, possess several antioxidant systems in place. The antioxidant systems encompass several intracellular enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferases(150). These enzymes act to convert ROS into its non-reactive

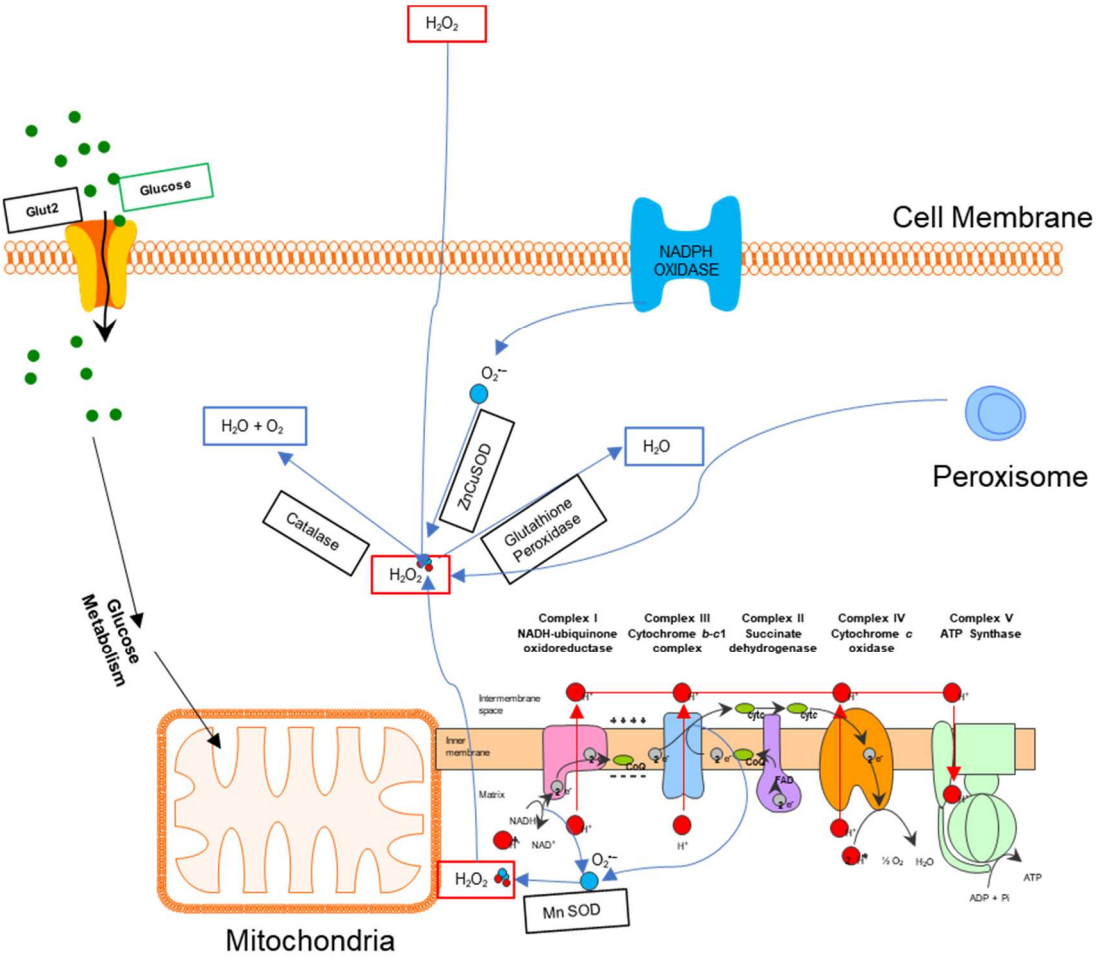

Figure 4. Sources of oxidative stress in pancreatic β cells

intermediate for elimination. Catalase is located primarily in peroxisomes and acts to convert H_2O_2 to water and oxygen. There are multiple isoforms of superoxide dismutase such as Mn-superoxide dismutase, Cu/Zn-superoxide dismutase that are found in the mitochondria, cytoplasm, and nucleus respectively(151). Glutathione peroxidases are located both in the cytoplasm and mitochondria of eukaryotic cells. Like catalase, Glutathione peroxidases also catalyzes the conversion of H_2O_2 to water and oxygen(152). Glutathione-S-transferases are a family of phase II detoxification enzymes that catalyze the conjugation of glutathione substrate allowing it to form thioether bonds with a variety of reactive metabolites for detoxification(153). Together these enzymes provide a protective mechanism against excessive oxidative stress generation during normal biological functions. ROS formed during normal biological activity function as important signaling molecules to regulates numerous processes such as cell division, inflammation, and immune function. Excessive expression of anti-oxidants enzymes can disrupt normal redox homeostasis leading to cellular dysfunction. Conversely, oxidative stress can occur if redox homeostasis is disturbed due to presence of excessive oxidants that overwhelm the antioxidant systems (Figure 5). Therefore, it is essential for a regulatory system that controls expression of antioxidant enzymes in response to reactive species in order to maintain redox homeostasis.

The nuclear factor erythroid 2 related factor 2 (Nrf2) has been identified as the master regulator of cellular antioxidant response(154). Nrf2 is a member of the cap n collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors(155). It regulates the expression of key antioxidant enzymes with anti-oxidant response element (ARE) motif. The importance of regulation of anti-oxidant enzymes is further highlighted by the tight regulation of Nrf2 itself. Kelch-like ECH-associated protein 1 (Keap1) tightly regulates the protein levels of Nrf2. Keap1 forms a homodimer and binds to Nrf2 in the cytosol acting as a substrate adaptor for cullin-based E3 ubiquitin ligase (CUL3). This then

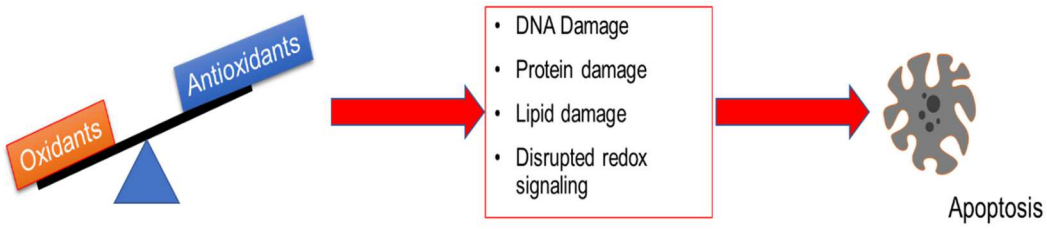

Figure 5. Consequences of disruption of redox homeostasis in cells

leads to polyubiquitination of Nrf2 by CUL3 and subsequent proteasomal degradation(156). This allows maintenance of low levels of Nrf2 protein expression that can be swiftly upregulated during oxidative stress. In states of oxidative stress specific cysteinyl residues of Keap1 are modified and disrupts its binding to Nrf2(157). This leads to accumulation of Nrf2 protein and its translocation to the nucleus. In the nucleus Nrf2 interacts with MAF proteins and bind to promoter of ARE genes such as NAD (P)H: quinine oxidoreductase-1, heme oxygenase 1, glutathione reductase, catalase, and glutathione peroxidase (158) (Figure 6). While this Keap1 regulatory model is the canonical mechanism by which Nrf2 levels is regulated the existence of Keap1-independent Nrf2 regulation and non-canonical p62 mediated regulation have also been demonstrated(159).

Dysfunction of redox homeostasis is involved in a variety of diseases including diabetes(138). Disruption of normal β cell function, and viability by excessive oxidative stress has been demonstrated in multiple studies. The main function of β cells is to produce and secrete insulin in response to glucose. The glucose sensing mechanism of β cells relies on changes in the ATP-to-ADP ratio within the cytoplasm of the β cell. This is mainly changed by increased glycolytic flux ultimately leading to an increase in mitochondria ATP production. As previously described, complex I and III of the electron transport chain generate highly reactive superoxide ions as a byproduct of their normal function. Recent evidence has demonstrated a necessity of mitochondria produced ROS for normal β cells glucose-stimulated insulin secretion possible through its regulation of ryanodine receptor-mediated calcium release(160–162). Additionally, glucokinase subcellular localization has been demonstrated to be regulated by insulin through production of NO and S-nitrosylation(163). Excessive levels of reactive species also induce a host of deleterious effects in β cells. Reactive species can cause damage to nucleic acids, proteins and lipids through nitration, carbonylation, peroxidation and

nitrosylation, leading to changes in enzymatic activity, signal transduction, gene expression and ultimately apoptosis.

With the importance of maintaining redox homeostasis for β cell function, it would be expected for β cells to have a well enriched antioxidant regulatory strategy. However, investigation of the ability of rodent β cells to counteract oxidative stress elucidated a relatively limited capacity. Studies have demonstrated that rodent islets exhibit significantly lower expression of antioxidant enzymes such as SOD, glutathione peroxidase and catalase compared to other tissues(164). This feature was first attributed only to rodent β cells as human islets were shown to have higher expression of antioxidant enzymes compared to rodent islets. However, recent studies have also demonstrated that relative to other human tissues human islets also exhibit low expression of antioxidant enzymes(165). This low expression of antioxidant enzymes makes the β cell very vulnerable to oxidative stress-mediated dysfunction in disease states such as diabetes.

As discussed above, T1D is a chronic autoimmune disease that involves an autoimmune attack against pancreatic β cells. Generation of ROS/RNS is a well-established mechanism employed by immune cells to destroy pathogens. During the onset of T1D, the islet is infiltrated by T-cells and macrophages in response to autoantibodies. T-cells then activate phagocytic cells including macrophages, neutrophils and dendritic cells which synthesize H_2O_2 that can diffuse into targeted β cells(166). Additionally, ROS release from these phagocytic cells can cause collateral damage to surrounding cells and enhance the autoimmune attack against β cells(167). Another detrimental facet of autoimmune attack against β cells is the release of pro-inflammatory cytokines (PIC) such as TNF-α, IL-1β, and IFN-γ(168). These cytokines induce β cell dysfunction and apoptosis through activation of complex signaling pathways that increase mitochondria ROS and RNS production and eventually inducing apoptosis(169,170). The presence of dysfunction of redox homeostasis has been demonstrated in humans with T1D(171). Assessment of

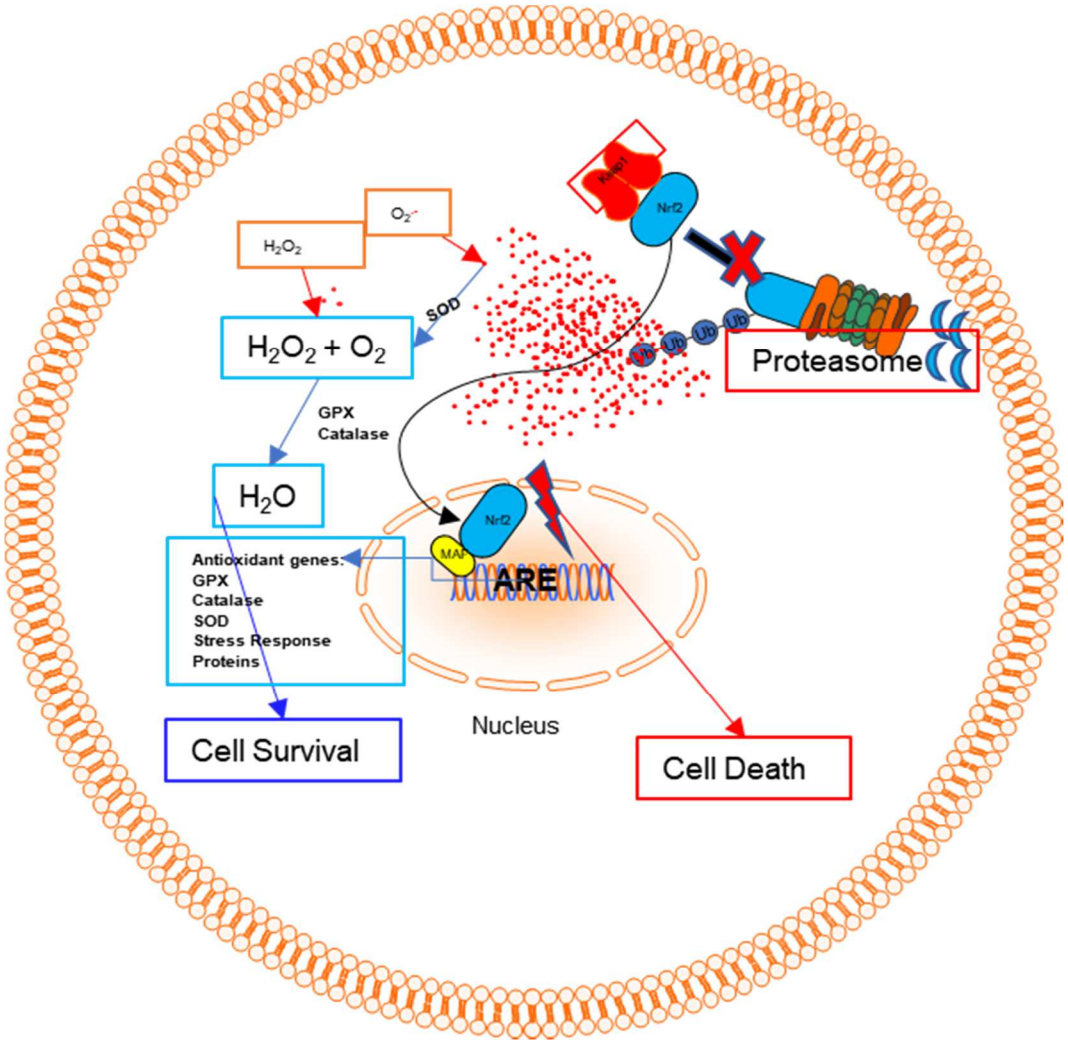

Figure 6. Diagram of NRF2 mediated antioxidant response in cells

the antioxidant enzymes catalase and glutathione peroxidase activity in plasma of T1D patients show a significant decrease in enzymatic activity in T1D individuals as compared to controls(172). Furthermore, the circulating levels of endogenous synthesized molecules with antioxidant activity such as glutathione, and uric acid were significantly decreased in patients with T1D compared to controls(172,173). The results of these studies highlight an overall depletion of antioxidant capacity in patients with T1D.

The role of oxidative stress in T2D pathogenesis is quite clear when the features of T2D development is considered. One of the hallmarks of T2D is a chronic history of obesity. Numerous studies have demonstrated a link between obesity and inflammation. Obese individuals have excess adipose tissue that has been demonstrated to be the source of proinflammatory cytokines such as TNF-α, chemokines, and eicosanoids(174). This leads to a chronic low-grade inflammation in obese individuals that contributes to β cell dysfunction in T2D. Additionally, high levels of glucose and fatty acids in obese and insulin intolerant individuals has a direct effect on pancreatic β cells. NADPH oxidase (NOX) is activated by glucose, fatty acids, and PIC(175). Increased NOX activity in β cells leads to increase generation of $O2^{\cdot-}$ and H_2O_2, resulting in mitochondrial dysfunction and subsequent impairment of insulin secretion(176). In the setting of increased carbohydrate consumption, this impairment of insulin secretion leads to a positive feedback loop involving increased circulating glucose and increased β cell dysfunction, that ultimately culminates in insulin-dependent T2D.

Several studies have investigated the total antioxidant capacity of individuals with T2D. Mirroring the results observed with T1D, patients with T2D also exhibit significantly lower total antioxidant capacity compared to controls(177,178). Additionally, patients with T2D exhibit increased nitrotyrosine, a marker of oxidative stress, levels during fasting and postprandial compared to matched healthy controls(179). Studies investigating the beneficial effects of antioxidant supplementation on glycemic control in T2D humans have

shown varied results that did not prove to be significant following comprehensive meta-analyses(180–182). This shows that oxidative stress is one component of the overall disease pathology of T2D and that further investigation into methods to augment the initial protection of β cells from oxidative stress is warranted.

1.7 The Role of Lipoxygenases in Redox Signaling

Lipoxygenases (LOXs) are lipid processing enzymes that catalyze the peroxidation of polyunsaturated fatty acids (PUFAs) such as arachidonic acid, Linoleic acid, and Docosahexaenoic acid(183). Depending on the specific substrate, LOXs produce a variety of different lipid metabolites that regulate a host of biological functions and disease state(184). LOXs can activate alternative signaling mechanisms through peroxidation of PUFAs esterified to phospholipids to generates oxidized phospholipids changing the properties of the plasma membrane(185). The nomenclature of LOXs is historically based on the location of oxygen insertion into arachidonic acid that it characterizes. Therefore 15-LOX catalyzes the oxygenation of the 15th carbon of arachidonic acid to produce 15S-hydroxyeicosatetraenoic acid (HETE) and 12/15-LOX catalyzes oxygenation at carbon 12 or 15 of arachidonic acid(186).

There are seven functional LOX genes in the murine genome (*Alox5*, *Alox12*, *Alox12b*, *Alox15*, *Alox15b*, *Aloxe3*, *Aloxe12*) and six functional genes in humans (*ALOX5*, *ALOX12*, *ALOX12B*, *ALOX15*, *ALOX15B*, and *ALOXE3*). Except for ALOX5 which is located on chromosome 10 in humans and chromosome 6 in mice the rest of the LOXs cluster on chromosome 11 in mice and chromosome 17 in humans(187,188). This thesis focuses on ALOX15 which encodes for human 15-LOX and murine 12/15-LOX and ALOX12 which encodes for 12-LOX in mouse and humans (Figure 7).

ALOX15 and *ALOX12* are expressed in a variety of tissues including reticulocytes, eosinophils, dendritic cells, epithelial cells, pancreatic islets, and peritoneal macrophages(189). Depending on the specific substrate LOXs catalyze a four-step

enzymatic peroxidation reaction to create its variety of substrates. In the case of arachidonic acid as a substrate 12/15-LOX forms 12-HETE, and 15-HETE in a 6:1 ratio, whereas 12-LOX forms only 12-HETE(190). 13S-Hydroxyoctadecadienoic acid (13S-HODE) is another major product of 12/15-LOX and 12-LOX that is formed when linoleic acid is used as a substrate(191). Additionally, Docosahexaenoic acid (DHA) can be used as a substrate to form 17S-HDHA which is then converted to the anti-inflammatory metabolites resolvins and protectins(192–194). Despite its ability to make anti-inflammatory metabolites, 12/15-LOX has mainly been demonstrated to have proinflammatory effects that are mediated through its major product 12-HETE(195). Due to 12-HETE being a lipid, it can freely pass through cell membranes and induce its effect on signaling, inflammation, and oxidative stress. Additionally, some effects of 12-HETE were also shown to be mediated through its interaction with the orphan G protein-coupled receptor 31 (GPR31)(196,197).12/15-LOX activity and 12-HETE levels have been linked to the pathogenesis of both T1D and T2D.

The activity of 12/15-LOX has been heavily investigated in its role in immune regulation(198,199). Activation of 12/15-LOX increases production of proinflammatory cytokines such as TNF α and IL-12(200,201). Additionally, 12/15-LOX levels have also been demonstrated to be increased by treatment with proinflammatory cytokines(202). 12/15-LOX activity has been demonstrated to modulate activation of macrophages(203).

In the context of T1D, 12/15-LOX has been analyzed in both mouse models of T1D and human tissues. Mice harboring whole-body knockout of *Alox15* are protected from hyperglycemia and β cell loss following multiple doses of the β cell toxin STZ(204). Additionally, non-obese diabetic (NOD) mice with whole-body knockout of *Alox15* also show protection from the development of T1D(205). This protective phenotype observed in whole-body knockout mice was also shown to be an intrinsic feature of the islet using pancreas-specific knockout mice of *Alox15*. Using this model, it was demonstrated that

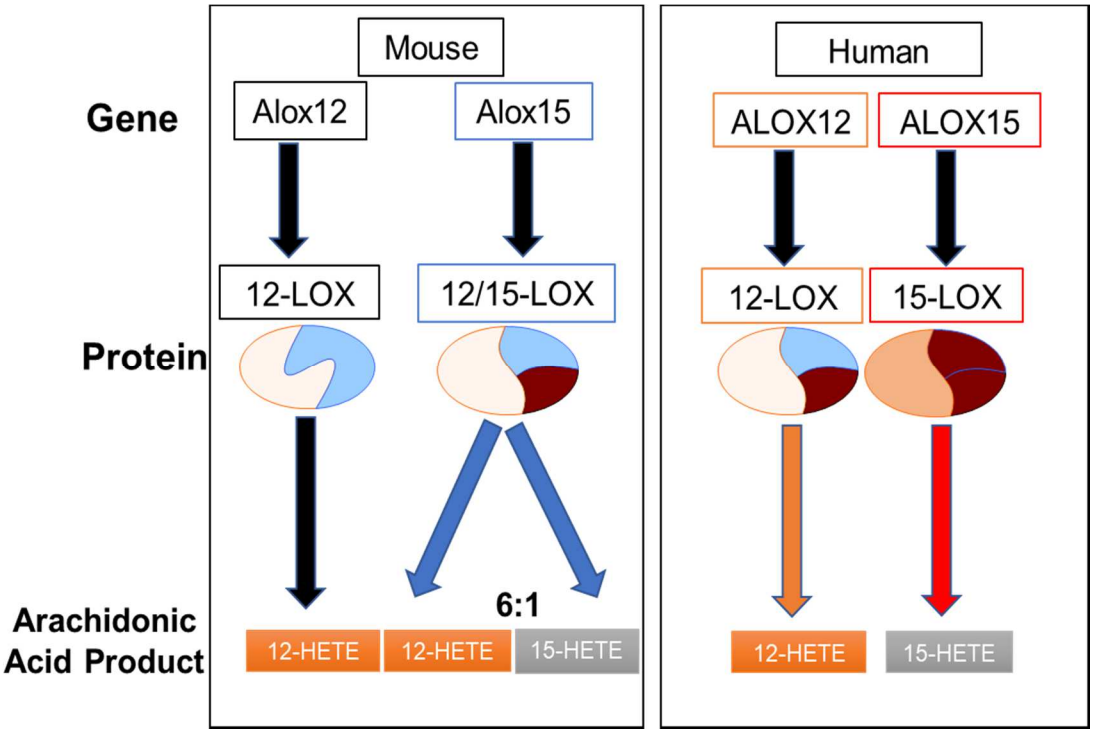

Figure 7. Lipoxygenase gene, protein and metabolic product in Mouse and Humans

mice harboring loss of *Alox15* only in pancreatic cells are protected from low dose STZ induced hyperglycemia(206). This shows that the detrimental effect of 12-LOX activity in the setting of T1D extends beyond its ability to modulate immune cell reactivity. Furthermore, studies in human islets have also corroborated the results observed in mouse models. Isolated human islets incubated with proinflammatory cytokine cocktail show upregulation of 12-LOX protein and activity(207).

The role of 12/15-LOX has also been investigated in the setting of T2D. Mice with whole body deletion of *Alox15* placed on a mimic of a western high-fat diet (45% kcal from saturated fat) exhibited significantly improved glucose tolerance, improved β cell function and reduce macrophage infiltration into adipose tissue compared to wild type mice(208). Similar protection was also found in mice placed on a high-fat diet with 60% kcal from saturated fat(209). Mice with pancreas-specific deletion of 12/15-LOX also exhibited protection from high-fat diet-induced glucose intolerance(206). Studies in human tissues show that 12-LOX and 12-HETE are both upregulated in visceral white adipose tissue of obese humans with T2D(210). Altogether, these studies have shown the potential benefit of loss of 12/15-LOX activity in the setting of metabolic diseases. Hence the development of specific inhibitors of 12-LOX activity in humans could be very beneficial in the management of metabolic diseases such as T1D and T2D. Multiple small molecule inhibitors of LOX enzymes have been identified(211). Treatment of mouse and human islets with these inhibitors showed protection like mouse models with loss of 12/15-LOX activity. Of note is the discovery of potent and selective lipoxygenase inhibitors ML351, and ML355. ML351 is a highly selective small molecule inhibitor of 12/15-LOX activity, and ML355 is selective for 12-LOX activity(212–214). Due to the presence of 12/15-LOX in mouse β cells, ML351 is primarily suitable to study inhibition of 12/15-LOX in mice. On the other hand, human β cells primarily express 12-LOX, making ML355 a potent inhibitor for human 12-LOX activity. The potency of these two inhibitors has been demonstrated

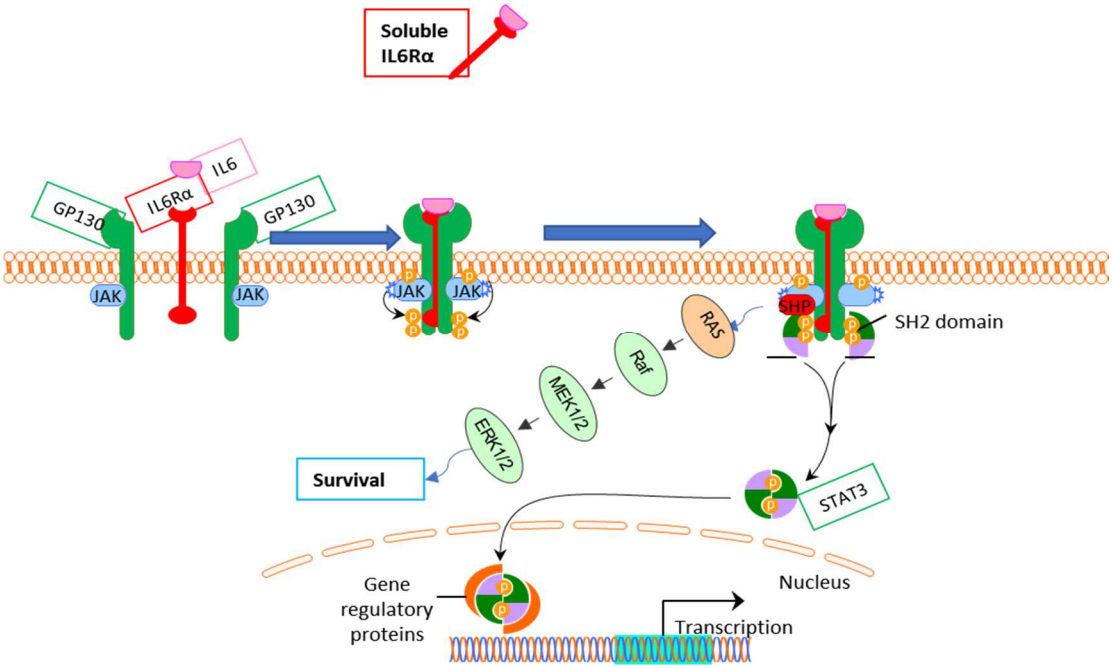

Figure 8. Classical IL-6 signaling pathways in cells

by *in vitro* studies of isolated islets and in the case of ML351 *in vivo* in mouse models(215).

The specific mechanisms by which 12/15-LOX activity induces β cell dysfunction remains unclear. However, recent studies suggest that 12-LOX activity inhibits the normal nuclear translocation of Nrf2 in response to oxidative stress(206). With the low expression of β cell antioxidant enzymes, processes that inhibit the antioxidant response mechanism would be devastating to β cells in the setting of metabolic diseases. Additionally, the generation of 12-HETE has been identified as the primary culprit by which 12/15-LOX causes its detrimental effects on β cells. This finding is supported by evidence that islet exposure to 12-HETE alone can reduce glucose-stimulated insulin secretion (GSIS) and increases islet death(195,207). By contrast, a role for 12-LOX, which also produces 12-HETE, has never been studied in the context of diabetes pathogenesis.

1.8 The Role of Interleukin 6 in Redox Signaling

Interleukin-6 (IL-6) is a pleiotropic cytokine that is secreted by a variety of cell types(216). IL-6 signaling has been primarily linked to immune responses and inflammation. IL-6 is a secreted helical polypeptide composed of four α-helix chains that together form the knot-like structure of the cytokine. IL-6 gene expression is under a complicated regulatory scheme involving multiple transcription factors including STAT3, CREB, and Nrf2, that can act in a cell-type-specific manner(217–220). Additionally, the activity of these transcription factors is controlled by cAMP in a cell-type-specific manner, which can act through CREB, by inducing expression in some cell types and inhibiting expression in others(221). To further add to the complexity of IL-6 signaling, a splice variant of the IL-6 gene has been identified that encodes for a possible non-signaling isoform(222). Newly synthesized IL-6 protein has a molecular weight ranging from 20-30 KD due to post-translational modification by either O-glycosylation or both N-glycosylation and O-glycosylation and subsequent phosphorylation(223–225). IL-6 is made by a variety of cell types ranging from immune cells to adipocytes and can act in an autocrine or

paracrine manner once secreted(226–229). Upon reaching its destination, IL-6 signals through a multicomponent receptor tyrosine kinase. IL-6 binds to the transmembrane IL-6 receptor alpha (IL-6Rα), a type I transmembrane protein with an extracellular N-terminus and a single transmembrane domain(230). The IL-6Rα subunit is not directly involved in the intracellular signal cascade induced by the cytokine due to its lack of any cytosolic signaling components(231). Upon binding of IL-6 to the IL-6Rα, the signal transducing component GP130 is recruited and heterodimerizes with the IL-6Rα-IL6 complex (232). This dimerization with IL-6Rα induces activation of GP130 through the associated kinases Jak1, Jak2, and Tyk2(233,234). These kinases phosphorylate residues of GP130 that are then able to act as a docking site for other proteins such as signal transducers and activators of transcription (e.g., STAT1 and STAT3). Upon phosphorylation, STAT1 and STAT3 can form homo or heterodimers and translocate to the nucleus to regulate the transcription of a variety of target genes(235). Additionally, phosphotyrosine residues of GP130 also act as docking sites for SHP2 proteins. Binding of SHP2 to GP130 leads to the activation of the ERK-MAPK signal cascade and downstream regulation of gene expression(236). These two robust signaling pathways have been identified as the essential signaling avenues by which IL-6 mediates most of its biological effects (Figure 8).IL-6Rα is expressed only in specific cell types such as macrophages, neutrophils, hepatocytes, β cells. On the other hand, gp130 is ubiquitously expressed in all cell types. Classically, this specificity in the expression of the IL-6Rα should limit the scope of IL-6 mediated signaling to cell types that express both components of the signaling complex. However, this paradigm has proven to be imperfect with the identification of soluble IL-6Rα (sIL-6Rα) in plasma of humans and rodents. Most of the soluble receptor is due to the translation of an alternatively spliced transcript of the IL-6Rα, while a small amount is generated by the proteolysis mediated shedding of the membrane-bound receptor(237–239). Additionally, soluble IL-6Rα retains the capacity to bind to circulating IL-6. This

complex is then able to induce IL-6 signaling in cells that express GP130 but do not endogenously express IL-6Rα, establishing an alternative trans-signaling to the classic IL-6 mediated pathway(240). Interestingly soluble gp130 (sGP130) has also been identified in plasma and has been demonstrated to inhibit the sIL6Rα – IL-6 complex(241). The complete signaling of IL-6 remains to be fully elucidated.

The initial activity of IL-6 was attributed mainly to the maintenance of normal immune system functions. This was supported by mouse studies that showed that lack of IL-6 leads to a decreased immune response to injury(242). Furthermore, IL-6 is implicated in the pathogenesis of inflammatory diseases such as hepatocellular carcinoma, and colitis-associated cancer in inflammatory bowel disease(243,244). One of the major roles of IL-6 immune activity is the mediation of the acute phase response(245,246). During the initial phase of an acute infection, immune cells release IL-6 to recruit neutrophils to the site of infection. Additionally, IL-6 regulates T cell differentiation and promotes $CD4^+$ T cell induction of B cells to produce antibodies(247). Due to its crucial role in modulating immune reactivity, it is not surprising that IL-6 has been implicated in the development of T1D(248). The role of IL-6 in T1D is contentious as results from multiple studies have established both a deleterious and protective role of IL-6 in T1D pathogenesis. *In vivo* studies in NOD mice show elevated IL-6 expression in islets of NOD mice before and during islet immune infiltration(249). On the other hand, studies of human tissues show no correlation between IL-6 islet expression and insulitis(250). Additionally, β cell-specific overexpression of IL-6 induces islet inflammation but delays diabetes development(251). The evidence of IL-6 being a critical factor in T1D is inconclusive, and further studies are required to delineate the role of IL-6 in T1D pathogenesis accurately. The effect of IL-6 on T1D pathogenesis may be through its actions on immune regulation, or directly on β cells.

Moreover, recent studies have highlighted the role of IL-6 in immune independent events such as glucose metabolism and muscle insulin sensitivity during exercise. An

increase in circulating IL-6 in obese humans and mice has been reported in numerous studies. Due to the increased adiposity that is associated with obesity, adipose tissue is postulated to be the primary source of the increase in circulating IL-6 levels observed in obese individuals. This is supported by multiple studies showing increased mRNA and protein expression of IL-6 in the adipose tissue of obese individuals(252). Additionally, IL-6Rα expression is also increased in adipose tissue of obese individuals(253). Adipose tissue in obese individuals is associated with increased infiltration of inflammatory cells that might be the actual source of IL-6 found in adipose tissue. Obesity is a critical factor in the development of insulin resistance and T2D. The observed increased association of obesity and IL-6 expression has led to multiple studies dubbing IL-6 as one of the main culprits responsible for the induction of obesity and insulin resistance. This conclusion is supported by studies showing that incubation of adipocytes or hepatocytes with IL-6 caused a decrease in insulin activity(254,255). Furthermore, infusion of IL-6 into WT mice led to inhibition of insulin signaling in the liver via upregulation of SOCS3(256). On the contrary, recent studies have also highlighted a protective effect of IL-6 in the setting of obesity and insulin resistance. Infusion of rats with IL-6 caused improved glucose tolerance and insulin sensitivity(257). Additionally, IL-6 has also been demonstrated to have CNS mediated effects. Direct infusion of IL-6 into the CNS led to the suppression of feeding and improved glucose tolerance(258). *In vitro* and *in vivo* studies have also demonstrated the ability of IL-6 to directly enhance GSIS in islets (111). Studies using transgenic mouse models have been very informative about the role of IL-6 in obesity and insulin resistance. Mice harboring whole-body knockout of IL-6 gene developed mature-onset obesity and glucose intolerance(260). Additionally, it was demonstrated that IL-6 KO mice were significantly more likely to develop increase hepatic insulin resistance, HFD induced liver inflammation and mitochondria impairment(261). Furthermore, IL-6 KO mice show decreased levels of insulin clearance in the liver and skeletal muscle compared to

WT mice(262). Studies using overexpression models also corroborate the results of KO studies. Overexpression of IL-6 in both whole brain and specifically in astrocytes protected mice from HFD induced weight gain and glucose intolerance(263,264). Furthermore, gene transfer of IL-6 into obese mice caused reduction in weight and fat mass (265).

In summary, IL-6 classically has been associated with obesity and systemic insulin resistance. However recent evidence using transgenic mouse models suggests an actual protective role of IL-6. The results of these studies highlight the possibility that the increased IL-6 observed in obesity is a feedback mechanism to counter the effects of obesity. However, further studies are required to properly explore the role of IL-6 in the setting of metabolic diseases such as T2D. Another facet that these studies highlight is the possibility of tissue-specific regulatory effects of IL-6. Therefore, proper use of conditional transgenic mice would be beneficial in elucidating the role of IL-6 in metabolic tissues and cells such as β cells.

IL-6 is often used as a marker for oxidative stress and inflammation, although there is no evidence of IL-6 directly increasing ROS production in mammalian cells. Several studies have raised the possibility of antioxidant effect of IL-6. IL-6 has been demonstrated to inhibit the activity and levels of TNFα, a cytokine that directly induces oxidative stress(266–268). Additionally, the IL-6 promoter has been demonstrated to have an ARE that can be recognized by NRF2, allowing for NRF2 induction of IL-6 expression(220). IL-6 has also been demonstrated to induce expression of antioxidant enzymes GPX1 and ref-1, to decrease oxidative injury following liver resection(269). Further evidence of an IL-6 protective effect in setting of oxidative stress comes from studies investigating oxidative stress in chemotherapeutic agents(270,271).

1.9 Summary

In summary, both T1D and T2D are diseases that are associated with an increase in oxidative stress in β cells. Assessment of islets from animal models of diabetes, and human samples from patients with diabetes show a marked increase in oxidative stress associated with disease. Hence, uncovering targets that modulate oxidative stress in β cells would be beneficial for both T1D and T2D. Lipoxygenases are enzymes that catalyze the oxygenation of polyunsaturated fatty acids to form lipid metabolites that are involved in a variety of biological functions including oxidative stress response. Increases in 12/15-LOX activity has been demonstrated to lead to an increase in oxidative stress in β cells through the production of the lipid metabolite 12-HETE. 12-LOX is another member of the lipoxygenase family, which also produces the detrimental metabolite 12-HETE. The activity of 12-LOX, which also produces 12-HETE, has never been studied in the context of diabetes pathogenesis. Additionally, the role of IL-6 in diabetes is contentious as some studies show a detrimental role while others have shown protective functions. Both, 12-HETE and IL-6 have been demonstrated to modulate oxidative stress response. Due to 12-HETE showing a detrimental effect of inducing oxidative stress while IL-6 has been demonstrated to suppress oxidative stress further studies are required to determine whether targeting of these two pathways can improve β cell function and survival through regulation of oxidative stress. Hence the objective of this thesis is to determine whether loss of 12-LOX(Alox12) and activation of IL-6 signaling will increase β cell function and survival in the setting of oxidative stress. The results from this study will highlight the actions of 12-LOX and IL-6 in the modulation of pancreatic β cell function and survival. Furthermore, these studies will provide novel mechanistic insight into the modulation of oxidative damage in the β cell. Identification of the mechanisms by which IL-6 and 12-LOX regulate β cell response to oxidative stress will provide novel therapies that can be used to reduce the loss of β-cells due to oxidative damage in the pancreatic islet.

2 Materials and methods

2.1 Cell Lines

Rat Insulinoma cell line INS1 (832/13) were maintained in RPMI 1640 media containing 10 mM HEPES, 2 mM glutamine, 1mM sodium pyruvate, 50 μM β-mercaptoethanol, 10% fetal bovine serum and 1% antibiotic/antimycotic.The mouse insulinoma cell line MIN6 was maintained in high glucose DMEM with 15% FBS, 1% Pen/Strep, and supplemented with 10 mM HEPES and sodium pyruvate.Cell media for both INS1 and MIN6 were changed every 3-4 days and cells were split at confluency.

2.2 Islet isolation and culture

Human islets were obtained through the integrated islet distribution program (IIDP) and cultured in suspension in Prodo Labs islet media containing 5% human AB Serum (Prodo Labs), 1% glutamine/glutathione supplement (Prodo Labs), and 10 μg/mL Ciprofloxacin (Fisher Scientific). Islets were cultured for up to 3 days with media changed each day.

Mouse Islets from both male and female mice were isolated from collagenase-perfused pancreata and cultured in RPMI medium by the Diabetes Center Islet Core as previously described(272). Briefly, mice were sacrificed by cervical dislocation and pancreata were inflated with 2.0 ml of collagenase. Pancreata were then incubate at 37 C for 15 min followed by dissociation in Hank's Balanced Salt Solution (HBSS) and Bovine Serum Albumin (BSA) solutions. Islets were hand-picked and allowed to recover overnight in complete media (8 mM glucose RPMI) before experimentation.

2.3 Animals

All experiments involving mice were performed with approval by the Indiana University Institutional Animal Care and Use Committee (IACUC). Mice were maintained in pathogen free conditions under a standard 12-hour light-dark cycle and provided unlimited access to water and a standard rodent chow. B6.129S2-Alox12tm1Fun/J (*Alox12*$^{-/-}$) and B6.129S2-Alox15tm1Fun/J (*Alox15*$^{-/-}$) mice were purchased from Jackson

Laboratories and maintained in the Indiana University School of Medicine animal facilities. For the lipoxygenase experiments wild-type (WT) mice were littermates from both *Alox15* and *Alox12* litters. In order to generate β cell specific IL6Rα knockout mice mixed C57BL/6J/6N mice containing LoxP sites surrounding exons 4-6 of the *Il6ra* gene were purchased from Jackson laboratory (#012944). Mice were then backcrossed to C57BL/6J wild type mice (Jackson laboratory 002650) to generate *IL6rα* floxed mice on a pure C57BL/6J background. Floxed mice were then bred to B6(Cg)-Ins1$^{tm1.1(cre)Thor}$/J (Jackson Labs #026801) to create β-cell specific IL6rα knockout mice(IL6Rα$^{\Delta\beta)}$.

2.4 Streptozotocin in IL-6 experiments

Male Wild type and IL6Rα$^{\Delta\beta}$ mice at 8 weeks of age were injected intraperitoneally (IP) with 45mg/kg of streptozotocin (Sigma S0130) in 300 μL saline daily for 5 consecutive days. All STZ solutions prepared immediately before injection and control mice were injected with equal volume of saline. On day 8 (3 days after the final injection), overnight fasted mice were given intraperitoneal glucose tolerance tests (IP-GTTs). Briefly, animals were given IP injections of 2g/kg glucose (in saline), with dosing based on total body mass. Blood glucose was measured with an AlphaTRAK2 glucometer (Abbott) before glucose injection and at 5 min, 15 min, 30 min, 60 min, 90 min, and 120 min post injection. The following day, mice were euthanized by cervical dislocation and tissues were collected for analysis.

2.5 Streptozotocin in Lipoxygenase experiments

Male WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice were injected intraperitoneally daily for 5 consecutive days with saline or streptozotocin at 55 mg/kg/day at 8 weeks of age. A cohort of mice received 12/15-LOX inhibitor, ML351, at 10 mg/kg body weight for 5 days prior, during, and 5 days post-STZ-treatment(213). IP-GTTs with 2 g/kg body weight of glucose were performed as described above. At the end of the respective studies, mice were euthanized by cervical dislocation and tissues were collected for analysis.

2.6 Alloxan treatments in IL-6 experiments

Overnight fasted mice were injected with 150 mg/kg alloxan monohydrate (Sigma A7413) in 300 μL saline prepared immediately before injection. Control injections consisted of equal volumes of saline. Following injections, mice were given free access to food and water for the duration of the experiment. Six hours after injection, mice were euthanized by cervical dislocation and tissue was collected for analysis. Blood glucose was measured before fasting, immediately before alloxan injection, and terminally.

2.7 High fat diet experiments

Weights and blood glucose of male mice at 8 weeks of age on regular chow diet (Research Diets; 5008) were measured prior to starting HFD. Regular chow diet of mice was then switched to high fat diet (60% kcal from fat; Research Diets; D12492) for 10 weeks (IL-6 experiments) or 4 weeks (Lipoxygenase experiments). Weights and random fed blood glucose were measured weekly. At the end of the diet period mice were fasted overnight for IPGTT as described above. Mice were then given a week to recover and insulin tolerance test (ITT) was performed. Mice were fasted 2 hours and then injected intraperitoneally with 0.75 U/kg regular Humulin-R insulin (Eli Lilly) diluted in sterile saline. Blood glucose was measured with an AlphaTRAK2 glucometer before insulin injection and at 15 min, 30 min, 45min, 60 min, 90 min, and 120 min post injection. Animals were given free access to HFD following ITT procedures.

2.8 Assessment of insulin signaling in skeletal muscle

Male wild type and IL6R$\alpha^{\Delta\beta}$ mice were placed on HFD for 10 weeks as described above. Mice were then injected intraperitoneally with saline or 30 units of Insulin 5 minutes prior to sacrifice by cervical dislocation. Tissues were harvested immediately following euthanasia and flash frozen in liquid nitrogen. Protein was isolated from frozen skeletal muscle tissue using a mortar and pestle to crush tissue. Powdered tissue was resuspended in RIPA buffer (20 mM Tris-HCl (pH 7.5) 150 mM NaCl, 1 mM Na_2EDTA,1 mM EGTA, 1% NP-40,1% sodium deoxycholate, 2.5 mM sodium pyrophosphate,1 mM β-

glycerophosphate,1 mM Na_3VO_4), and protein levels was quantified using a Bradford assay.

2.9 INS-1 and mouse islet live-cell imaging

Analysis of mitochondrial membrane potential was performed using Tetramethylrhodamine Methyl-ester (TMRM, 100 nM, ThermoFisher). INS-1 cells in phenol free RPMI were incubated with TMRM and Mito-Tracker Green (200 nM, ThermoFisher) for 30 minutes. Cells were washed with 3x with PBS followed by replacement with phenol free RPMI. Cells were incubated with vehicle or IL-6 followed by live cell imaging using a Nikon TiE spinning disk equipped with a CO_2-controlled 37 ºC stage. Image intensity was quantified using ImageJ Fiji.

For real-time imaging of autophagy stimulation by IL-6, INS-1 cells were transfected with pBABE-puro mCherry EGFP-LC3B (Addgene plasmid 22418). Cells were incubated with vehicle or IL-6 followed by live cell imaging using a Nikon TiE spinning disk equipped with a CO_2-controlled 37 ºC stage. Colocalization was determined using CellProfiler software version 2.2.

For real-time imaging of cAMP dynamics, isolated mouse islets were infected with a mNeon cADDis cAMP biosensor (Montana Molecular). Islets were imaged in a Krebs-Ringer bicarbonate solution with 20mM HEPES and 16.7 mM glucose. Islets were treated with Exendin-4 (100 nM), followed by IL-6, and finally Isoproterenol (1 μM). Image intensity was quantified using ImageJ Fiji.

2.10 Measurement of β Cell redox state

Mouse islets were isolated as described above. After overnight recovery, islets were briefly washed with PBS and distended by incubation with Accutase (STEMCELL Technologies) for 1 minute at 37° C. Accutase was then inactivated, and islets were resuspended in complete islet media containing serum. Islets were then transduced with roGFP2 adenovirus for 6 hours in complete islet media. Following transduction, islets were

then transferred to virus-free islet media containing a vehicle or proinflammatory cytokine cocktail (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 100 ng/ml IFN-γ) for 16 hours. Islets were then imaged on a Zeiss LSM 700 confocal microscope. The biosensor was sequentially excited with 405, and 488 nm excitation and GFP fluorescence were collected and ratioed. Image intensity of 405 and 488 nm images was quantified using ImageJ Fiji and ratio was calculated as 405nM/488nM intensity. Ratiometric images were made using a custom pipeline in Cell Profiler 2.2.

2.11 Flow cytometry sorting of pancreatic islets

Islets were isolated using standard isolation protocol described above. Following isolation islets were incubated for 24 hours in standard islet media. Islets were then dissociated by suspending in Accumax (2000 IEQ/mL) with 0.0015% DNase and incubated at room temperature with agitation on a thermoblock for 10 mins. Cells were then triturated until single cells were observable using a tissue culture inverted microscope. A sample of cells (~2000) were then aliquoted for use as unstained controls. Cells were then incubated in 25 μM of Newport Green (ThermoFisher Catalog # N24191) in culture media and incubated at 37 ºC for 90 minutes in an incubator. Unstained controls were resuspended in culture media and incubated at 37 ℃ for 90 minutes. Following incubation cells were washed 3x in PBS and resuspended in PBS. Cells were then filtered using 70 μM mesh into flow cytometer tubes and sorted using negative control cells to gate.

2.12 RNA Seq

Islets from male wild type and IL6R$\alpha^{\Delta\beta}$ mice (n ≥ 3) on normal chow were isolated as described above. Islets were cultured overnight in islet media and RNA was collected using RNeasy micro kit (Qiagen). RNA was used to prepare dual-indexed nonstranded cDNA library using SMART- Seq v4 Ultra Low Input RNA Kit (Takara Bio). Libraries were sequenced using a HiSeq4000 (Illumina). All sequenced libraries were mapped to the

mouse genome (UCSC mm10) using STAR RNA-seq aligner (273). The reads distribution across the genome was assessed using bamutils (from NGSUtils)(274). Uniquely mapped sequencing reads were assigned to mm10 refGene genes using featureCounts (from subread)(275). Data were normalized using trimmed mean of M values method. Differential expression analysis was performed using DESeq2(276). P-values were adjusted for multiple hypothesis testing using the Benjamini-Hochberg procedure. For volcano plots, the fold change (log2 scale) differences in genes between groups were plotted on the x-axis, and the p-value (–log10 scale) were plotted on the y-axis using R (version 3.2) and the ggplot2 package (version 2.2.1). Pathway enrichment was determined using Ingenuity Pathway Analysis (IPA)(Qiagen) software with threshold at padj ≤ 0.05.

2.13 Proteomic Analysis

Human islets obtained through the IIDP were cultured as described above. Following one day of culture, islets were treated with 100ng/mL recombinant Human IL-6(Miltenyi Biotec; Cat#130-093-929) (~1500 IEQ per condition) for 5 minutes in a 1.5mL tube. Islets were then centrifuged (800g x 3mins) and supernatant was collected. Islet pellet was then resuspended in 8M urea in 50mM Tris-HCL. Islets were then titurated using a 25-gauge needle, followed by sonication at stored at -80 C. Samples were submitted to the Indiana University School of medicine proteomics core for TMT peptide assay using their in house protocol below:

2.13.1 Mass Spec Prep, Digestion and Clean up

Samples were first treated with 8 M urea in 50mM Tris-HCl, then sonicated with sonicate tip and incubate for 2hr at RT. Protein concentration of each sample was determined using Bio-Rad protein assay (Bio-Rad Laboratories Inc). Equal amounts (30µg) of protein samples were reduced with 5 mM tris(2-carboxyethyl) phosphine hydrochloride (TCEP), and alkylation with 10 mM chloroacetaminde (CAM), then digested

with trypsin/LysC mix (Promega) over night, the Sep-Pak C18 purification system (Waters) was used to clean up the tryptic peptide.

2.13.2 TMT labeling and peptide assay

Samples were labeled with the different isobaric TMT tagging reagents (Thermo Fisher Scientific) and following manufacturer's instruction, peptide concentration was measured using Pierce Quantitative Colorimetric Peptide Assay Kit (Thermo Scientific) and combine samples at equal amounts.

2.13.3 Fractionation

The mixed samples were fractionated using Pierce High pH Reversed-Phase Peptide Fractionation Kit (Thermo Scientific) to improve protein sequence coverage and increases the number of identified proteins. All liquid from fractions were completely removed using vacuum centrifuge and fractions were re-suspend in 0.1% formic acid before LC-MS analysis.

2.13.4 Instrument Method

Thermo-Fisher Scientific Oribitrap Fusion Lumos coupled with Thermo –Fisher Scientific Easy-nLC1200, 10µl of enriched phosphopeptide were loaded onto an Acclaim PepMap C18 trapping column (3 µm particle size, 100 Å pore size, 2 cm length, 75 µm outer diameter) and eluted on a PepMap™ RSLC C18 column (2 µm particle size, 100 Å pore size, 50 cm length, 75 µm outer diameter) with a linear gradient from 3 to 35% acetonitrile (in water with 0.1% FA) developed over 180 min at room temperature at a flow rate of 400 nL/min, and effluent was electro-sprayed into the mass spectrometer.

2.13.5 LC-MS/MS data analysis

The resulting nano-LC-MS/MS data were analyzed using Proteome Discoverer (Version 2.2, ThermoFisherScientific™). SEQUEST HT (as a node in PD 2.2) was utilized to perform database searches with a few modifications: trypsin digestion, 2 maximum missed cleavages, precursor mass tolerance of 10 ppm, fragment mass tolerance of 0.8 Da, a fixed modification of +57.021 Da on cysteine, and a variable modification of +15.995

Da on methionine. The delta mass for the TMT 6-plex reagent is 229.163 dalton and is added as a static modification to peptide N-termini as well as lysine side chains, the spectral false discovery rate (FDR) was set to ≤ 1%. The FASTA database used was a human proteome downloaded from Uniprot with additional common contaminants. Protein data was analyzed for pathway enrichment using IPA. Threshold for proteins to include in the analysis was set using padj ≤ 0.1.

2.14 Eicosanoid Analysis

Mouse serum collected from 8-week-old WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. Banked human plasma from subjects with, normal glucose tolerance, impaired glucose intolerance, and T2D and mouse samples were sent to Washington University Mass Spectrometry core for eicosanoid analysis. The eicosanoid panel consisted of 5-HETE, 12-HETE, 8-HETE, 15-HETE, 12-HEPE, 13-HODE, 17-HDHA, and LTB4. Eicosanoids were extracted from 50 µL of serum with 200 µL of methanol, containing 2ng each of deuterated 5-HETE-d_8, 13-HODE-d_4, and LTB4-d_4, as the internal standards. The supernatant was reconstituted with 250µL of water for mass spectrometry analyses. 4 point to 7-point calibration standards of all eicosanoids, containing the deuterated internal standards were prepared for the absolute quantification. The sample analysis was performed with a Shimadzu 20AD HPLC system and a SIL-20AC autosampler coupled to a tandem mass spectrometer (API-6500+: Applied Biosystems) operated in MRM mode. The negative ion ESI mode was used for detection of these eicosanoids. The plasma extracts were injected in duplicate for data averaging. Data processing was conducted with Analyst 1.6.3 (Applied Biosystems).

2.15 Immunostaining

Cells grown on coverslips were fixed with 3.7% paraformaldehyde and immunostaining performed as in Linnemann et al. (10). Alternatively, freshly isolated pancreata were fixed with 3.7% paraformaldehyde for 3 hours then transferred to a 70%

ethanol before paraffin embedding. Antigen retrieval was performed on 5 μm sections using a citrate-based antigen unmasking solution (Vector Laboratories), samples were blocked with Dako blocking solution, then incubated overnight with primary antibodies diluted in Dako antibody diluent. For immunofluorescence, slides were incubated with fluorescently conjugated secondary antibodies, and Dapi (ThermoFisher) diluted in antibody diluent, then mounted with Vectashield (Vector Laboratories). Images were collected on a Zeiss LSM 700 scanning laser confocal microscope for standard confocal or a Zeiss ELYRA for structured illumination microscopy (SIM; used for co-localization studies). For β-cell mass analysis, tissue sections were stained as described (277) then mounted with Permount (ThermoFisher) and scanned using a Zeiss Axioscan imager.

2.16 In vitro oxidative stress measurements

For analysis of oxidative stress, treated cells were incubated with 1μM CellROX Deep Red (ThermoFisher) for 30 min at 37°C and then stained with Hoescht (ThermoFisher) for 15 min. Cells were washed and imaged in RPMI 1640 lacking phenol red. Islets were incubated with 5 μM CellROX Deep Red (ThermoFisher) for 30 min at 37°C, then stained with Hoescht (ThermoFisher) for 15 min, and fixed with 3.7% paraformaldehyde for 20 min at room temp. Samples were imaged on a Zeiss LSM 700 scanning laser confocal microscope.

2.17 Cell Fractionation and Western Blotting

Cellular lysates were separated into cytoplasmic, mitochondrial, and nuclear fractions using the Subcellular Fractionation Protocol from Abcam. Briefly, INS-1 cells treated with vehicle or 200 ng/mL IL-6 were lysed in fractionation buffer (20mM Hepes (pH 7.4), 10 mM KCl, 2 mM MgCl2, 1 mM EDTA, 1mM DTT, and phosphatase and protease inhibitors. Nuclei were pelleted by spinning at 3000rpm for 5 minutes. Mitochondria were separated from the cytoplasm by spinning at 8000rpm for 5 minutes. Nuclear and mitochondrial pellets were resuspended in TBS+0.1% SDS. Proteins were resolved using

4-20% SDS-PAGE gels (Bio-Rad) and transferred to polyvinylidene difluoride membranes (EMD Millipore). Membranes were blocked with LiCOR Odyssey Blocking buffer (LiCOR) then probed overnight at 4°C with primary antibodies in Tris-buffered saline containing 0.1% Tween-20 and 5% bovine serum albumin. Membranes were then incubated with IRdye conjugated secondary antibodies (LiCOR) and imaged on a LiCOR Odyssey imager.

2.18 Real-time RT-PCR.

Reverse-transcribed RNA was analyzed by real-time PCR using SYBR Green or Taqman technology. Primers include: *Alox15* and *Alox12* (Qiagen). All samples were corrected for input RNA by normalizing to *Actb* message. All data represent the average of independent determinations from at least three separate mice.

2.19 Immunohistochemistry, immunofluorescence, and β-cell mass

Pancreata were fixed in 4% paraformaldehyde, sectioned, and immunostained for insulin as described(278). Pancreata were stained by immunofluorescence using the following primary antibodies: anti-4-HNE antibody (Ab464545, Abcam; 1:100), anti-GPX1 (Santa Cruz), anti-CAT (Santa Cruz) and anti-insulin antibody (A0564, Dako; 1:500). Alexa Fluor 568 donkey anti-rabbit antibody and Alexa Fluor, 488 donkey anti-guinea-pig antibody, were used as secondary antibodies (Invitrogen). Images were acquired using a Zeiss LSM 700 or LSM 800 confocal microscope. 4-Hydroxynononeal (4-HNE) immunostainings were quantified by measuring pixel density per insulin-positive cell. β-cell mass was calculated as described previously using at least 3 pancreas sections 70 μm apart from 5 pancreata per group(279).

2.20 Measurement of β-cell redox state

Mouse islets were isolated as described above. After overnight recovery, islets were briefly washed with PBS and distended by incubation with Accutase (STEMCELL Technologies) for 1 minute at 37° C. Accutase was then inactivated, and islets were resuspended in complete islet media containing serum. Islets were then transduced with

roGFP2 adenovirus for 6 hours in complete islet media. Following transduction, islets were then transferred to virus-free islet media containing a vehicle or proinflammatory cytokine cocktail (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 100 ng/ml IFN-γ) for 16 hours. Islets were then imaged on a Zeiss LSM 700 confocal microscope. The biosensor was sequentially excited with 405, and 488 nm excitation and GFP fluorescence were collected and ratioed.

2.21 Statistical analysis

All data are presented as the mean ±SEM. One-way ANOVA (with Holm-Sidak's post-test) was used for comparisons involving more than two conditions, a two-way ANOVA was used for comparisons with multiple time points, and a two-tailed Student's t-test was used for comparisons involving two conditions. Prism 8 software (GraphPad) was used for all statistical analyses. Statistical significance was assumed at $p < 0.05$.

3 Deletion of platelet-type 12-lipoxygenase exacerbates islet β-cell oxidative stress and dysfunction

3.1 Introduction

Lipoxygenases (LOXs) are processing enzymes that catalyze the oxygenation of polyunsaturated fatty acids (280) . Depending on the specific substrate, lipoxygenases produce a variety of different lipid metabolites that regulate a host of biological functions and disease states (189,281,282). In mice, the gene Alox15 encodes for “leukocyte-type” 12-lipoxygenase (known as 12/15-LOX), which catalyzes oxygenation of lipids at carbon 12 or 15 (187,283). The related gene Alox12 encodes “platelet-type” 12-lipoxygenase (known as 12-LOX), which oxygenates lipids at carbon 12. Both enzymes catalyze the oxygenation of the polyunsaturated fatty acid arachidonic acid, with 12/15-LOX forming 12-, and 15- hydroxyeicosatetraenoic acid (HETE) in a 6:1 ratio, whereas 12-LOX forms only 12-HETE. The activity of 12/15-LOX and its major lipid metabolite, 12-HETE, have been linked to the pathogenesis of T1D. Mice harboring whole-body knockout of *Alox15* show protection from low-dose streptozotocin (STZ)-induced diabetes (204). Likewise, non-obese diabetic (NOD) mice with whole-body knockout of *Alox15* also show protection from the development of T1D (205) . This protective effect of loss of Alox15 is likely due to pancreas expression of the enzyme, as mice with a pancreas-specific deletion of *Alox15* are also protected from low-dose STZ-induced diabetes (204). The specific mechanism underlying this protection has not been identified, but studies have highlighted the loss of the lipid metabolite 12-HETE as one possible mechanism. This possibility is supported by evidence that islet exposure to 12-HETE alone can reduce glucose-stimulated insulin secretion (GSIS) and increase islet death (207,284). By contrast, a role for 12-LOX, which also produces 12-HETE and related eicosanoids, has never been studied in the context of diabetes pathogenesis. We reasoned that since both 12-LOX and 12/15-LOX can make 12-HETE and related eicosanoids, then the loss of Alox12 should show similar protection

as loss of Alox15. In this study, we sought to directly compare the effect of loss of Alox12 versus Alox15 in the setting of diabetes.

3.2 Results

3.2.1 Deletion of Alox12 exacerbates, while deletion of Alox15 protects against STZ-induced diabetes

We sought to assess the metabolic effects of whole-body deletion of the genes encoding 12/15-LOX and 12-LOX (*Alox15* and *Alox12*, respectively) in mice on the C57BL6/J background. As shown in Figure 9A-D, Alox15$^{-/-}$, Alox12$^{-/-}$, and their WT control littermate mice at 8 weeks of age exhibited no differences in body weight, glucose tolerance (by intraperitoneal glucose tolerance tests (GTTs)), or β-cell mass (by histomorphometric analysis of immunostained pancreata). These results are consistent with prior findings (206,208) that loss of Alox12 or Alox15 does not appear to affect the normal development of β-cells or whole-body glucose homeostasis.

To assess if the loss of Alox15 and Alox12 negatively or positively affect β-cell function during the development of diabetes, we leveraged the multiple low-dose streptozotocin (STZ) model (55 mg/kg body weight STZ intraperitoneally daily for 5 days) to induce diabetes. In this β-cell toxicity model, mice develop a T1D-like phenotype with local islet inflammation and consequent hyperglycemia over 4 weeks(285,286). As expected, WT mice developed overt diabetes (blood glucose ≥300 mg/dl) within 14 days following STZ injections (Figure 10A, closed circles). Consistent with previously published data (205), Alox15$^{-/-}$ mice were protected from overt diabetes following STZ (Figure 10A, grey circles). In striking contrast, Alox12$^{-/-}$ mice exhibited more severe hyperglycemia than WT mice by 14 days following STZ and continuing through the conclusion of the study at 28 days (Figure 10A, open circles). GTTs performed 4 days following STZ revealed that Alox12$^{-/-}$ mice were significantly more glucose intolerant than WT and Alox15$^{-/-}$ mice, whereas Alox15$^{-/-}$ mice showed protection from STZ-induced glucose intolerance compared to WT mice (Figure 10B-C).

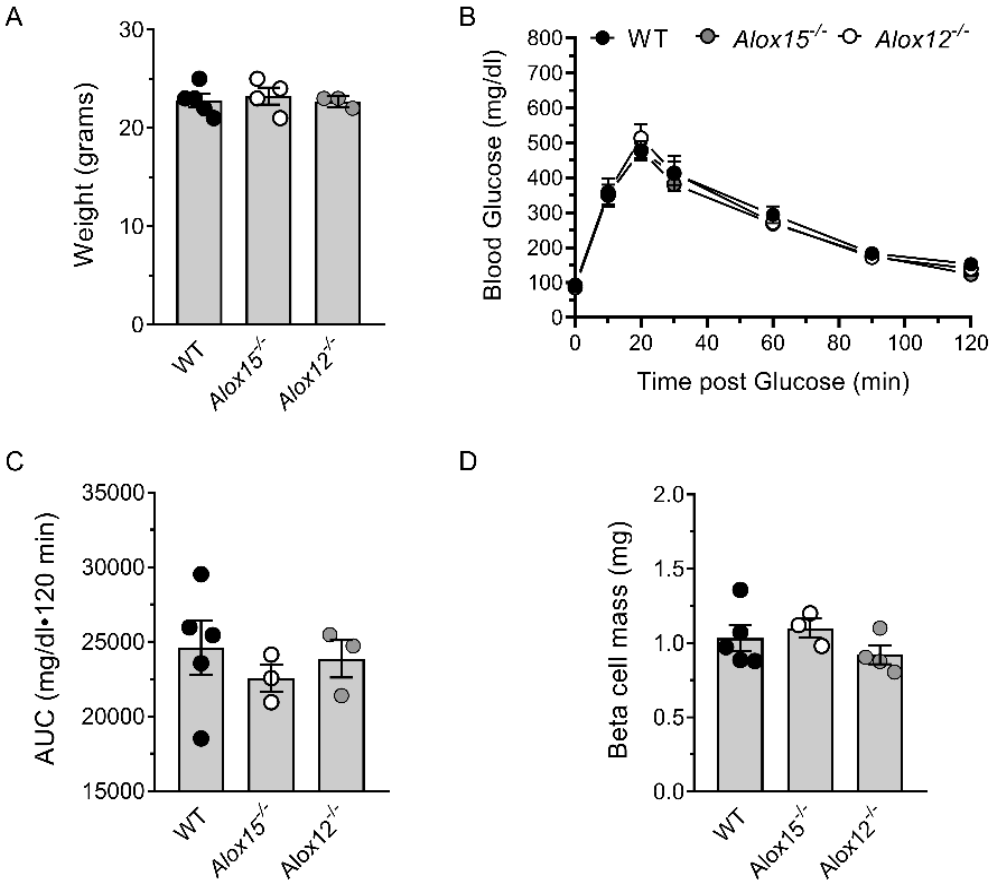

Figure 9. Deletion of Alox12 or Alox15 does not affect normal β cell development and function (A) Bodyweight measurements of WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. (B) Analysis of glucose tolerance (GTT) in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. (C) Area under the curve analysis of GTT. (D) Analysis of β-cell mass in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. N ≥ 3 mice per experimental group for all experiments.

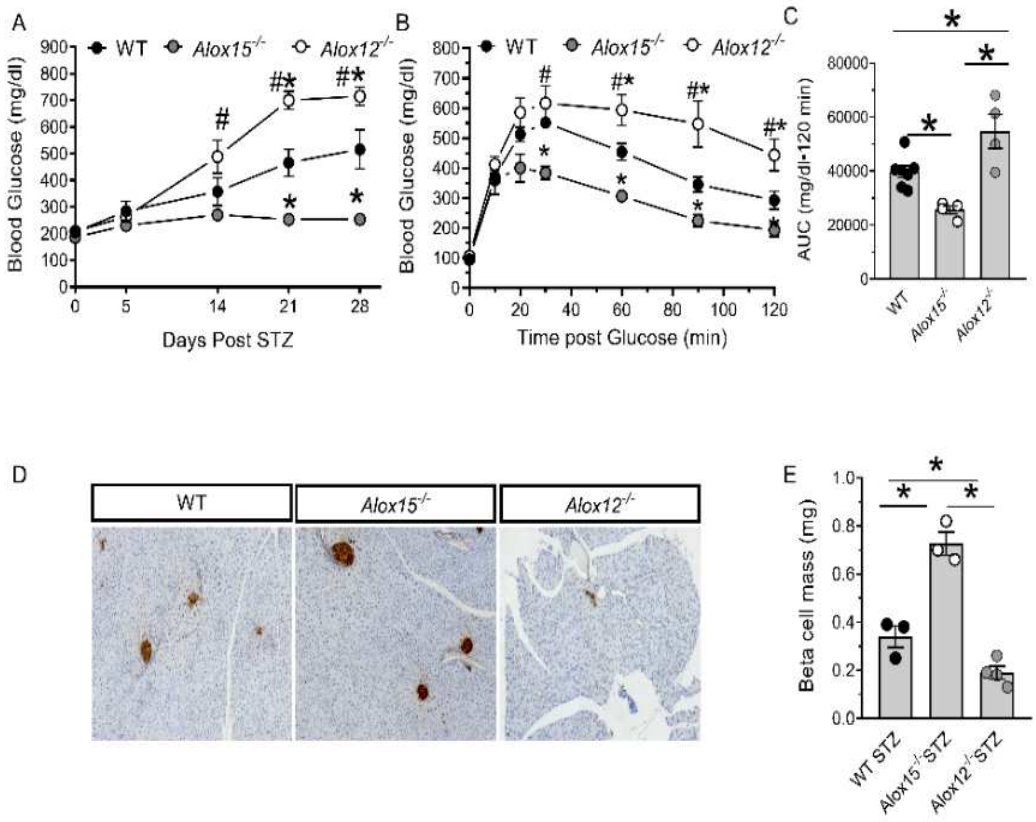

Figure 10. Deletion of Alox12 exacerbates, while deletion of Alox15 protects against STZ-induced diabetes (A) Random feed blood glucose levels of WT and Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ regime. (B) Analysis of glucose tolerance (GTT) in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice 4 days following STZ regime. (C) Area under the curve analysis of GTT post STZ. (D) Representative images of Insulin immunohistochemistry staining of pancreata for WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ used for of β-cell mass. (E) Analysis of β-cell mass in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ. N ≥ 3 mice per experimental group for all experiments *p<0.05 compared to WT, #p<0.05 compared to Alox15$^{-/-}$.

We next evaluated β-cell mass in STZ-treated mice. As shown in Figure 10D-E, STZ-treated Alox12$^{-/-}$ mice exhibited a significant 1.8-fold reduction in β-cell mass compared to STZ-treated WT mice. By contrast, β-cell mass was more than 2-fold higher in STZ-treated Alox15$^{-/-}$ mice compared to STZ-treated WT mice (Figure 10D-E). Together, these data suggest that loss of *Alox12* exacerbates inflammation-induced β-cell dysfunction, whereas loss of *Alox15* is protective in this setting.

3.2.2 Deletion of Alox12 exacerbates inflammation-induced oxidative stress in β cells

12-HETE, a lipid product of 12-LOX and 12/15-LOX, is linked to oxidative stress in islets (287). We, therefore, asked if oxidative stress in β cells differed in WT, Alox15$^{-/-}$, and Alox12$^{-/-}$ mice after STZ treatment. We examined oxidative stress in tissue sections from STZ-treated control and knockout mice by analysis of islet 4-hydroxynoneal (4-HNE) (288,289) by immunofluorescence. STZ-treated Alox15$^{-/-}$ mice exhibited approximately 2-fold reduced 4-HNE immunostaining intensity compared to STZ-treated WT mice (Figure 11A and B). However, in agreement with their exacerbated diabetic phenotype, *Alox12*$^{-/-}$ mice exhibited a significant 1.4-fold increase in 4-HNE immunostaining intensity compared to STZ-treated WT mice (Figure 11A and B). This demonstrates that *Alox12*$^{-/-}$ mice have increased STZ induced oxidative stress that could lead to β cell dysfunction.

3.2.3 Deletion of *Alox12* decreases antioxidant enzyme production in β cells

To determine if the opposing effects on oxidative stress observed between *Alox15*$^{-/-}$ and *Alox12*$^{-/-}$ mice could be due to differential production of antioxidant enzymes, we co-immunostained pancreatic sections from STZ-treated WT, *Alox15*$^{-/-}$ and *Alox12*$^{-/-}$ mice for insulin and the antioxidant enzymes glutathione peroxidase 1 (GPX1), and catalase (CAT). We observed significant increases in β-cell immunostaining intensities of both GPX1 and CAT in *Alox15*$^{-/-}$ mice compared to both WT and *Alox12*$^{-/-}$ mice (Figure 12A-D). Conversely, *Alox12*$^{-/-}$ mice showed a significant decrease in β-cell GPX1 immunostaining compared to WT and *Alox15*$^{-/-}$ mice (Figure 12A-D). These results suggest that loss of

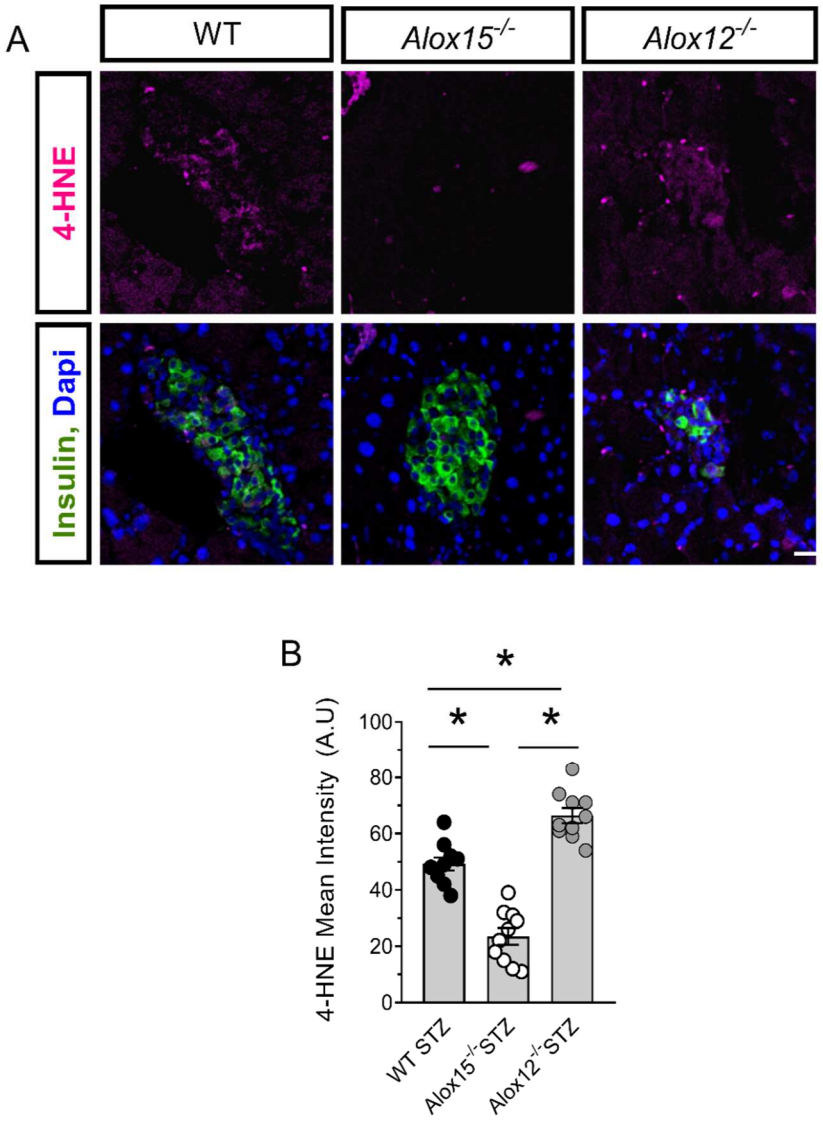

Figure 11. Deletion of Alox12 exacerbates inflammation-induced oxidative stress in β cells (A) Representative images of staining for Insulin (green), the oxidative stress marker, 4-hydroxynononeal (4-HNE) (pink) and nuclei (blue) in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ induced diabetes. (B) Quantification of 4-HNE mean intensity in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ induced diabetes. N ≥ 3 mice per experimental group for all experiments *p<0.05.

Alox15 and *Alox12* result in changes in antioxidant protein levels in β cells that may explain their observed effects on β-cell oxidative stress.

3.2.4 Alox12 deletion exacerbates reactive oxygen species accumulation in β cells

To determine if the increased oxidative stress was a result of elevated reactive oxygen species (ROS) generation in the islet, we employed the redox biosensor reduction-oxidation sensitive green fluorescent protein (roGFP) to measure ROS accumulation dynamically (290,291). Islets were transduced with adenovirus harboring roGFP then treated for 16 hours with a proinflammatory cytokine cocktail (IL1-β, TNF-α, IFN-γ) to approximate inflammation-induced oxidative stress. As shown in the representative ratiometric image in Figure 13A and quantified in Figure 13B, islets from WT mice exhibited an increased redox ratio after cytokine treatment, signifying that cytokines increased ROS. In support of our observations *in vivo*, cytokine-treated *Alox15*$^{-/-}$ islets exhibited significantly less ROS elevation in response to cytokines compared to WT islets. By contrast, cytokines elicited significantly increased ROS in *Alox12*$^{-/-}$ islets when compared to WT islets (Figure 13A-B). Collectively, these results suggest that deletion of *Alox15* protects against ROS accumulation, whereas deletion of *Alox12* promotes enhanced oxidative stress.

3.2.5 Alox12 deletion decreases circulating eicosanoid levels in mice

The products of LOXs are biologically active lipid metabolites, and loss of LOXs are expected to alter the levels or ratios of these metabolites that may account for the effects observed in our mice. To determine the changes in eicosanoid profile of *Alox12*$^{-/-}$ and *Alox15*$^{-/-}$ mice we performed liquid chromatography-tandem mass spectroscopic (LC-MS/MS) analysis of key eicosanoids in the circulation of mice. There was no statistically significant difference in circulating levels of 5-HETE, 15-HETE, 13-HODE, 17-HDHA, and LTB4 in *Alox12*$^{-/-}$ and *Alox15*$^{-/-}$ mice compared to WT mice (Table 1). Levels of 12-HEPE were significantly increased in *Alox15*$^{-/-}$ mice compared to controls. There were expected

reductions in 12-HETE, which reached statistical significance in *Alox12*$^{-/-}$ mice (P=0.02) and approached significance in *Alox15*$^{-/-}$ mice (P=0.06) compared to WT controls. These data confirm that our knockout mice exhibited reductions in the major 12-LOX and 12/15-LOX metabolite in the circulation.

3.2.6 Alox12 deletion increases STZ-induced oxidative damage through reciprocal upregulation of Alox15

We next asked how the loss of *Alox12* could render mice more sensitive to STZ-induced diabetes. Because *Alox15* and *Alox12* are functionally related, we analyzed the expression of each gene in both *Alox15*$^{-/-}$ and *Alox12*$^{-/-}$ islets and compared them to expression levels in WT controls. qPCR analysis of *Alox12* mRNA in WT, *Alox15*$^{-/-}$ and *Alox12*$^{-/-}$ islets show the expected decrease in *Alox12*$^{-/-}$ islets and no significant change in *Alox15*$^{-/-}$ islets (Figure 14A). Strikingly, *Alox12*$^{-/-}$ mice exhibited a 28-fold increase in *Alox15* mRNA expression compared to WT (Figure 14B), suggesting that loss of *Alox12* leads to a compensatory upregulation of *Alox15*, but not vice-versa. We hypothesized that the striking reductions in 12-HETE observed in *Alox12*$^{-/-}$ mice (Table 1) might cause a

Table 1. Alox12 deletion decreases circulating eicosanoid levels in mice

	Wild Type		**Alox15$^{-/-}$**		**Alox12$^{-/-}$**	
	Mean(ng/mL) ± SEM	pvalue vs. WT	Mean(ng/mL) ± SEM	pvalue vs. WT	Mean (ng/mL) ± SEM	pvalue vs. WT
5-HETE	2.88 ± 0.48	-	1.75 ± 0.99	0.270	1.31 ± 0.30	0.264
12-HETE	3433 ± 1143	-	1266 ± 234	0.063	1.01 ± 0.07	**0.023***
15-HETE	7.85 ± 2.53	-	3.55 ± 1.60	0.140	2.32 ± 0.81	0.137
12-HEPE	0.89 ± 0.04	-	60.2 ± 6.1	**0.001***	Not Detected	*
13-HODE	77.33 ± 24.93	-	35.43 ± 8.88	0.140	29.97 ± 5.24	0.140
17-HDHA	515.0 ± 237.6	-	118.2 ± 25.79	0.090	1.46 ± 0.06	0.080
LTB4	9.78 ± 6.50	-	4.032 ± 2.00	0.165	2.36 ± 2.73	0.145

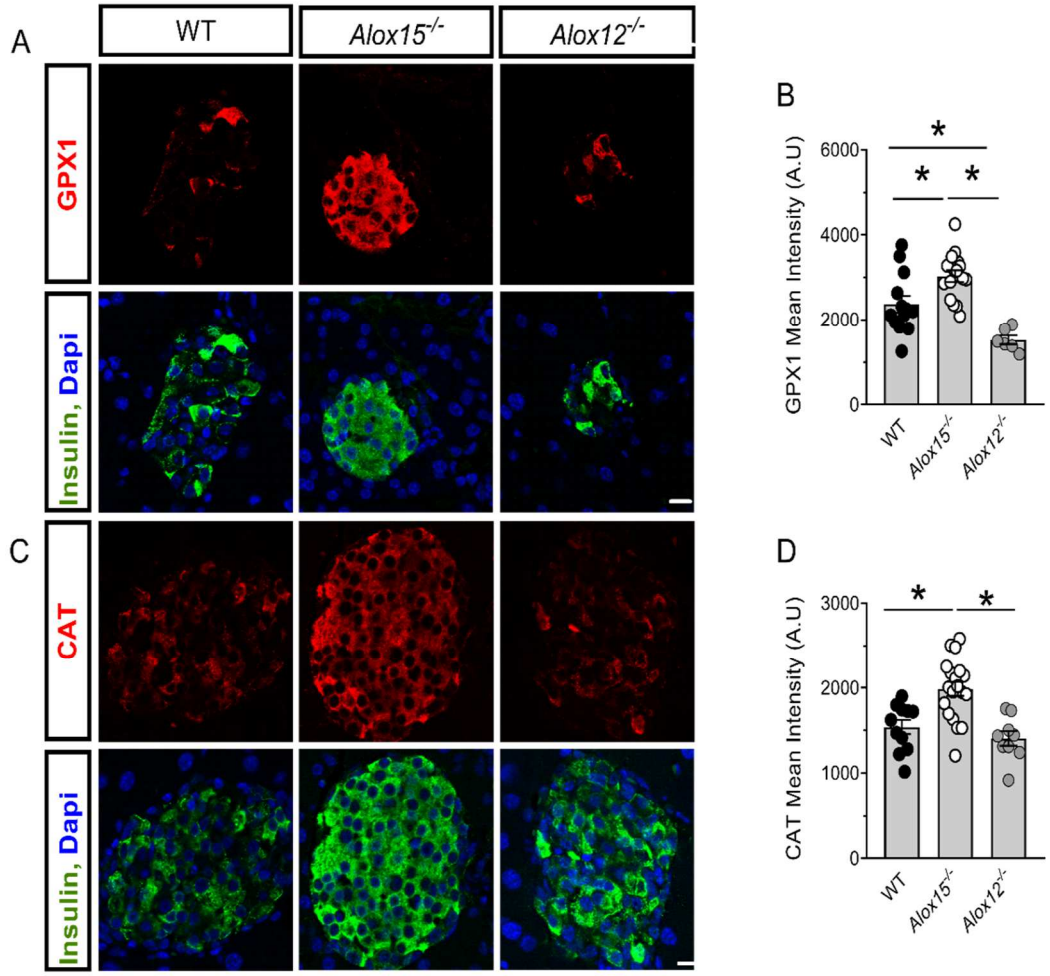

Figure 12. Deletion of Alox12 decreases antioxidant enzyme production in β cells (A) Representative images of staining for Insulin (green), the oxidative stress marker, Glutathione Peroxidase 1 (GPX-1) (red) and nuclei (blue) in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ induced diabetes. (B) Quantification of GPX-1 mean intensity in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ induced diabetes. (C) Representative images of staining for Insulin (green), the oxidative stress marker, Catalase (CAT)(red) and nuclei(blue) in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ induced diabetes. (D) Quantification of CAT mean intensity in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice following STZ induced diabetes. N ≥ 3 mice per experimental group for all experiments *p<0.05.

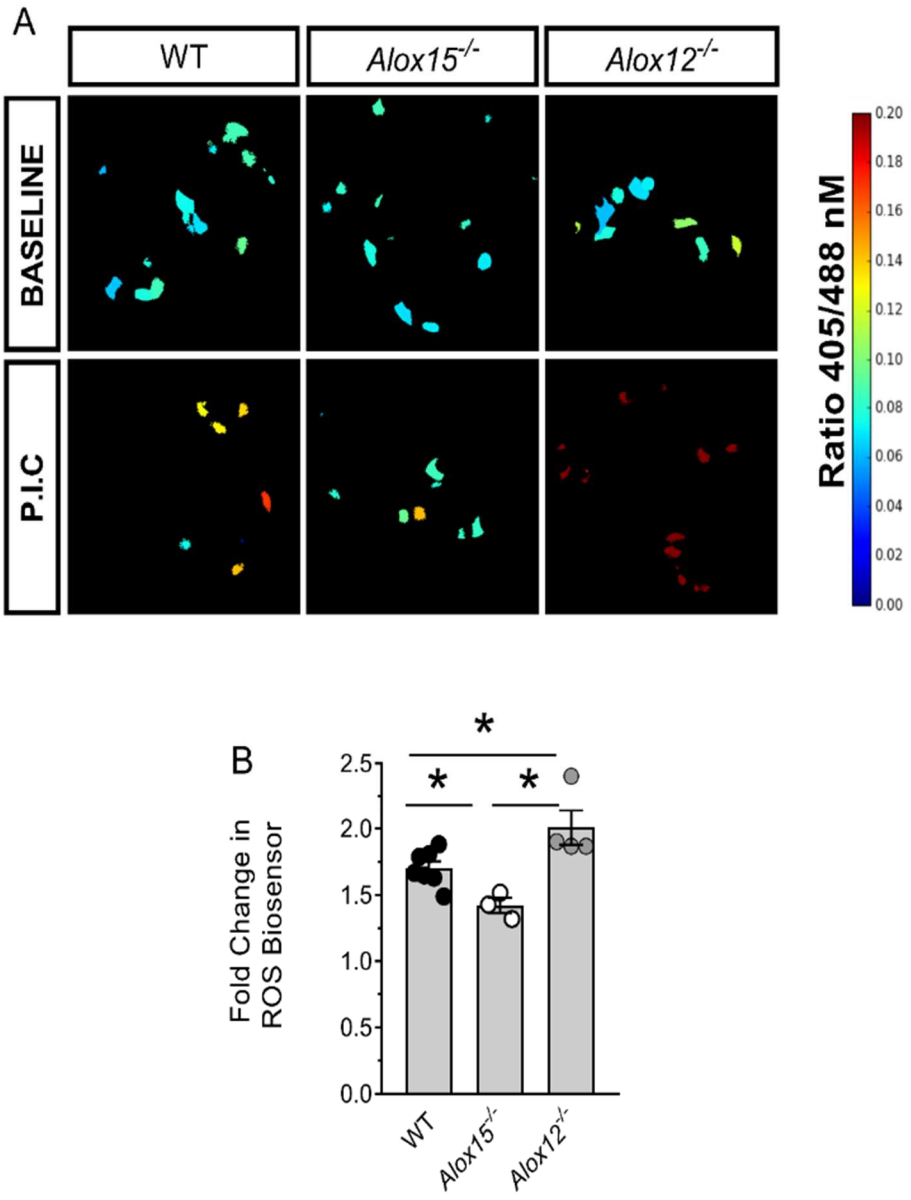

Figure 13. Alox12 deletion exacerbates reactive oxygen species accumulation in β cells (A) Ratiometric image of Islets from WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice expressing ROGFP2 ROS sensor at Baseline and following overnight treatment with a pro-inflammatory cocktail (P.I.C) (IL1β, TNFα, IFNγ). (B) Fold Change of 405/488 ratio of islets treated with P.I.C. N ≥ 3 mice per experimental group for all experiments *p<0.05.

compensatory increase in *Alox15* in this setting implying that 12-HETE levels might inversely regulate *Alox15*. To test this hypothesis, we incubated Min6 β cells overnight with 100 nM 12-HETE and performed gene expression analysis by qPCR. Treatment with 12-HETE caused a significant reduction in *Alox15* expression compared to control (Figure 14C), a result that supports our hypothesis. We also sought to determine the effect of 12-HETE on the expression of antioxidant genes. Incubation of Min6 cells with 12-HETE caused a significant decrease in Gpx1 expression (Figure 14D). The expression of Catalase was increased following 12-HETE incubation but was not statistically significant (Figure 14E).

3.2.7 Inhibition of 12/15-LOX with ML351 prevents STZ induced β cell dysfunction in Alox12$^{-/-}$ mice

To determine if the observed increase in *Alox15* expression contributes to the increased sensitivity of *Alox12*$^{-/-}$ mice to STZ mediated β cell dysfunction, we employed the 12/15-LOX-specific small molecule inhibitor ML351 (215). *Alox12*$^{-/-}$ mice treated with ML351 were protected from STZ-induced glucose intolerance compared to vehicle-treated *Alox12*$^{-/-}$ mice (Figure 15A-B). These results suggest that 12/15-LOX activity in *Alox12*$^{-/-}$ mice augment the sensitivity of these mice to STZ-induced dysfunction.

3.2.8 Alox12 mRNA expression is decreased while ALOX15 mRNA is increased in humans with T2D

Our results so far have focused on the role of 12-LOX vs. 12/15 LOX in models of T1D. 12/15-LOX activity has also been demonstrated to be involved in the pathogenesis of T2D (206,208,209). Therefore, our next objective was to determine the role of 12-LOX vs. 12/15-LOX in T2D. We have observed reciprocal regulation of Alox12 and Alox15 in genetic knockout mice. Hence, we hypothesized that in humans we will observe similar regulation of ALOX12 and ALOX15. Therefore, we analyzed the expression of *ALOX12* and *ALOX15* in islets from normoglycemic and T2D human donors. qPCR analysis showed a significant decrease of ALOX12 mRNA levels in T2D islets compared to controls

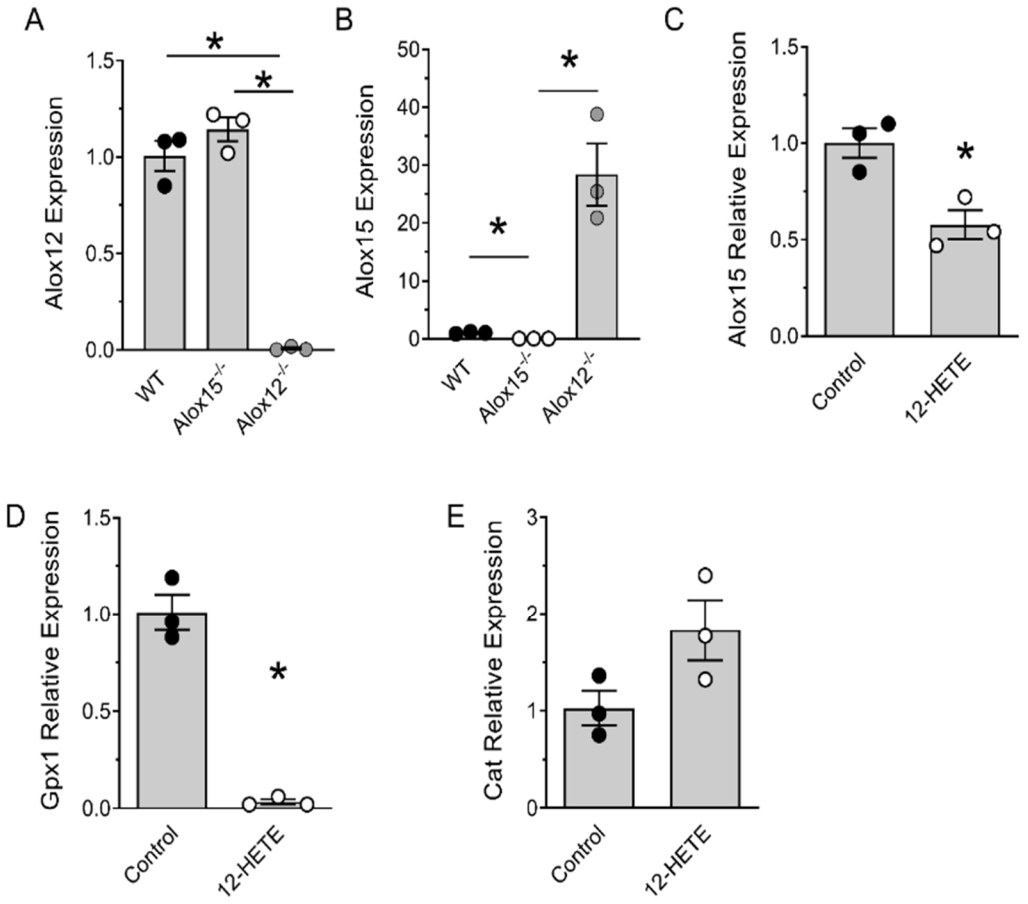

Figure 14. *Alox12* deletion increases STZ-induced oxidative damage through reciprocal upregulation of *Alox15* (A) Alox12 gene expression in islets from WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. (B) Alox15 gene expression in islets from WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. (C) Alox15 gene expression in Min6 cells following overnight incubation with vehicle or 12-HETE. (D) Gpx1 gene expression in Min6 cells following overnight incubation with vehicle or 12-HETE. (E) Catalase gene expression in Min6 cells following overnight incubation with vehicle or 12-HETE. N ≥ 3 mice per experimental group for all experiments *p<0.05.

(Figure 16A). Interestingly, we observed a significant increase in ALOX15 mRNA expression in T2D individuals compared to normoglycemic controls (Figure 16B). This pattern of expression of Alox12 and ALOX15 in T2D humans further supports the results observed in Alox12 knockout mice.

3.2.9 Changes in eicosanoid profile of individuals with T2D

Based on these results we explored the possibility of altered eicosanoid expression profile in the setting of T2D due to a decrease in ALOX12 and reciprocal increase in ALOX15. We performed Lc-MS/MS to evaluate the levels of serum eicosanoid in normoglycemic and T2D humans. Circulating 12-HETE levels were significantly decreased in T2D individuals compared to normoglycemic controls (Figure 17A). The levels of 15-HETE were similar in both conditions (Figure 17B). Furthermore, we observed a non-significant decrease in 12-HEPE levels in T2D individuals compared to controls (Figure 17C). Collectively these results show the possibility of a protective role of 12-LOX in the setting of T1D and possibly in T2D. Further studies are required in order to firmly establish the role of 12-LOX in T2D setting.

3.2.10 Loss of *Alox15* protects while loss of Alox12 exacerbates HFD induced metabolic disorder

Preliminary studies exploring the effect of loss of Alox15 versus loss of Alox12 in HFD mouse models of T2D show similar results as observed in T1D models. Wild Type, Alox15$^{-/-}$, and Alox12$^{-/-}$ mice were placed on high fat diet for 5 weeks. Initial results show body weight of Alox12$^{-/-}$ mice were higher compared to WT and Alox15$^{-/-}$ mice (Figure 18A). Additionally, fasted blood glucose was higher in Alox12$^{-/-}$ mice compared to WT or Alox15$^{-/-}$ (Figure 18B). Furthermore, we performed insulin tolerance test to assess peripheral insulin resistance in WT, Alox15$^{-/-}$ and Alox12$^{-/-}$ mice. Alox15$^{-/-}$ mice exhibited improved insulin tolerance compared to WT mice (Figure 18C-D). Alox12$^{-/-}$ showed marked insulin desensitization compared to WT mice (Figure 18C-D). Based on these preliminary data we observed similar protective effect of 12-LOX in HFD model of T2D.

3.3 Discussion

Lipoxygenases (LOXs) form a family of enzymes that catalyze the oxygenation of cellular polyunsaturated fatty acids to form lipid inflammatory mediators (282,283). LOXs have been shown to contribute to the pathogenesis of T1D and T2D. *Alox12* encodes for 12-LOX (also commonly called "platelet-type" 12-LOX), which catalyzes the oxygenation of the 12th carbon of its substrates, while *Alox15* encodes for 12/15-LOX (also known as "leukocyte-type" 12-LOX) that oxygenates substrates at either the 12th or 15th carbon (187). 12-LOX-catalyzed oxygenation of arachidonic acid leads to the formation of the proinflammatory lipid 12-HETE, and 12/15-LOX forms both 12-HETE and 15-HETE (in a 6:1 ratio) (292). In the setting of diabetes, the loss of 12/15-LOX has been shown to be protective in both STZ and non-obese diabetic (NOD) mouse models of T1D, effects that have been ascribed to reductions in the levels of 12-HETE (204,205,207,292). The proinflammatory actions of 12-HETE may be due to its properties as a lipid peroxide and via its interactions with its receptor Gpr31 (196,197). Although 12-HETE is also produced by 12-LOX, a role for the *Alox12* gene in β-cell oxidative stress during diabetes pathogenesis has not been interrogated. In this study, we interrogated the roles of 12/15-LOX and 12-LOX in the development of oxidative stress in pancreatic islets by studying their respective gene knockouts (*Alox15* and *Alox12*) in the context of T1D models *in vivo* and *in vitro* and T2D. Whereas our data confirms a pro-diabetogenic role for *Alox15*, we found a protective effect for *Alox12*. Our data demonstrate that loss of *Alox12* leads to compensatory hyperactivity of 12/15-LOX encoded by *Alox15* (Figure 19).

We observed that knockout of either *Alox12* or *Alox15* did not appear to affect β-cell mass or glucose homeostasis, findings that are in concordance with prior studies that showed no gross phenotype in unchallenged animals(204,206). However, a striking and discordant phenotype between the two knockouts was observed when islets of these animals were subjected to proinflammatory stress using the multiple low-dose STZ. STZ

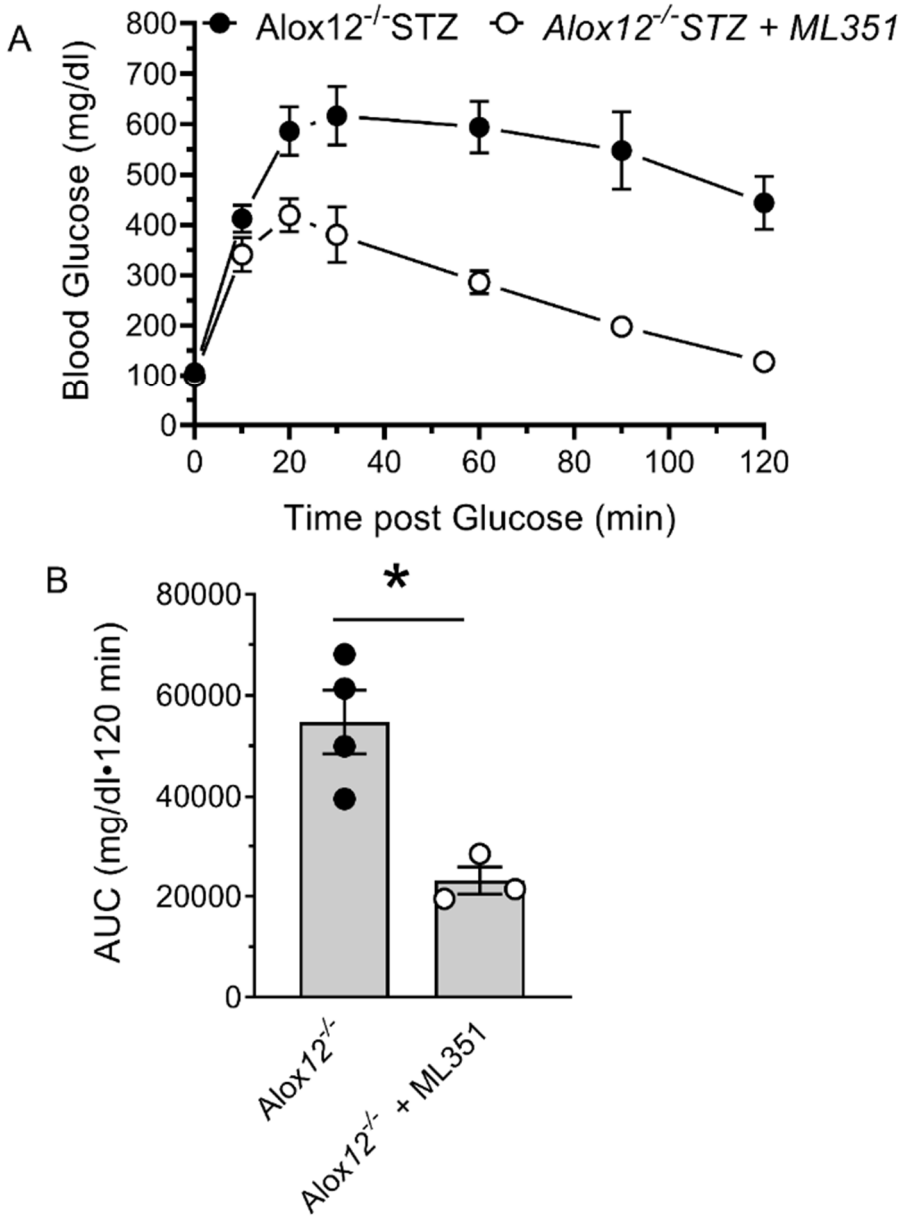

Figure 15. Inhibition of 12/15-LOX with ML351 prevents STZ induced β cell dysfunction in Alox12$^{-/-}$ mice (A) Analysis of glucose tolerance (GTT) in vehicle or ML351 treated Alox12$^{-/-}$ mice 4 days following STZ regime. (B) Area under the curve analysis of GTT. N ≥ 3 mice per experimental group for all experiments *p<0.05.

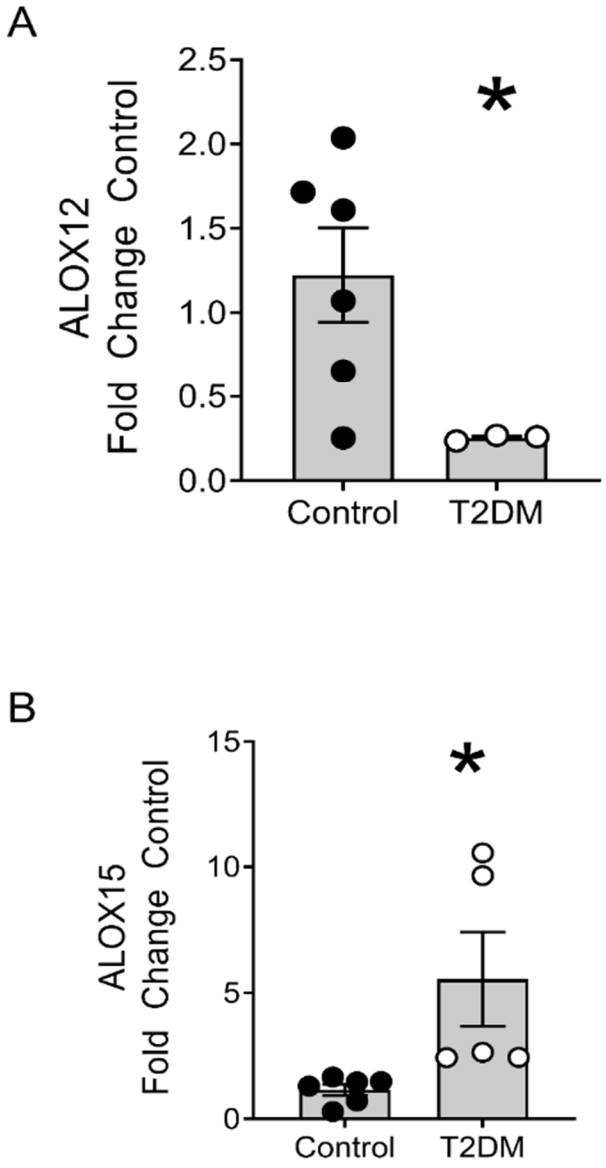

Figure 16. ALOX12 mRNA expression is decreased while ALOX15 mRNA is increased in T2D (A) mRNA expression of *ALOX12* in islets of normoglycemic controls and individuals with T2D. (B) *ALOX15* mRNA expression in islets of normoglycemic controls and individuals with T2D. N ≥ 3 individuals per group for all experiments *p<0.05.

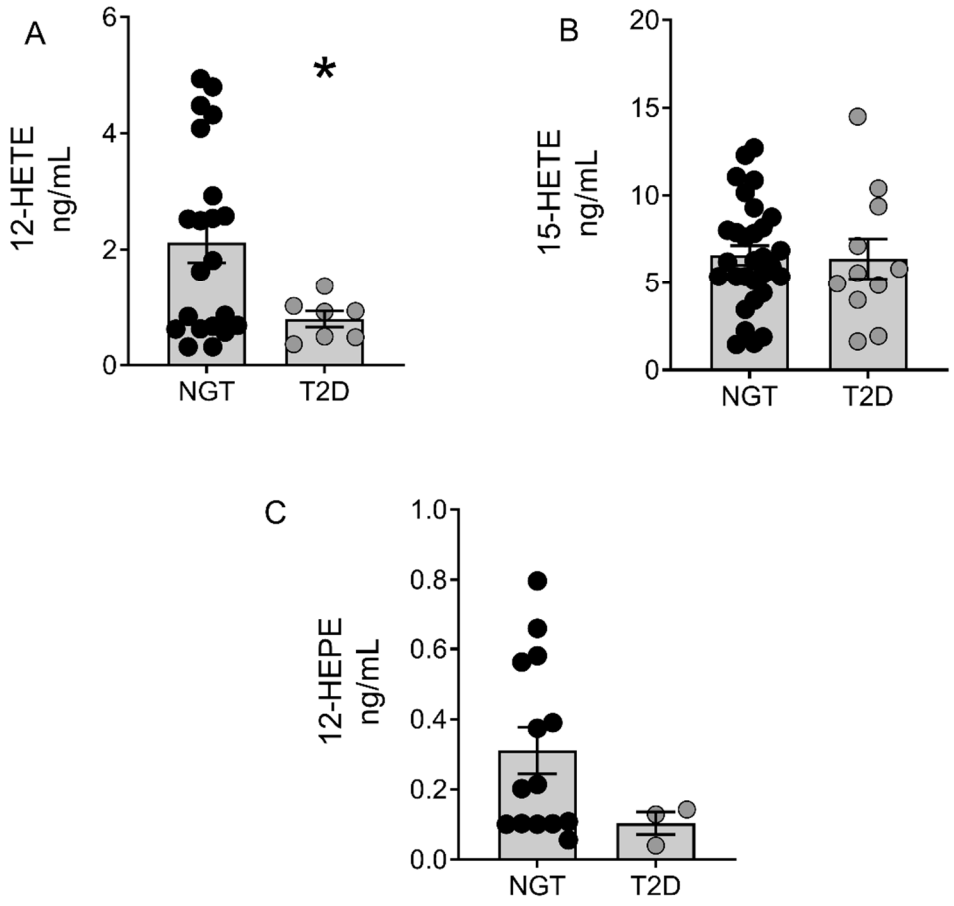

Figure 177. Changes in eicosanoid profile of individuals with T2D (A) Circulating 12-HETE levels in individuals with normoglycemia (NGT), and T2D individuals. (B) Circulating 15-HETE levels in individuals with normoglycemia (NGT), or T2D. (C) Circulating 12-HEPE levels in individuals with normoglycemia (NGT), or T2D. N ≥ 3 individuals per group. *p<0.05.

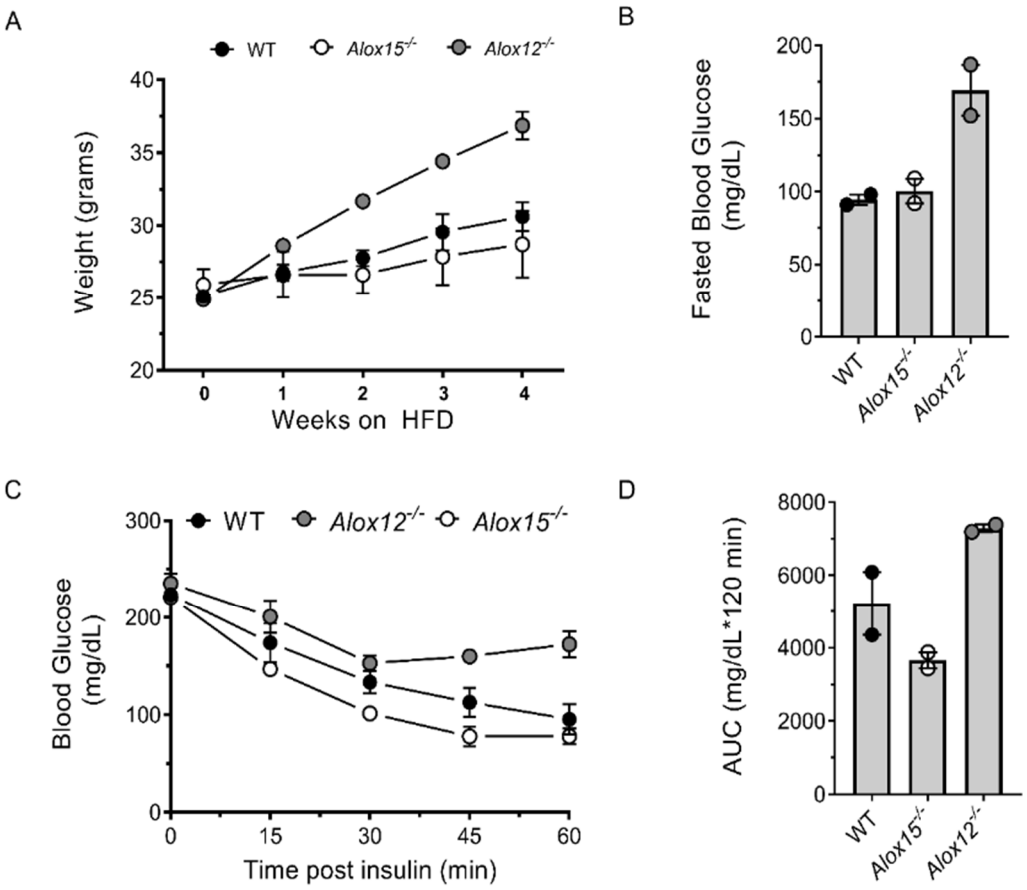

Figure 188. Loss of Alox15 protects while loss of Alox12 exacerbates HFD induced metabolic disorder (A) Weekly weight measurements in HFD-fed mice. (B) Fasted blood glucose levels in WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice levels after 4 weeks of HFD. (C) Insulin tolerance test (ITT) follow 4 weeks of HFD. (D) AUC of ITT show increase insulin desensitization in Alox12$^{-/-}$ but not Alox15$^{-/-}$ mice following 4 weeks of HFD. N = 2 mice per experimental group for all experiments

is selectively taken up by β cells through the Glut2 transporter and leads to DNA alkylation and eventual cell death (293). Low doses of STZ trigger low frequency of β-cell killing, which is exacerbated by macrophage influx, proinflammatory cytokine release, and oxidative stress (294). Under these conditions, prior studies have shown that *Alox15*$^{-/-}$ mice are protected from β-cell destruction and hyperglycemia (204). Our results with *Alox15*$^{-/-}$ mice confirm these prior studies but add the vital observation that protection from β-cell dysfunction may be caused by reductions in oxidative stress, as judged by reduced immunostaining for 4-HNE accompanied by enhanced immunostaining for antioxidant enzymes GPX1 and CAT. By contrast, we show that *Alox12*$^{-/-}$ mice exhibit exacerbated hyperglycemia compared to WT controls, with enhanced immunostaining for 4-HNE in β cells, accompanied by reduced immunostaining for GPX1 and CAT. Our studies provide the first documented evidence that the enzymes encoded by *Alox15* and *Alox12* display apparently opposing roles in β-cell oxidative stress, notwithstanding that both enzymes produce identical major products (12-HETE).

At least two possibilities might be invoked to account for the disparate phenotypes of *Alox15*$^{-/-}$ mice and *Alox12*$^{-/-}$ mice. First, it is possible that the products that each enzyme produces and/or their relative ratios can affect the net production of ROS. In this regard, while arachidonic acid and its metabolites are important in cytokine induced β-cell dysfunction (295) it is not the only substrate for these enzymes, and the preferential utilization of other key substrates, such as dihomo-γ-linolenic acid or eicosapentaenoic acid (EPA), form products that may be protective (189,296). Indeed, we observed an increase in circulating levels of the EPA derived metabolite 12-HEPE in *Alox15*$^{-/-}$ mice that was undetected in the *Alox12*$^{-/-}$ mice. This finding raises the possibility that loss of *Alox15* leads to a preferential use of EPA over arachidonic acid as a product. This switch to EPA metabolism has been shown to be protective of β cell function (297). Second, it is possible that the catalytic similarities between the two enzymes might allow one enzyme to

compensate to produce the products of the other. We show here that whereas no upregulation of *Alox12* was observed in *Alox15*$^{-/-}$ mice, there was a nearly 30-fold increase in *Alox15* levels in *Alox12*$^{-/-}$ mice. The near-reversal of the diabetogenic phenotype of *Alox12*$^{-/-}$ mice through the concurrent pharmacologic inhibition of 12/15-LOX (with ML351) supports that notion that 12/15-LOX activity contributes to the phenotype of these animals. We recognize, however, that we cannot rule out a contribution resulting from the loss of potentially protective lipid products emanating from 12-LOX activity in *Alox12*$^{-/-}$ mice. Based on the lipidomics analysis we observed a decrease in 12-HETE in the *Alox12*$^{-/-}$ mice. This suggests that the observed detrimental effects observed due to loss of *Alox12*$^{-/-}$ is not caused by an increase in 12-HETE levels. Additionally, we show that 12-HETE inhibits the expression of *Alox15* in Min6 cells. Taken together, the observed decrease in 12-HETE in the *Alox12*$^{-/-}$ mice could then lead to the observed upregulation of *Alox15*.

In conclusion, our data suggest that *Alox15* and *Alox12* play disparate roles in the pathogenesis of β-cell oxidative stress and dysfunction. The observation that *Alox15* levels are elevated in *Alox12*$^{-/-}$ islets also highlights a deficiency in our understanding of the regulation of the genes encoding LOX enzymes. As inhibitors of LOX enzymes begin to gain traction for disease modification (211,215), it will be especially relevant to understand how inhibition of specific enzymes might influence the expression and activities of other related LOXs. Future studies unraveling the effect of complete loss versus inhibition of a lipoxygenase enzyme and possible compensatory effects are thus warranted.

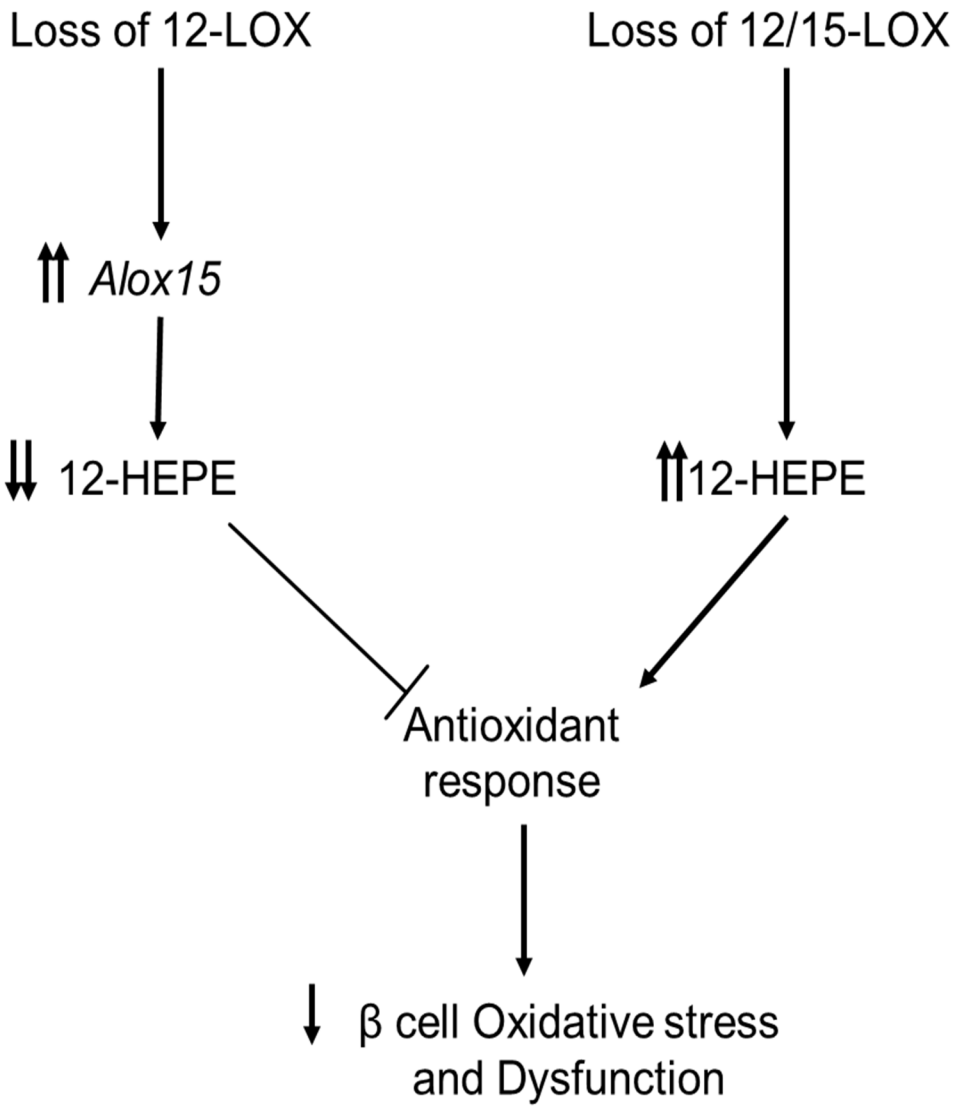

Figure 19. Proposed model of 12-LOX vs. 12/15-LOX. Deletion of 12/15-LOX(Alox15) leads to a decrease in 12-HETE levels, an increase in 12-HEPE, augmentation of antioxidant response in β cells during oxidative stress, leading to increase survival. Deletion of 12-LOX(Alox12) on the other hand causes a compensatory increase in 12/15-LOX(Alox15) expression, loss of 12-HEPE production, inhibition of antioxidant response leading to β cell dysfunction.

4 Direct Beta-cell Signaling by Interleukin-6 Couples Autophagy with Antioxidant Response via NRF2 to Reduce Oxidative Stress

4.1 Introduction

All forms of diabetes result from either dysfunction and/or death of islet β-cells, and increasing evidence has implicated the generation of reactive oxygen species (ROS) in this process (298). The typical response to increased production of ROS includes activation of the master transcriptional regulator Nrf2, which in turn activates a host of antioxidant genes and thus plays a critical role in the protection against ROS-mediated injury (299). An inadequate response to ROS leads to uncontrolled oxidative stress and subsequent cell death. Thus, a maladaptive response to ROS likely plays a significant role in diabetes pathogenesis (300), and the activation of antioxidant pathways in the face of increased ROS may be key to β-cell survival.

Multiple links between the antioxidant response and autophagy have been demonstrated in recent years (301). Importantly, there is evidence that stimulation of the autophagy machinery leads to activation of Nrf2 (302) whereas β-cell specific loss of Nrf2 leads to increased sensitivity to oxidative stress (303). Autophagy has been shown to promote survival as an adaptive response to cellular stress (304), and plays a critical role in antioxidant response in degenerative diseases (305). In addition, autophagy is crucial to β-cell function and homeostasis (306).Together, these observations suggest that activation of islet autophagy may bolster the antioxidant response leading to reduced oxidative stress and thus reduced apoptosis.

One potential endocrine target in islet ROS response is the pleiotropic cytokine interleukin 6 (IL-6), which we have recently found to stimulate β-cell autophagy both *in vitro* and *in vivo* and to protect β-cells from apoptosis (307). Interestingly, the IL-6 promoter contains an antioxidant response element (ARE) and its expression can be stimulated by Nrf2 (308), implying regulatory links between the signaling pathways. The link between IL-6-mediated stimulation of β-cell autophagy and ROS mitigation has not been tested. Here,

we show that IL-6 rapidly activates a cascade of events that leads to reduction of proinflammatory cytokine-induced ROS generation in both cultured β-cells and human islets. Loss of function studies *in vivo* further demonstrate that β-cell IL-6 signaling plays a key role in the response to ROS generated by the toxic glucose analogs STZ and alloxan. Collectively, we show that acute upregulation of signaling events by IL-6 in the β-cell orchestrates activation of autophagy and antioxidant response to reduce ROS and promote survival under diabetogenic conditions.

4.2 Results

4.2.1 IL-6 reduces ROS through direct action on the β-cell

We previously found that IL-6 was able to protect INS-1 β-cells from proinflammatory cytokine induced apoptosis. Since proinflammatory cytokines stimulate islet ROS buildup (309), we wanted to determine if IL-6 played a role in preventing or reducing the ROS generated under these conditions. We find that in both INS-1 β-cells (Figure 20A) and whole human islets (Figure 20B), treatment with IL-6 reduces the ROS buildup generated by proinflammatory cytokines.

4.2.2 Loss of the β-cell IL-6 receptor renders mice more susceptible to Streptozotocin-induced oxidative damage and development of diabetes

To determine if IL-6 driven ROS reduction is mediated via the β-cell IL-6 receptor, we generated pancreatic β-cell-specific IL-6 receptor knockout (IL6R$\alpha^{\Delta\beta}$) mice. Flow-sorted β-cells from islets isolated from these mice had significantly reduced expression of the *Il6ra* gene (>9-fold reduction), whereas expression of *Gp130*, the common component of the IL6 receptor family, was unaffected (Figure 21A). Intact isolated islets also had reduced STAT3 activation in response to exogenous IL-6 treatment (Figure 21B). IL6R$\alpha^{\Delta\beta}$ mice exhibited weight gain and glucose tolerance that were indistinguishable from control littermates (Figure 21C-D).

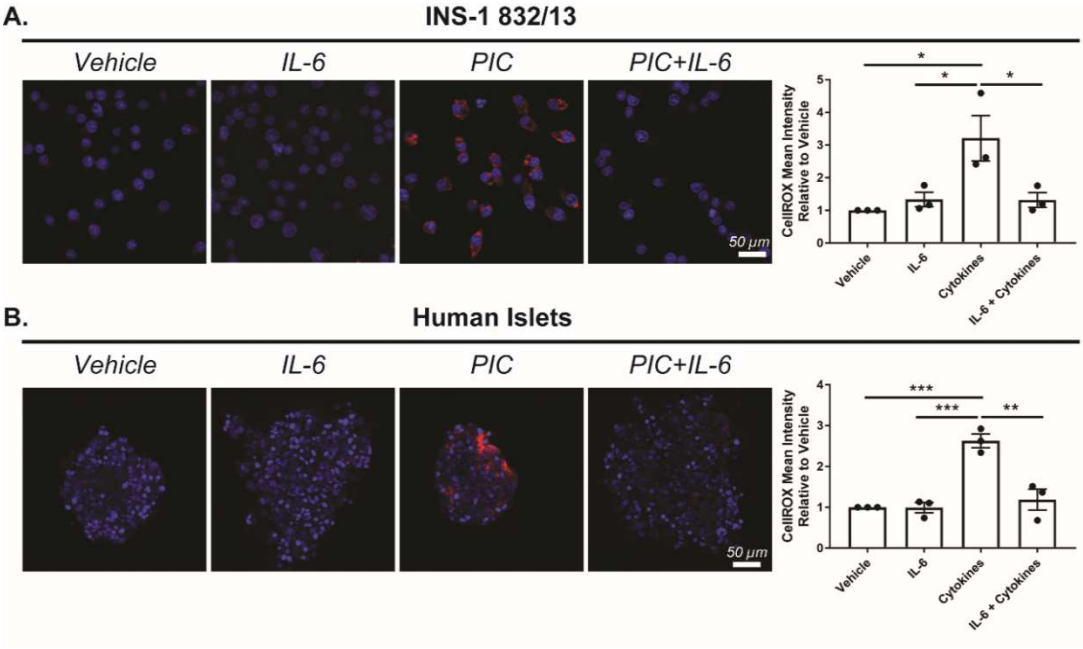

Figure 20. IL-6 reduces the ROS generated by proinflammatory cytokines INS-1 cells (A) and human islets (B) were treated with vehicle, 200 ng/mL recombinant mouse IL-6, a proinflammatory cytokine cocktail (PIC) consisting of 100 ng/mL IFN-γ, 50 ng/mL TNFα, and 10 ng/mL IL1-β, or the combination of PIC and IL-6 for 24 hours then reactive oxygen species (ROS) accumulation was measured using the fluorescent dye CellROX Deep Red. Nuclei are stained with DAPI in blue. N=3. *p<0.05, **p<0.01, ***p<0.001.

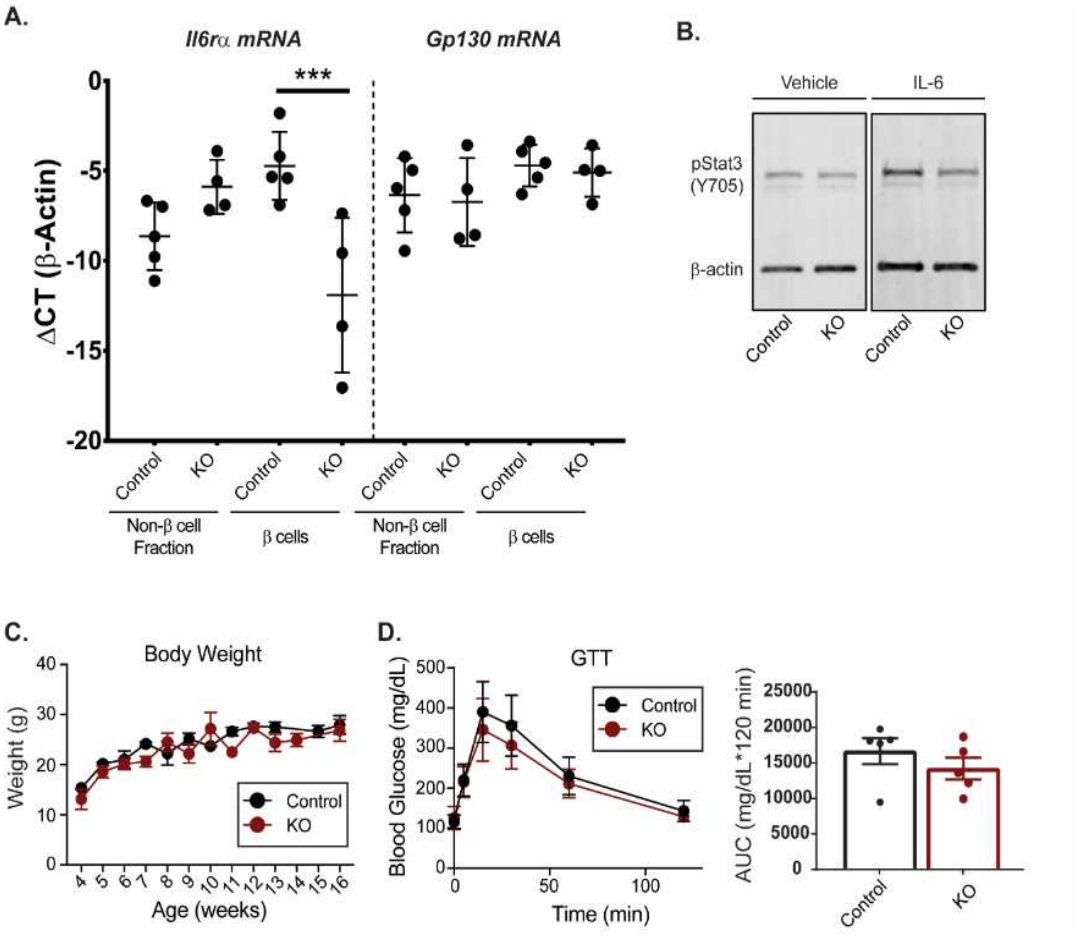

Figure 21. Characterization of β-cell specific IL-6Rα knockout mice (A) *Il6rα* and *gp130* expression in flow sorted β-cells from knockout mice and littermate controls were measured in the sorted β-cells and the non-β-cell fraction (i.e., the cells that were not sorted by high Newport Green positivity). (B) Representative western blot showing STAT3 phosphorylation at Tyrosine 705 in IL-6Rα KO and littermate control islets treated with vehicle or IL-6. (C) Bodyweight measurements of IL-6Rα KO mice and control littermates between 4 and 16 weeks of age. (D) Glucose tolerance tests of IL-6Rα KO mice and littermate controls at 12 weeks of age (left panel) with area under the curve (AUC, right panel). N≥3. ***p<0.001

To test if islets from IL6Rα$^{\Delta\beta}$ mice are more susceptible *in vivo* to ROS-induced β-cell death, we used a multiple low-dose Streptozotocin (STZ)-induced diabetes model (Figure 22A), which generates β-cell ROS and DNA damage leading to apoptosis (310–312). To determine if loss of IL-6 signaling makes β-cells more susceptible to oxidative stress, we analyzed a cohort of STZ-injected animals prior to extensive β-cell death and development of overt diabetes. Blood glucose and glucose tolerance were monitored over time in a second cohort of mice. IL6Rα$^{\Delta\beta}$ mice developed hyperglycemia more rapidly in response to STZ vs. control mice (Figure 22B), and there was a trend toward earlier development of diabetes with no change in weight (Figure 22A-B). STZ-treated knockout mice were, however, more glucose intolerant at both day 15 and day 30 (Figure 22C), but not at day 8, prior to the development of hyperglycemia (Figure 22C). STZ treatment resulted in significantly decreased β-cell mass in both wild type and knockout mice relative to saline injected mice (Figure 22D). However, consistent with our observation of the development of exacerbated glucose intolerance, IL6Rα$^{\Delta\beta}$ mice had a significantly greater loss of β-cell mass when measured early in response to STZ treatment, suggesting that IL-6 signaling in the β-cell protects against STZ-induced apoptosis (Figure 22D). At this early timepoint, STZ-treated knockout mice displayed evidence of greater oxidative stress in the islet as shown by increased 4-hydroxynononeal (4-HNE) staining relative to STZ-treated wild type mice (Figure 22E).

4.2.3 Loss of the β-cell IL-6 receptor renders mice more susceptible to Alloxan-induced oxidative damage

To further assess if IL6Rα$^{\Delta\beta}$ mice are more susceptible to ROS buildup, we treated wild-type and knockout mice with alloxan, which produces ROS as a direct metabolite (311,312). To assess early responses to alloxan treatment, tissues from some mice were analyzed 6 hours post injection, at the peak of ROS production, prior to widespread β-cell death (311), while a second cohort was followed for 8 days to assess the development of

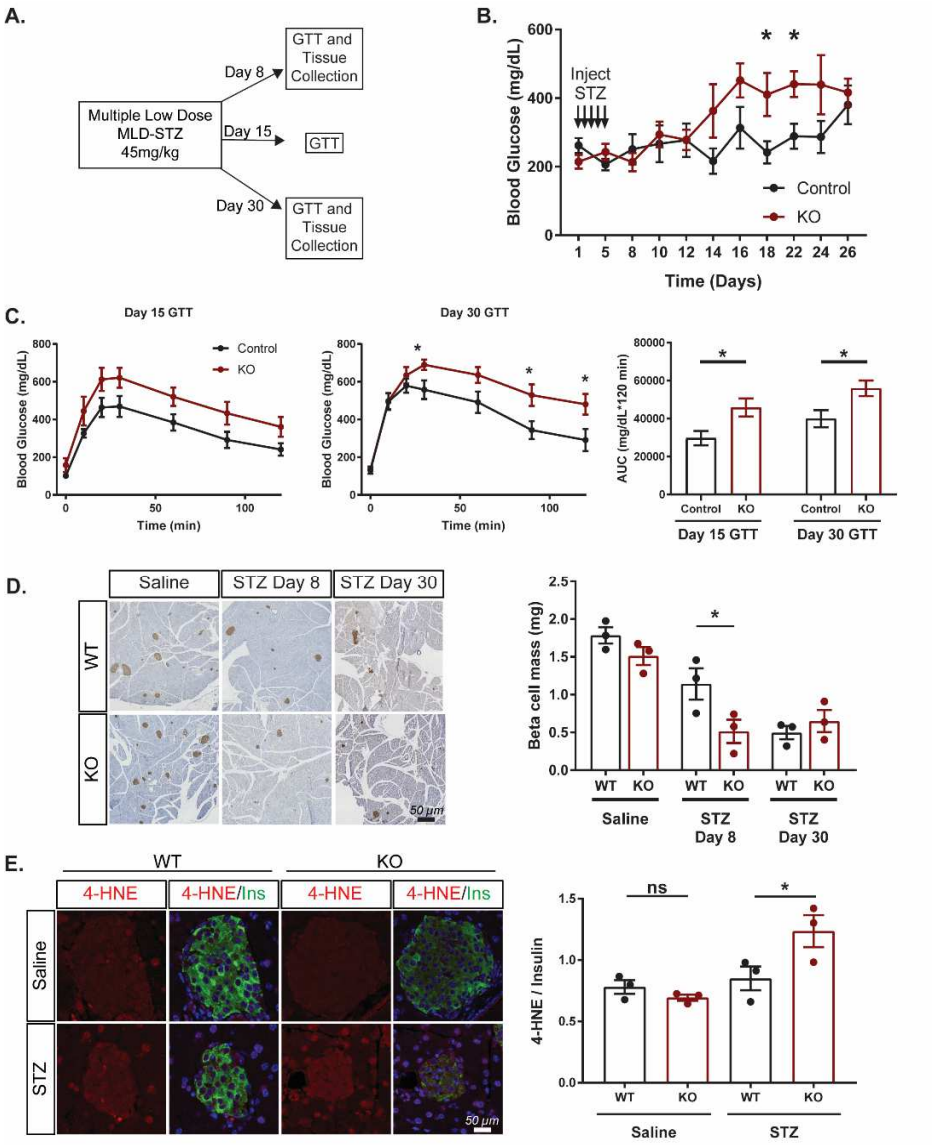

Figure 22. IL-6Rα$^{\Delta\beta}$ mice are more susceptible to STZ-induced β-cell oxidative stress and apoptosis (A) Multiple low-dose STZ treatment experimental design. (B) Random-fed blood glucose levels were monitored every other day for 26 days after the first STZ injection. (C) Glucose tolerance tests performed on day 15 and 30 with AUC. (D) Representative images of pancreata, collected either at STZ day 8 or STZ day 30, and quantification of β-cell mass. (E) Representative images of pancreata from STZ day 8 immunostained for insulin (green) and 4HNE (red), with DAPI stained nuclei in blue, and quantification of 4HNE. N≥3 mice per experimental group. *p<0.05

diabetes (Figure 23A). We did not observe significant differences in the random-fed blood glucose levels of IL6R$\alpha^{\Delta\beta}$ mice relative to controls (Figure 23B). However, consistent with the previous observation that alloxan induces a characteristic increase in circulating insulin due to β-cell degranulation and cell membrane rupture (311), alloxan injected IL6R$\alpha^{\Delta\beta}$ mice had significantly increased circulating insulin 6 hours post-injection (Figure 23C). Similarly, while we did not observe differences in β-cell mass at the early or later timepoint (Figure 23D), 4-HNE levels were significantly increased in alloxan injected IL6R$\alpha^{\Delta\beta}$ mice (Figure 23E), indicating early increases in oxidative damage to the islet. Together, these data suggest that loss of β-cell IL-6 receptor signaling renders the islet more susceptible to *in vivo* alloxan-induced oxidative damage.

4.2.4 IL-6 stimulates the master antioxidant response factor NRF2 to reduce ROS

Antioxidant response is primarily controlled through the rapid action of the transcription factor NRF2, which activates a network of immediate-early genes involved in ROS mitigation (313). We find that IL-6 stimulates a rapid accumulation of NRF2 protein within 5 minutes of treatment in both INS-1 β-cells (Figure 24A) and human islets (Figure 24B). This accumulation of NRF2 is followed by a reduction in levels of the NRF2 inhibitor, KEAP1, within 15 minutes of IL-6 treatment of INS1 cells (Figure 24A).

In the cytoplasm, KEAP1 binds to NRF2 and targets it for degradation by the proteasome. Disruption of the interaction between KEAP1 and NRF2 leads to the accumulation and activation of NRF2. To determine if IL-6 disrupts the interaction between KEAP1 and NRF2, we immunoprecipitated NRF2 in the presence and absence of exogenous IL-6. IL-6 treatment increased the amount of NRF2 that was immunoprecipitated, while decreasing the amount of KEAP1 that was coimmunoprecipitated with NRF2 by approximately 4-fold (Figure 24C).

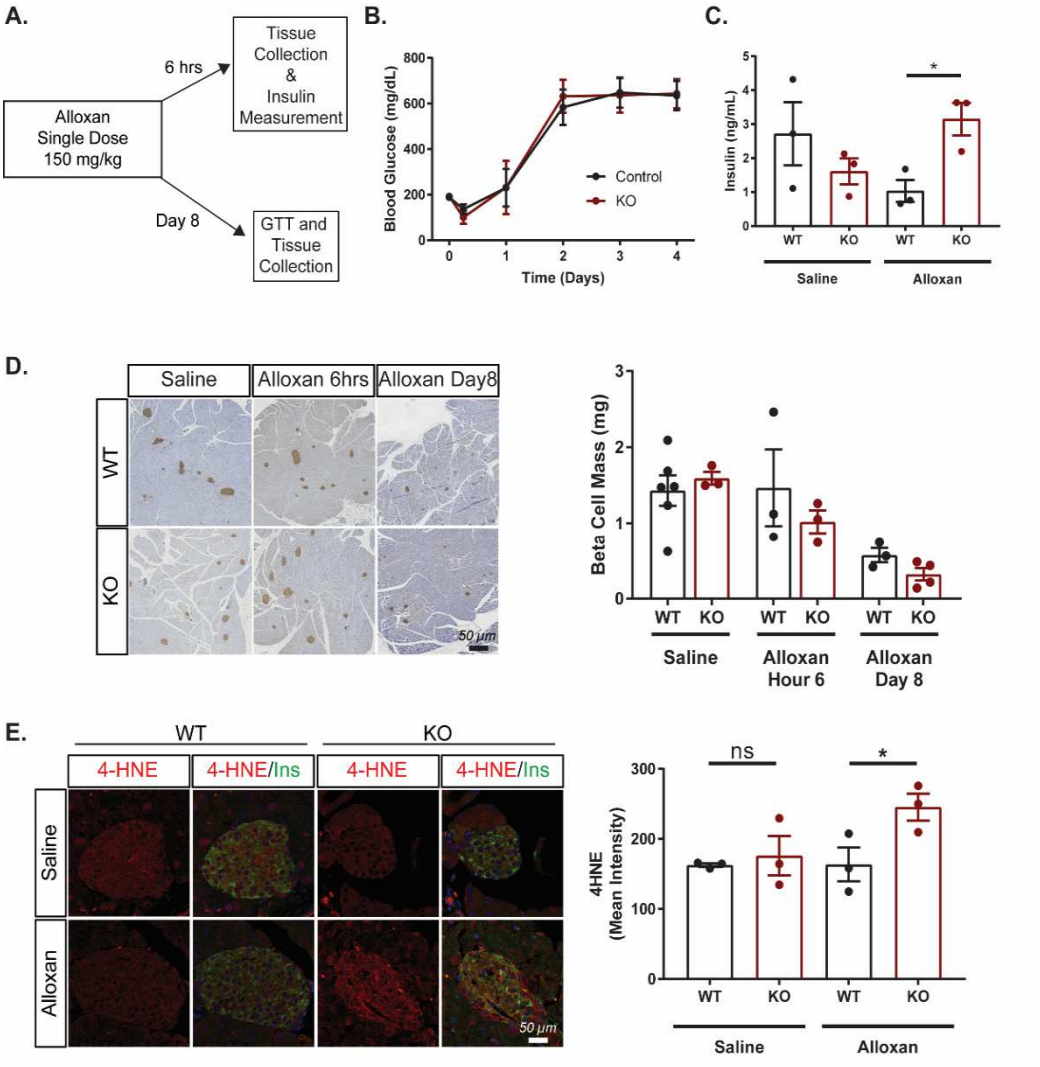

Figure 23. IL-6Rα$^{\Delta\beta}$ mice are more susceptible to Alloxan-induced β-cell oxidative stress (A) Alloxan experimental design. (B) Random-fed blood glucose levels were measured daily for 5 days post alloxan injection. (C) Terminal serum insulin levels from samples collected 6 hours after injection. (D) Representative images of pancreata, collected either at alloxan 6hrs or alloxan day 8, from which β-cell mass was calculated showing insulin staining in brown, and quantification of β-cell mass. (E) Representative images of pancreata, collected 6 hours after injection, immunostained for insulin (green) and 4HNE (red), with DAPI stained nuclei in blue, and quantification of islet 4HNE. N≥3 mice per experimental group for all.

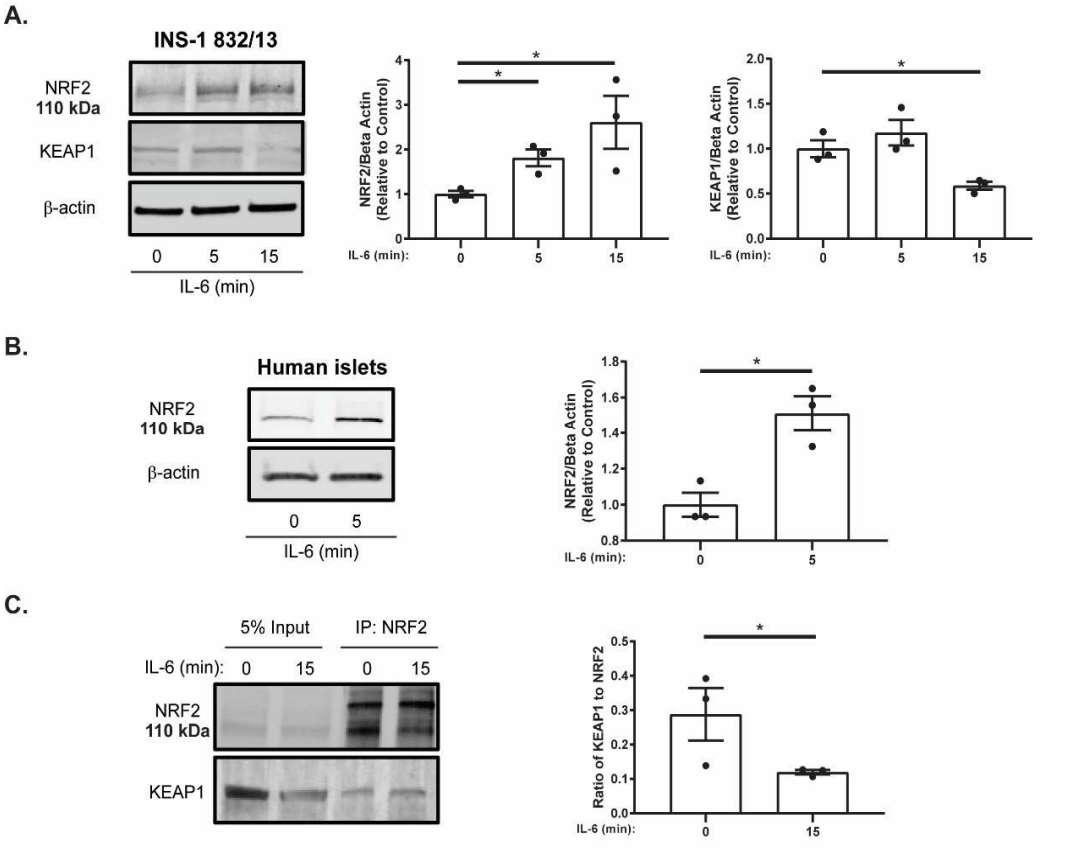

Figure 24. IL-6 rapidly stimulates NRF2 (A-B) Representative western blots and quantification of NRF2 and KEAP1 levels in response to acute treatment with IL-6 in INS-1 β-cells (A) and human islets (B). (C) Representative western blot of NRF2 and KEAP1 levels in lysates from INS-1 cells treated with IL-6 immunoprecipitated with anti-NRF2 antibody, and quantification of coimmunoprecipitated KEAP1 relative to NRF2. N=3. *p<0.05.

4.2.5 IL-6 signaling links autophagy to antioxidant response in the β-cell

We previously observed β-cell autophagy stimulation within one hour of IL-6 treatment (307). To determine the kinetics of autophagy activation, we transfected INS-1 β-cells with a dual-color-tagged Map1lc3 (LC3) that coalesces into puncta that are green/yellow as autophagosomes form but become red as the autophagosomes fuse with lysosomes and autophagy proceeds (314). We find that IL-6 stimulates autophagy within minutes (Figure 25A), and this is accompanied by a rapid increase in LC3-II and phospho-p62 (Figure 25B-C). Phosphorylation of the autophagy protein p62 at serine 351 causes it to bind to KEAP1 and to target it for degradation by autophagy (302). We find that IL-6 stimulates phosphorylation of p62 (S351) within 5 minutes, followed by a reduction in both phospho- and total-p62 after 15 minutes of IL-6 treatment (Figure 25C), further supporting increased autophagic flux.

We hypothesized that IL-6-induced disruption of the NRF2-KEAP1 interaction may be mediated by stimulation of p62 phosphorylation and autophagy by IL-6. To test this hypothesis, we used structured illumination microscopy (SIM) to determine if phospho-p62 and KEAP1 are targeted to lysosomes by IL-6. Indeed, within 15 minutes of IL-6 treatment, there is colocalization of both phospho-p62 and KEAP1 with lamp1-positive puncta (Figure 25D-E) suggesting targeting of these proteins for degradation by IL-6 stimulated autophagy.

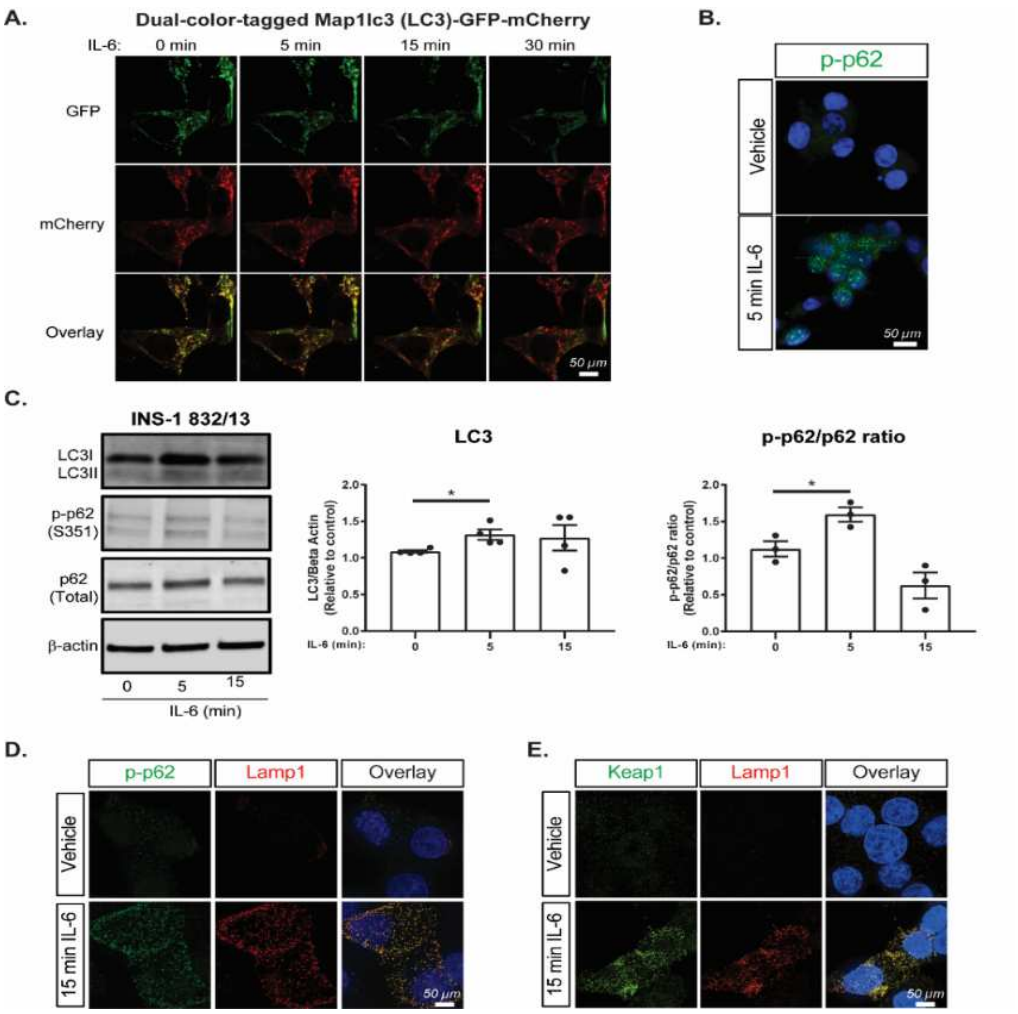

Figure 25. IL-6 rapidly stimulates the degradation of phospho-p62 and KEAP1 by autophagy (A) Representative snapshots from live cell imaging of mCherry-EGFP-LC3B in INS-1 β-cells stimulated with IL-6. (B) Representative image showing acute stimulation of p62 phosphorylation in INS-1 cells within 5 minutes of IL-6 treatment. (C) Representative western blot and quantification of autophagy markers (LC3-II and phospho-p62) in INS-1 acutely treated with IL-6. (D) Representative images showing colocalization of phospho-p62 (green) with Lamp1 (red) after 15 minutes of IL-6 treatment. (D) Representative images showing colocalization of Keap1 (green) with Lamp1 (red) after 15 minutes of IL-6 treatment. N≥3 for all experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

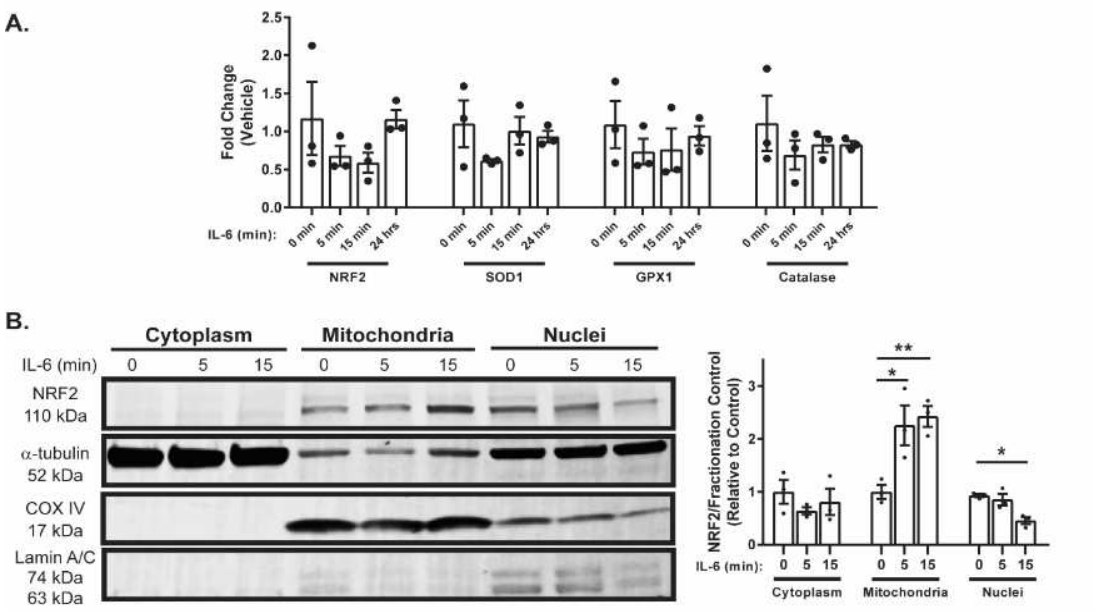

Figure 26. IL-6 induces NRF2 mitochondria translocation (A) qPCR analysis of expression of NRF2 target genes in INS-1 cells following IL-6 treatment for indicated times. (B) Representative western blot showing NRF2 levels in fractionated INS-1 cells treated with IL-6 for indicated times. Fractionation controls include α-tubulin (cytoplasm), COXIV (mitochondria), and Lamin A/C (nuclei). N≥3 for all experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

4.2.6 IL-6 induces NRF2 mitochondria translocation

Activation of the transcription factor NRF2 typically involves its translocation to the nucleus where it regulates transcription of antioxidant response genes. However, there were no changes in NRF2-target gene expression at either the early timepoints that we observed NRF2 protein accumulation in response to IL-6 treatment or after long-term exposure to IL-6 (Figure 26A). To determine if IL-6 influences NRF2 translocation after IL-6 stimulation, we separated the cytosolic, nuclear, and mitochondrial protein fractions from IL-6-treated INS-1 β-cells. Unexpectedly, stimulation of cells by IL-6 led to a robust increase in mitochondrial NRF2 localization and a decrease in nuclear NRF2 levels (Figure 26B).

4.2.7 IL-6 regulates mitochondrial function through NRF2

NRF2 mitochondrial translocation has been reported in other cell types, but the mechanism and function of this translocation are not well understood (315–317). Synthetic NRF2 activators have been found to stimulate mitochondrial degradation by autophagy, a process known as mitophagy, thereby contributing to mitochondrial homeostasis (318). To determine if IL-6 also stimulates mitophagy, we assessed translocation of the key mitophagy protein PARKIN to the mitochondria and found that IL-6 rapidly induces increased mitochondrial PARKIN levels within minutes (Figure 27A). This is accompanied by an initial rapid increase LC3 and p62 in the mitochondrial fraction. The subsequent decrease in PARKIN, LC3, and p62 is consistent with the progression of mitophagy (Figure 27A).

Loss of mitochondrial membrane potential is also associated with the initiation of mitophagy. We imaged live cells stained with the membrane potential-dependent dye, tetramethylrhodamine methylester (TMRM) and found that IL-6 treatment results in decreased mitochondrial membrane potential (Figure 27B). In both untransfected and

control siRNA-transfected cells, IL-6 treatment led to a decrease in mitochondrial membrane potential, which was blunted when *Nrf2* was knocked down (Figure 27B).

Mitochondrial fragmentation is an early event in mitophagy. cAMP levels affect the regulation of mitochondrial fusion and fission, with decreased cAMP being associated fission (319). We found that IL-6 triggered a transient drop of cAMP in intact mouse islets transfected with the mNeon cADDis cAMP biosensor (Figure 27C-D).

4.2.8 IL-6 induces mitophagy in INS-1 cells

To directly test the ability of IL-6 to induce mitophagy we employed the fluorescent mitophagy sensor mt-Keima. MitoKeima is composed of a pH-sensitive, dual-excitation ratiometric fluorescent protein Keima with added mitochondria localization sequence(320). INS-1 832/13 cells were transiently transfected with mt-Keima and incubated with vehicle or IL-6. Treatment with IL-6 caused a rapid induction of mitophagy as seen with an increase in 561nm/488nm ratio (Figure 28A-B).

4.2.9 IL-6 rapidly regulates protein expression in human islets

Our previous results showed that IL-6 can rapidly regulate the protein expression of Nrf2 within minutes following addition of IL-6 in both primary islets and INS-1 832/13. Our next objective was to determine whether incubation of islets with IL-6 causes rapid induction of other proteins. Therefore, we treated human islets with IL-6 or vehicle for 5 minutes and collected protein for mass spectrometry analysis. Incubation of IL-6 caused rapid changes of protein levels in islets. We then performed pathway analysis using ingenuity pathway software to determine which pathways are rapidly regulated by IL-6. Verifying our previous observations, we found that mTOR signaling was one of the significant pathways affected by IL-6 treatment (Figure 29A). Additionally, pathway analysis also shows that IL-6 regulated pathways involved in T2D such as Sirtuin Signaling Pathway and Type II Diabetes Mellitus Signaling (Figure 29A). These results show that

IL-6 rapidly regulates multiple signaling pathways in islets that are involved in diabetes. Specifically, it shows that IL-6 is also regulate pathways that are involved in T2D.

Following discovery of rapid induction of important diabetes related signaling pathways by IL-6, our next objective was to determine the consequences of chronic loss of IL-6 signaling in β cells. To this end RNA was isolated from IL6Rα$^{\Delta\beta}$ and WT mouse islets and RNA sequencing was performed. Loss of IL-6Rα in β cells induced a host of changes in mRNA expression in mouse islets. We performed ingenuity pathway analysis to further identify which signaling pathways are affected by loss of IL-6 signaling in β cells. Loss of IL-6Rα affected numerous pathways including iNOS, eNOS signaling and Type II Diabetes Mellitus signaling (Figure 29B). The involvement of iNOS and eNOS signaling is interesting as this pathway has been demonstrated to be involved in cellular oxidative stress (321) and further explains the susceptibility of IL6Rα$^{\Delta\beta}$ mice to oxidative damage. Additionally, we observed the involvement of Type II Diabetes Mellitus signaling pathways during induction of IL-6 signaling and loss of IL-6 signaling. These results led us to test the involvement of β cell IL-6 signaling in the pathogenesis of diabetes.

4.2.10 IL-6 rapidly regulates pathways involved in T2D

Based on the observed rapid induction of NRF2 protein levels by IL-6 in β cells, we hypothesize that IL-6 could potentially acutely regulate the protein expression of numerous targets. To test this hypothesis, we incubated human islets acutely (5 mins) with recombinant human IL-6 and performed Mass spectrometry analysis to detect unbiased changes in protein expression. We found over a 100 significantly expressed proteins following incubation with IL-6. Interestingly the protein RICTOR (rapamycin-insensitive companion of mTOR) was significantly upregulated (FC = 2.85 versus vehicle; p = 0.017) following incubation with IL-6. RICTOR is a critical component of the mTORC2 complex and plays a role in cellular survival, metabolism and proliferation(322). To further elucidate the effects of protein changes in β cell due to acute IL-6 we performed ingenuity

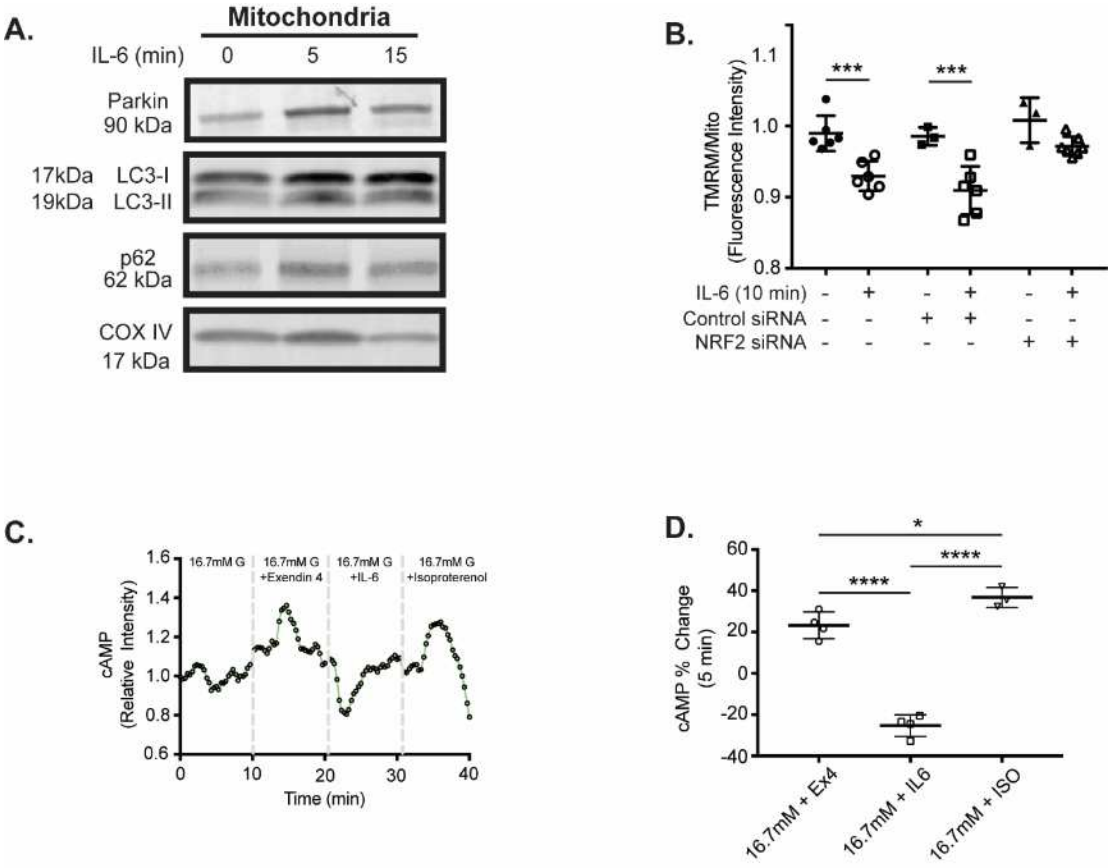

Figure 27. IL-6 regulates mitochondrial function through NRF2 (A) Representative western blot showing PARKIN, p62, and LC3 levels in fractionated INS-1 cells treated with IL-6 for indicated times. Mitochondrial fractionation control is COXIV. (B) Mitochondrial membrane potential measurements in IL-6 treated INS-1 cells that were either untransfected or transfected with nontargeting or nrf2-specific siRNA. TMRM was normalized to the stable Mitotracker green levels. (C) Representative single islet trace showing real-time Cyclic AMP measurements from mouse islets infected with the mNeon cADDis cAMP biosensor in the presence of glucose, Exendin-4, IL-6, or Isoproterenol. (D) Quantification of percent change across all replicates relative to baseline. N≥3 for all experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

pathway analysis of the mass spectrometry results (Figure 28A).Interestingly, mTOR signaling, Type II Diabetes signaling and Sirtuin signaling were highlighted as pathways that are significantly modulated by IL-6. The involvement of mTOR signaling further corroborates the results observed of acute induction of autophagy by IL-6. The acute modulation of Type II Diabetes signaling and Sirtuin signaling, two pathways involving T2D, lead us to hypothesize that IL-6 may also be protective in the setting of T2D. To test this hypothesis, we performed RNA sequencing of RNA from islets from WT and β cell ILRα knockout mice. Ingenuity pathway analysis of the sequencing results also showed the involvement of Type II Diabetes signaling pathway (Figure 28B). Based on these results we tested the hypothesis that loss of IL-6 signaling in the β cell leads to increase susceptibility to HFD induced T2D.

4.2.11 Loss of β cell IL6Rα had no effect of HFD mediated metabolic disorder

To test the involvement of β cell IL-6 signaling in development of T2D we placed WT and IL6R$\alpha^{\Delta\beta}$ mice on HFD (60% Kcal from fat) for 10 weeks. Over the course of the HFD we observed no differences in weight gain or random fed blood glucose between WT and IL6R$\alpha^{\Delta\beta}$ mice (Figure 30A-B). Additionally, we performed glucose tolerance test following 10 weeks of HFD and observed no statistically significant changes between the two groups (Figure 30C). In order to assess peripheral insulin resistance, we performed insulin tolerance test (ITT) on mice following 10 weeks of HFD. Again, we observe no significant difference between WT and IL6R$\alpha^{\Delta\beta}$ mice (Figure 30D).

4.2.12 Loss of β cell IL6Rα decreases insulin mediated signaling in skeletal muscle

Numerous studies have observed an increase in IL-6 levels in obese individuals(323). Additionally, the soluble IL-6 – IL6Rα complex have been shown to induce signaling in cells lacking IL-6Rα(229,237). Due to the HFD induced obesity observed in our mice, which could upregulate IL-6 levels and the ability of soluble IL6Ra signaling there is a high capacity of compensatory IL-6 signaling in our IL6R$\alpha^{\Delta\beta}$ mice that

would blunt effects of loss of IL-6. Hence, we hypothesized that changes to peripheral insulin signaling might be too subtle to observe due to these compensatory changes and the use of a more sensitive technique to assess peripheral insulin signaling was warranted. Therefore, following 10 weeks of HFD, we administered 30 units of insulin or saline to WT and IL6R$\alpha^{\Delta\beta}$ mice, and sacrificed mice after 5 minutes for tissue collection. We then assessed insulin signaling in the skeletal muscles of WT and IL6R$\alpha^{\Delta\beta}$ mice by immunoblotting for P-AKT(S473) and P-Erk1/2 (Figure 31A). Analysis of P-AKT signaling showed a significant increase in phosphorylation in control mice by insulin and a blunted increase in phosphorylation in IL6R$\alpha^{\Delta\beta}$ mice (Figure 31B). Additionally, P-ERK1/2 signaling also showed a significant increase in insulin injected control mice that was blunted in IL6R$\alpha^{\Delta\beta}$ mice. These results show that there is development of low-grade peripheral insulin resistance in IL6R$\alpha^{\Delta\beta}$ mice.

4.3 Discussion

IL-6 is a pleiotropic cytokine that is produced by and acts on several tissues throughout the body. This cytokine plays a well-known role in cancer cell survival, but its role in metabolism and pancreatic islet biology is more complicated (324). Exercise acutely stimulates circulating IL-6 to levels that are ~50-100 times above those observed during resting conditions (325–327). When increased to these levels, IL-6 is associated with heightened insulin sensitivity and nutrient availability (328,329). A series of recent studies demonstrated that IL-6 signaling might play a decisive role in both anti-inflammatory M2 macrophage polarization (330,331) and insulin secretion (332). However, circulating IL-6 is also modestly increased 2 to 4-fold in humans with both T1D and T2D (333,334), where its role as a positive or negative regulator of metabolic homeostasis remains controversial (335). In fact, numerous studies have reported proinflammatory roles of IL-6 signaling and

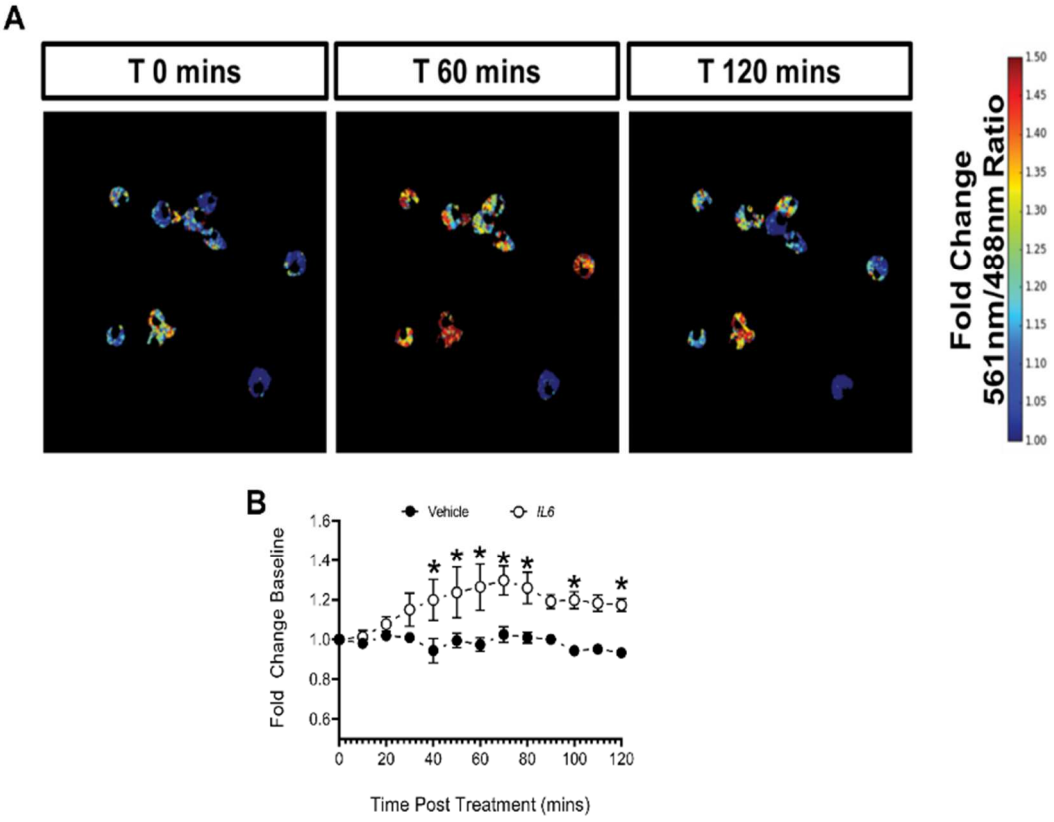

Figure 28. IL-6 induces mitophagy in INS-1 cells (A) Representative ratiometric image of INS1832/13 cells transfected with mt-Keima, at various time points following treatment. The colors represent fold change of 561nm/488nm ratio (B) Fold change quantification of the intensity ratio of 561nm/488nm, after IL-6 treatment in comparison to vehicle. N≥3 for all experiments. *p<0.05

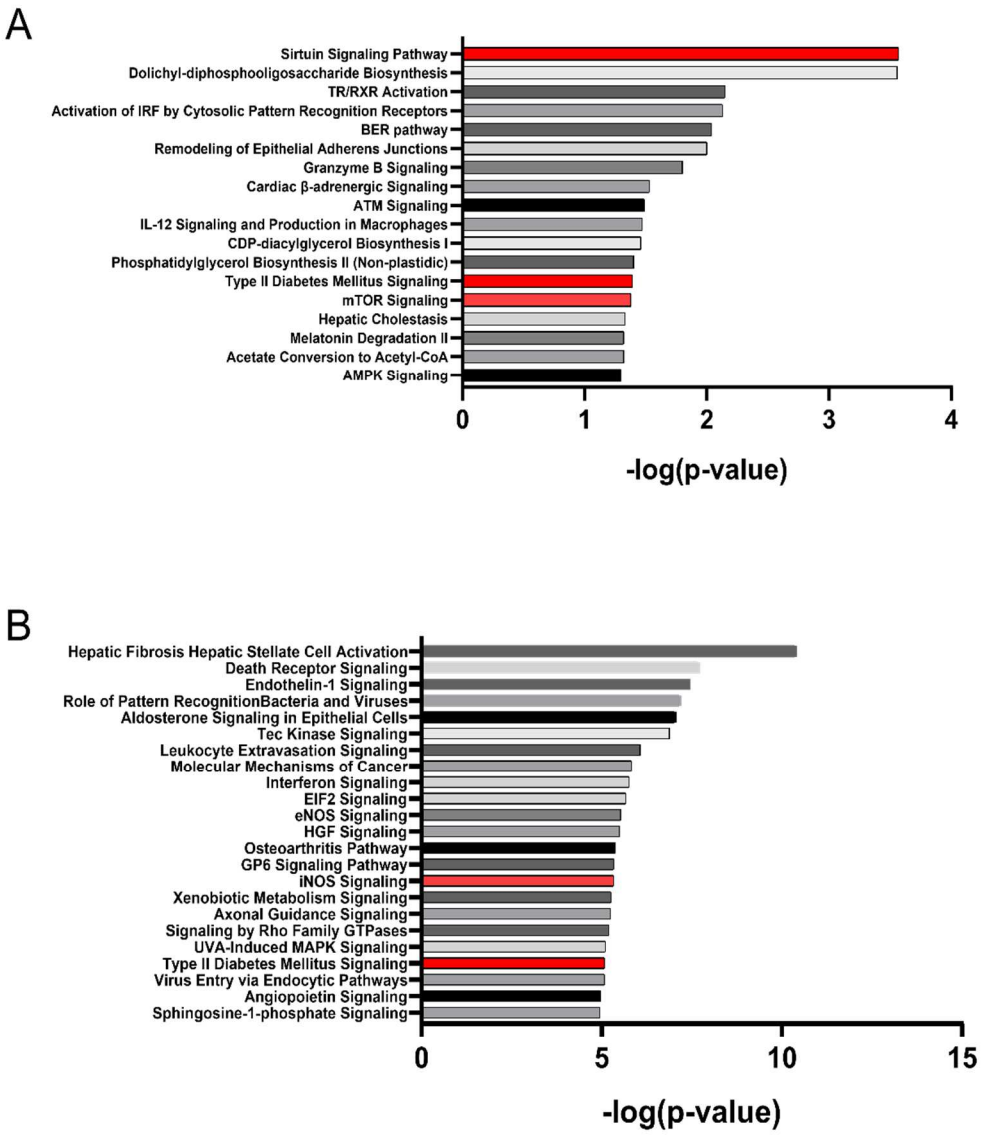

Figure 29. IL-6 rapidly regulates pathways involved in T2D (A) IPA analysis of pathways affected in human islets incubated for 5 minutes with IL-6. (B) IPA analysis of differentially expressed genes from islets of WT versus IL6R$\alpha^{\Delta\beta}$ mice.

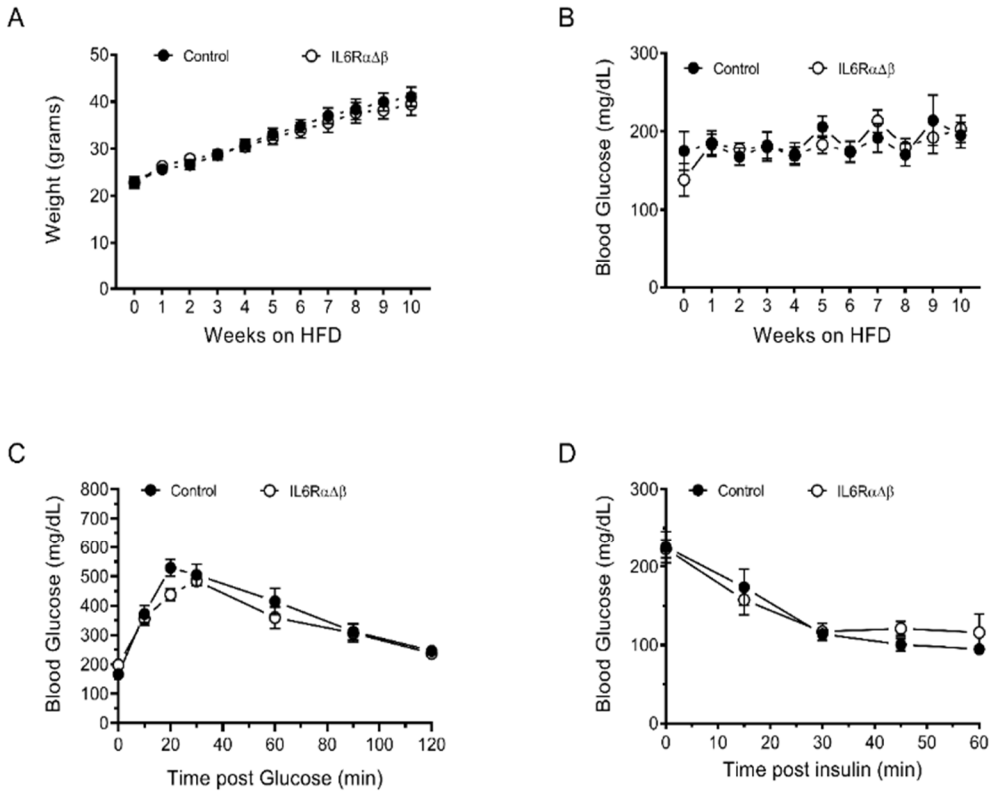

Figure 30. Loss of β cell IL6Rα had no effect of HFD mediated metabolic disorder (A) Bodyweight measurements of WT and IL6R$\alpha^{\Delta\beta}$ mice on HFD. (B) Random fed blood glucose measurements of WT and IL6R$\alpha^{\Delta\beta}$ mice on HFD. (C) Analysis of glucose tolerance (GTT) in WT IL6R$\alpha^{\Delta\beta}$ mice following 10 weeks of HFD. (D) Analysis of insulin tolerance (ITT) in WT IL6R$\alpha^{\Delta\beta}$ mice following 10 weeks of HFD. N ≥ 3 mice per experimental group for all experiments.

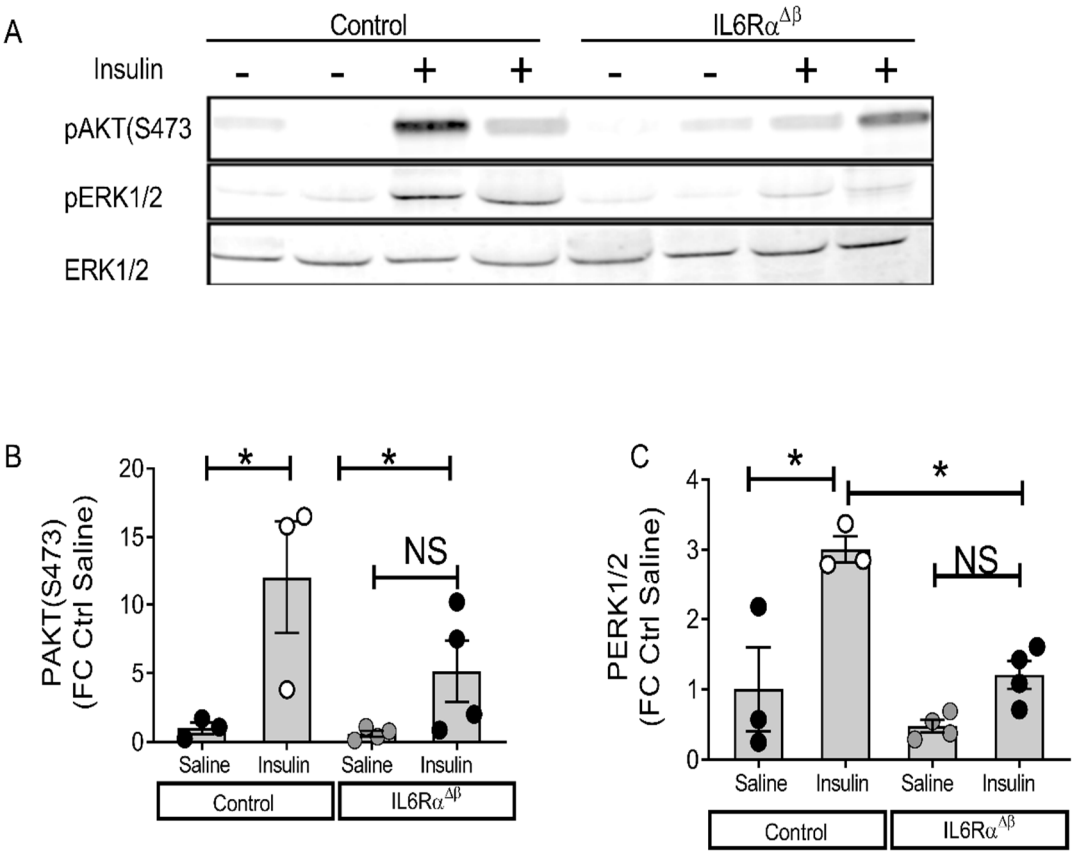

Figure 31. Loss of β cell IL6Rα decreases insulin mediated signaling in skeletal muscle (A) Representative western blot showing PAKT(s473) and P-ERK1/2 in Control and IL6Rα^{Δβ} mice injected with saline or 30 units of Insulin 5 minutes prior to sacrifice. (B) Quantification of change in PAKT(S473) in skeletal muscle of mice following insulin injection normalized to total ERK1/2 (C) Quantification of change in P-ERK1/2 in skeletal muscle of mice following insulin injection normalized to total ERK1/2. *p<0.05

the promotion of insulin resistance in some tissues (336–339). It has been proposed that this apparent discrepancy in IL-6 action may result from cell-type specific effects, differences in acute versus chronic signaling by this cytokine, and/or differential effects of signaling through soluble and membrane-bound variants of IL6Rα (335). We recently found that increases in IL-6 had a protective effect on β-cells through the stimulation of autophagy (307). Like IL-6, autophagy has also been reported to have conflicting roles in promoting versus preventing inflammation and cell death depending on context and duration of activation (340,341).

Here, we present data supporting the hypothesis that acute IL-6 signaling in the β-cell couples antioxidant response to autophagy for β-cell mass preservation under conditions of increased oxidative stress. We find that the IL-6 signaling pathway functions to reduce oxidative damage both *in vitro* and *in vivo*. β-cell-specific knockout of IL6Rα in mice exacerbated oxidative stress, β-cell death, and hyperglycemia in response to STZ. However, it is worth noting that in our model, it is still possible that soluble IL6Rα from other cell types could be present and bind to/activate signaling through β-cell GP130. This could potentially diminish the effects of a β-cell IL6Rα knockout, suggesting that the contribution of β-cell IL-6 signaling to oxidative stress response may be underestimated in our study.

Our data supports that IL-6 protects β-cells from oxidative stress through the stimulation of autophagy and NRF2, a key regulator of the antioxidant response. We show that IL-6 stimulates translocation of NRF2 to the mitochondria and provide evidence suggesting that mitochondrial NRF2 may play a vital role in the rapid response to elevations in β-cell ROS. Thus, we propose a model in which IL-6 signaling rapidly stimulates autophagy through the phosphorylation and activation of the autophagy chaperone p62, which, in turn, stimulates the antioxidant response through the phospho-

p62-mediated targeting of the NRF2 inhibitor, KEAP1, to the autophagosome for degradation. Liberated NRF2 rapidly translocates to the mitochondria, where it functions to promote redox homeostasis, similar to previous observations (342).

Mitochondria are a primary source of cellular ROS. Therefore, pathways that promote mitochondrial homeostasis are critical for preventing cellular damage and death. One established mechanism of maintaining mitochondrial homeostasis is through selective degradation of dysfunctional mitochondria by mitophagy. Increases in ROS stimulate mitophagy through PINK1-mediated kinase activation of the ubiquitin ligase PARKIN (343,344). Decreases in mitochondrial membrane potential and cAMP, like those observed here in response to IL-6, are associated with the stimulation of PINK1/PARKIN-mediated mitophagy (319,345,346). Indeed, we observe that IL-6 rapidly stimulates mitochondrial translocation of PARKIN. Furthermore, using a fluorescent sensor that is used to assess mitophagy we were able to establish a direct ability of IL-6 to induce mitophagy in β cells. We also show that IL-6 induces the mitochondrial translocation of NRF2, where it has been reported to function in the modulation of respiration and to counteract ROS (315,342). Therefore, we propose that IL-6 stimulates mitochondrial turnover to reduce ROS and prevent oxidative damage, and this contributes to the restoration of homeostasis within the β-cell. This protection from oxidative stress likely plays a crucial role in reducing apoptosis and thus preserving β-cell mass in conditions where large amounts of ROS are generated in the β-cell.

We have shown by immunoblotting that IL-6 rapidly regulates protein levels of NRF2 in β cells. Using mass spectrometry analysis, we show that numerous proteins are acutely regulated by IL-6. These proteins are involved in many pathways, showing the broad effect of acute IL-6 signaling. Interestingly one of the significant pathways involved in acute addition of IL-6 is the Type II Diabetes signaling pathway. Ingenuity pathway

analysis predicted a downregulation of this pathway in IL-6 treated human islets. Numerous studies have associated IL-6 with obesity and T2D pathogenesis(253,347). Based on the results of our previous experiments showing a protective ability of IL-6 in the setting of β cell oxidative stress, we predicted similar protection in mouse models of T2D. Further analysis of mRNA expression from islets of Wild Type and IL6Rα knockout mice showed increased activation of Type II Diabetes signaling pathway in IL6Rα knockout mice. These results are further corroborating evidence for protection of IL-6 in the setting of T2D. Contrary to these predictions we found no effect of loss of β cell IL6Rα on the metabolic status of mice placed on HFD. These results were contrary to the numerous other studies that have linked IL-6 signaling to the development of diabetes. A point of consideration of this lack of effect is the tissue specificity of the knockout. The development of diabetes involves an intricate interplay between many organ systems that can lead to insulin resistance followed by overt T2D. Additionally, the ability of IL-6 to undergo trans-signaling could allow for compensation by other tissue sources of IL-6 to restore signaling in the IL6Rα β cells.

One fundamental limitation of our study is that the *in vitro* activation of autophagy and the antioxidant response we observed occur so rapidly that these early events would be difficult to detect in our *in vivo* STZ and alloxan studies. Future studies utilizing novel methods such as intravital microscopy could potentially be used to monitor these events in real-time. This would provide valuable insight into the *in vivo* coupling of autophagy with the antioxidant response to determine how perturbations in these pathways could contribute to diabetes development.

In conclusion, these data provide novel evidence that autophagy and the antioxidant response are linked in the pancreatic β-cell, and that mitochondrial NRF2 may play an essential role in these processes. Signaling through the IL-6 receptor rapidly activates these coupled pathways, and we show that IL-6 receptor signaling is critical for

reducing islet oxidative stress, preserving β-cell mass, and protecting from STZ-induced diabetes. Therefore, we suggest that acute activation of IL-6 receptor signaling may be beneficial for protection against the development of diabetes.

5 Conclusion and Future Directions

This thesis presents research providing novel insight into the modulation of β cell oxidative stress. The two general strategies explored in this thesis are the inhibition of pathways that enhance oxidative stress and induction of pathways that mitigate oxidative stress. In the first part of the thesis, the role of 12-LOX and 12/15-LOX in beta cell dysfunction and death were delineated in models of T1D and T2D. We present evidence of reciprocal regulation of 12-LOX and 12/15-LOX in the pancreatic islet and the show a novel mechanism by which 12/15-LOX activity induces oxidative stress in pancreatic islets. The second half of the thesis show that induction of IL-6 signaling in the beta cell is protective in conditions of oxidative stress. Additionally, we show a novel ability of IL-6 to cause NRF2 translocation to the mitochondria and induce mitophagy. In this final chapter a summary of the prominent results of the thesis, future research inquiries and the experiments to explore these questions are presented.

Lipoxygenase is enzymes that catalyze the oxygenation of polyunsaturated fatty acids. Numerous studies have demonstrated that the activity of 12/15-LOX contributes to the pathogenesis of diabetes possibly through the production of 12-HETE (280). In the first part of this dissertation the effect of loss of 12-LOX, an enzyme that also produces 12-HETE, is compared to a loss of 12/15-LOX. The underlying hypothesis tested was that loss of 12-LOX would also be protective in β cells due to a decrease in 12-HETE production.

Mice harboring whole-body knockout of Alox12(12-LOX) and Alox15(12/15-LOX) were utilized to compare the effect of loss of 12-LOX versus 12/15-LOX in the setting of T1D and T2D. Contrary to our hypothesis we observed that loss of 12-LOX exacerbates the development of diabetes while the loss of 12/15-LOX protected from diabetes-induced using multi low dose administration of STZ. Furthermore, an increase in oxidative stress and a corresponding decrease of antioxidant enzymes expression was observed in the

islets of Alox12$^{-/-}$ mice following STZ administration. In-vitro studies in isolated islets from Alox12$^{-/-}$ mice further showed that the observed decrease in antioxidant response in Alox12$^{-/-}$ mice is intrinsic to the β cell. Interestingly similar results were observed in HFD model of T2D, with Alox12$^{-/-}$ showing increase susceptibility to HFD induced metabolic disorder while Alox15$^{-/-}$ mice were protected. To elucidate the mechanism driving the pathogenic effect of the Alox12$^{-/-}$ mice we measured the expression of *Alox12* and *Alox15* mRNA in islets from Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. Surprising, *Alox15* mRNA was significantly increased in the islets of Alox12$^{-/-}$ mice. These results show a novel reciprocal regulation of the expression of Alox12 and Alox15. Additionally, it highlighted the possibility of an increase in 12/15-LOX activity in the Alox12$^{-/-}$ as the primary driver of the detrimental effect of loss of Alox12. To test this hypothesis Alox12$^{-/-}$ mice were treated with ML351 a specific small molecule inhibitor of 12/15-LOX. Inhibition of 12/15-LOX led to the protection of Alox12$^{-/-}$ mice from STZ induced diabetes. Based on these results we hypothesized that increased 12/15-LOX activity in the Alox12$^{-/-}$ mice leads to increase generation of 12-HETE that sensitizes islets of Alox12$^{-/-}$ mice to oxidative stress. To explore this possibility, we measured circulating eicosanoids in the serum of WT, Alox12$^{-/-}$ and Alox15$^{-/-}$ mice. 12-HETE levels were decreased in both Alox12$^{-/-}$ and Alox15$^{-/-}$ mice compared to WT mice. Interestingly, Alox15$^{-/-}$ mice showed a dramatic increase in 12-HEPE levels while Alox12$^{-/-}$ mice had no detectable level of 12-HEPE.

Altogether the results of this study show that loss of Alox12 sensitized islets to oxidative stress, potentially through increased Alox15 and decreased 12-HEPE generation. These results are of importance to the development of inhibitors of LOX enzymes in the treatment of diseases such as diabetes. It illustrates the importance of specificity of inhibition of LOX enzymes to avoid the possibility of feedback mechanisms that might influence the expression and activity of other lipoxygenase enzymes leading to a detrimental outcome.

In the second part of the thesis, we explored the role of IL-6 signaling in the pathogenesis of diabetes. Numerous studies have linked IL-6 to the pathogenesis of diabetes. The results of these studies have been contentious as some have demonstrated a pathogenic role of IL-6 while others have demonstrated a protective role of IL-6 in the development of both T1D and T2D. In this thesis, we explored whether IL-6 signaling is protective in the induction of oxidative stress in β cells. Using human islets and the INS-1 beta cell line, we showed that exogenous IL-6 leads to decreased proinflammatory cytokine-induced oxidative stress. To further demonstrate a protective effect of IL-6 we developed mice harboring a β cell-specific knockout of IL-6Rα. These mice exhibited increased sensitivity to both STZ and Alloxan-induced diabetes and increased levels of markers of oxidative stress. Based on these results we conclude that IL-6 signaling protects β cells from oxidative stress.

To determine the mechanism by which IL-6 signaling reduces oxidative stress we explored the effect of IL-6 on the transcription factor NRF2, the primary regulator of antioxidant response in mammalian cells. We demonstrated the novel finding that IL-6 causes a rapid induction of NRF2 protein levels in β cells. The mechanism of this rapid increase of NRF2 protein was determined to be through p62 mediated degradation of the NRF2 regulatory protein KEAP1. Surprisingly this increase in NRF2 levels by IL-6 did not cause the expected increase in antioxidant enzymes regulated by NRF2.

Due to the lack of change in mRNA of antioxidant enzymes by IL-6, we then determined whether IL-6 caused translocation of NRF2 to any specific cellular compartment. Results of immunoblotting of fractionated INS1 cells treated with IL-6 show an increase in NRF2 translocation to the mitochondria and a corresponding decrease in the nucleus. Furthermore, we show that IL-6 mediated mitochondria translocation of NRF2 induces a cascade of events that leads to mitophagy in β cells.

The results of these studies show a novel protective effect of IL-6 on β cells through modulation of NRF2 levels and translocation. The β cell is susceptible to oxidative stress due to its high metabolic activity and relatively low expression of antioxidant enzymes. The primary function of this cell is the production and secretion of insulin in response to glucose, a process that heavily relies on ATP generated by the mitochondria oxidative phosphorylation. The results of this study demonstrate the existence of a mechanism by which the cell can quickly remove malfunctioning mitochondria that can lead to a decrease in ROS and oxidative stress generation. Furthermore, it highlights the possibility that increased IL-6 levels observed in diabetes could be playing a protective role in the reduction of oxidative stress in β cells.

5.1 Future Directions

The results presented in this thesis provide a novel insight into the regulation of oxidative stress in the β cell. However, numerous studies are required to elucidate the mechanisms driving these effects.

5.1.1 Transcriptional control of lipoxygenase enzymes

The results of these studies highlight the importance of studying the transcriptional control of lipoxygenase enzymes. While we demonstrated a novel reciprocal regulation of Alox15 by Alox12 expression, the exact mechanism driving this regulation is yet to be delineated. Studies into the regulation of Alox15 expression to date have only been performed in immune cells in the context of maturation of macrophages. Considering the impact of lipoxygenase activity on β cell function studies are warranted into the regulation of lipoxygenase levels in models of diabetes. Studies of transcription regulation previously required known transcription factor candidates in order to test their ability to regulate a gene. The advent of CRISPR Cas9 gene editing allows for identification of de novo transcription factors binding to a sequence of DNA. Employing a catalytically inactive dCas9 and a specific guide RNA this system allows for purification of a specific area of

genomic DNA and its associated molecules (348). Using this system would allow for unbiased identification of regulators of lipoxygenase enzyme levels in the setting of diabetes, providing further insight into targets to modulate lipoxygenase levels in the treatment of diabetes.

5.1.2 Effect of 12-HEPE on β cell function

The results of the LC/MS measurement of circulating eicosanoids Alox12$^{-/-}$ and Alox15$^{-/-}$ mice identified 12-HEPE as a possible metabolite regulated by 12/15-LOX. Levels of circulating 12-HEPE were increased in Alox15$^{-/-}$ mice compared to WT and were virtually undetected in Alox12$^{-/-}$ mice. Based on these results we concluded that 12-HEPE is negatively regulated by 12/15-LOX activity and maybe be beneficial in protecting β cells from oxidative stress. While HEPES such as 5-HEPE have been demonstrated to be beneficial in the function of β cells (349), the role of 12-HEPE is unknown in the context of β cell function and diabetes. Future studies into the role of 12-HEPE in the modulation of oxidative stress could provide further insight into the treatment of diabetes.

5.1.3 Mechanisms regulating IL-6 mediated NRF2 mitochondrial translocation

We demonstrate the ability of IL-6 to rapidly increase NRF2 protein expression and induce its translocation to the mitochondria. The mechanism underlying IL-6 mediated NRF2 recruitment to mitochondria is yet to be elucidated. To date sequence analysis of NRF2 and localization prediction software have failed to identify a mitochondria localization signal in NRF2. This hints at the possibility of another chaperone protein that could form a complex with NRF2 to facilitate its translocation to the mitochondria. Experiments to determine the interactome of NRF2 following IL-6 treatment would allow for identification of candidate proteins that facilitate the observed IL-6 mediated NRF2 mitochondria translocation. Additionally, further studies are required to determine whether IL-6 induces NRF2 translocation to the outer mitochondria membrane or the inner

mitochondria matrix. Localization of NRF2 in the mitochondria is crucial for identification of the exact mechanisms by which it regulates mitophagy in β cells.

5.1.4 Role of β Cell IL-6 signaling in the pathogenesis of T2D

Obesity is a condition of insulin resistance and generalized inflammation that causes increased generation of reactive oxygen species (ROS) leading to oxidative stress and β cell death. We have demonstrated the ability of IL-6 to mitigate oxidative stress in β cells due to STZ, Alloxan, and pro-inflammatory cytokines. Additionally, levels of IL-6 has been demonstrated to be significantly elevated in obese individuals and T2D individuals (253). This increase has been used as evidence of a pathologic effect of IL-6 in obesity(350,351). This paradigm is under contention as studies have demonstrated an anti-obesogenic effect of IL-6 (260,261,352). Further studies are required to determine the role of β cell IL-6 signaling in obesity and T2D. Preliminary results presented in this thesis show that loss of IL-6 signaling in the β cell caused no effect on weight gain, glucose tolerance or insulin tolerance assessed by ITT following 10 weeks of HFD. Interestingly we observed a decrease in peripheral insulin signaling in skeletal muscle of β cell IL-6R α knockout mice following 10 weeks of HFD. This hints at possible development of low-grade insulin intolerance in IL6R$\alpha^{\Delta\beta}$ mice that is not detected by ITT. Hence, studies extending the duration of HFD are necessary to elucidate the role of β cell IL-6 signaling in obesity and T2D.

All together the discoveries presented in this dissertation provide novel insight into the role of lipoxygenase and IL-6 in the modulation of β cell oxidative stress. The pleiotropic effects of both lipoxygenase and IL-6 indicate that further studies are warranted to identify more specific therapeutic targets that allow for more regulated modulation of β cell survival. Improved understanding of pathophysiological mechanisms underlying β cell dysfunction in diabetes is vital to the development of potent therapies to combat this epidemic. Oxidative stress is a common cause of β cell dysfunction and death in both T1D

and T2D. Hence understanding pathways that can be targeted to improve β cell handling of oxidative stress is beneficial in the setting of both diseases.

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CURRICULUM VITAE

Abass M. Conteh

Education

2019 Doctor of Philosophy

Biochemistry and Molecular Biology Indiana University, Indianapolis, IN

2011 Bachelor of Science

Neurobiology and Physiology, Purdue University, West Lafayette,

Research Experience

2017-2019 The Role of Lipoxygenase and Interleukin-6 on Islet β-Cell Oxidative Stress and Dysfunction

Amelia Linnemann and Raghavendra Mirmira, Indiana University School of Medicine.

2015-2017 Concomitant Targeting of Met and Epidermal Growth Factor Receptor in Pancreatic Cancer

Murray Korc, Indiana University School of Medicine.

2013-2014 Cardiovascular effects of the Glucagon like peptide1 receptor activation in lean and obese animals

Johnathan Tune, Indiana University School of Medicine.

2009 Undergraduate Student Researcher, Summer Undergraduate Research Fellowships, A case study of dislodgeable residues from lawns and urinalysis of children and dogs from 30 households using lawn care services.

Wendy Peer, Purdue University

2009 Undergraduate Student Researcher, Short-Term Education Program for Underrepresented Persons, Altered Taste Preferences for Sweet in Dietary Obese Rats fed with High Fat or High Carbohydrate Diets (Summer 2009)

Andras Hajnal, Penn State Medical College.

2007 Analysis of CRIPT loss of function mutants

Wendy Peer, Purdue University

Other Experience and Professional Memberships

2018-2019 Endocrine Society

2013-2014 American Physiological Society

2012-present American Medical Student Association

Honors

2018 Annual Biochemistry Symposium certificate of excellence in research

2018 Annual Diabetes Symposium certificate of excellence in research

2018 Endocrine Society's Annual Meeting & Expo Abstract Travel Award

2016 American Physician Scientist Association Travel Award

2014 Cardiovascular Section Research Recognition Award

2008-2010 Purdue Black Caucus Award

2007-2008 Health Sciences Distinguished Student

Teaching experience

2009-2011 Tutoring for the Purdue HORIZONS student support Program

Selected Peer-reviewed Publications

1. **Abass M. Conteh,** Christopher A. Reissaus, Marimar Hernandez-Perez, Swetha Nakshatri, Ryan M. Anderson, Raghavendra G. Mirmira, Sarah A. Tersey, Amelia K. Linnemann: *Platelet-type 12-lipoxygenase deletion provokes a compensatory 12/15-lipoxygenase increase that exacerbates oxidative stress in islet β-cells*. Journal of Biological Chemistry 02/2019;, DOI:10.1074/jbc.RA118.007102
2. Michelle R. Marasco, **Abass M. Conteh**, Christopher A. Reissaus, John E. Cupit V, Evan M. Appleman, Raghavendra G. Mirmira, Amelia K. Linnemann: *Interleukin-6 Reduces β-Cell Oxidative Stress by Linking Autophagy With the Antioxidant Response*. Diabetes 05/2018; 67(8):db171280., DOI:10.2337/db17-1280
3. Abhishek A. Kulkarni, **Abass M. Conteh**, Cody A. Sorrell, Anjali Mirmira, Sarah A. Tersey, Raghavendra G. Mirmira, Amelia K. Linnemann, Ryan M. Anderson: *An In Vivo Zebrafish Model for Interrogating ROS-Mediated Pancreatic β -Cell Injury, Response, and Prevention*. Oxidative medicine and cellular longevity 03/2018; 2018(21):1-8., DOI:10.1155/2018/1324739
4. Marimar Hernandez-Perez, Gaurav Chopra, Jonathan Fine, **Abass M. Conteh**, Ryan M. Anderson, Amelia K. Linnemann, Chanelle Benjamin, Jennifer B. Nelson, Kara S. Benninger, Jerry L. Nadler, David J. Maloney, Sarah A. Tersey, Raghavendra G. Mirmira: *Inhibition of 12/15-Lipoxygenase Protects Against β Cell Oxidative Stress and Glycemic Deterioration in Mouse Models of Type 1 Diabetes*. Diabetes 08/2017; 66(11):db170215., DOI:10.2337/db17-0215
5. Daniel J Sassoon, Johnathan D Tune, Kieren J Mather, Jillian N Noblet, Mackenzie A Eagleson, **Abass M Conteh**, Joshua T Sturek, Adam G Goodwill:

Glucagon Like Peptide-1 Receptor Activation Augments Cardiac Output and Improves Cardiac Efficiency in Obese Swine Following Myocardial Infarction. Diabetes 05/2017; 66(8)., DOI:10.2337/db16-1206

6. Daniel J. Sassoon, Adam G. Goodwill, Jillian N. Noblet, **Abass M. Conteh**, B. Paul Herring, Jeanette N. McClintick, Johnathan D. Tune, Kieren J. Mather: *Obesity alters molecular and functional cardiac responses to ischemia/reperfusion and glucagon-like peptide-1 receptor agonism*. Archiv für Kreislaufforschung 07/2016; 111(4)., DOI:10.1007/s00395-016-0563-4
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