Diabetes Research Connection Project Number 46
6-Month Progress Report

Principal Investigator: Rehana Akter, Ph.D.
Period of Performance: 03/01/2022 - 02/28/2023
Project title: Role of Steroidogenic Acute Regulatory Protein in Cholesterol-Induced β-Cell Dysfunction in Type 1 diabetes

Cholesterol is commonly elevated in individuals with type 1 diabetes (T1D). The overall goal of this project is to understand whether in T1D, exposure of pancreatic β cells to excess cholesterol exacerbates β-cell dysfunction/loss.

Mitochondria are essential for the maintenance of normal β-cell function. Steroidogenic acute regulatory protein (StAR) facilitates cholesterol transport into mitochondria. Previously, we found that cholesterol accumulation in pancreatic islets results in elevated StAR expression, and that β-cell StAR overexpression *per se* results in β-cell dysfunction. Based on these observations, we hypothesize that in T1D increased exposure of β cells to cholesterol results in StAR-mediated mitochondrial dysfunction that exacerbates immune-based β-cell dysfunction and loss.

To address this hypothesis, we propose the following specific aims:
Specific Aim 1A: To determine whether StAR mediates cholesterol-induced β-cell dysfunction and loss *in vitro*.
Specific Aim 1B: To determine whether CYP27A1 overexpression can rescue StAR-mediated mitochondrial dysfunction and improve β-cell function in islets with elevated cholesterol *in vitro*.
Specific Aim 2: To determine whether β-cell specific StAR deletion reduces β-cell dysfunction and loss in mice with hypercholesterolemia.

Project Progress:
In the first 6 months of funding, we have focused our efforts largely on the *in vitro* experiments described in Specific Aim 1.

1) **Generation of β-cell specific StAR knock-out mice:** Our lab generated $Star^{fl/fl}$ mice in order to cross them with $Ins1^{cre/cre}$ mice to produce β-cell specific StAR knock-out mice. We previously verified that we successfully ablated StAR from β cells using the Cre-LoxP recombination strategy (Figure 1A). Therefore, in the last 6 months, we expanded the β-cell specific StAR knock-out mouse line (Figure 1B), which we will use for the *in vitro* and *in vivo* studies.

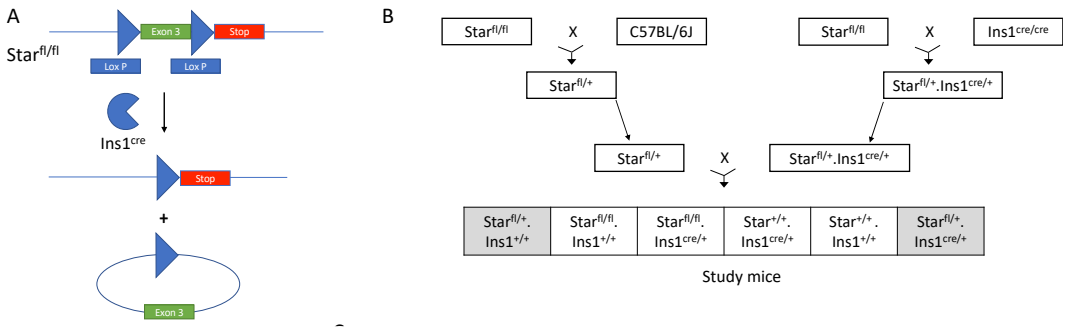

Figure 1: (A) Schematic representation of the Cre-LoxP strategy for conditional Star gene knock-out from β cells. (B) The breeding scheme for generation of β-cell specific StAR knock-out mice.

2) ***Ex vivo* treatment of islets from β-cell specific StAR knock-out mice with cholesterol:** After confirming a reduction of StAR mRNA expression in islets from β-cell specific StAR knock-out mice (Figure 2A), we treated islets with vehicle or 0.5 mM cholesterol for 24 h to evaluate changes in total islet cholesterol. Our data show that addition of exogenous cholesterol increased total islet cholesterol levels in both β-cell specific StAR knock-out and control islets (Figure 2B). In addition, StAR mRNA levels were elevated upon cholesterol accumulation in control, but not in β-cell specific StAR knock-out islets (Figure 2C), the latter suggesting no compensatory upregulation of StAR in non β-cells of the islet.

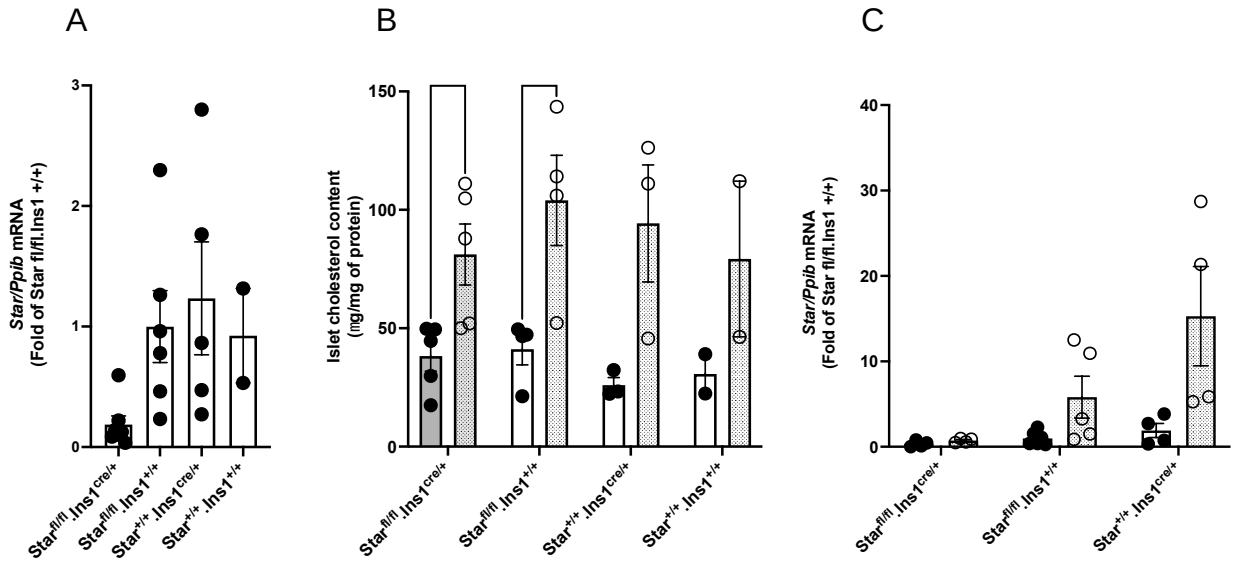

Figure 2: (A) StAR mRNA expression in islets from β-cell specific StAR knock-out and control mice. 0.5 mM cholesterol treatment (open circles) increases (B) total islet cholesterol content compared to vehicle treatment (solid circles), and (C) expression of Star mRNA in islets from control but not β-cell specific StAR knock out ($Star^{fl/fl}.Ins1^{cre/+}$) mice.

3) **Development of a model of early-stage T1D:** In the last 6 months, we have been working on developing a mouse model of early-stage T1D, wherein mice will exhibit mild β-cell dysfunction and/or loss before apparent hyperglycemia. We performed a pilot study in lean C57BL/6J male mice that received once daily i.p. injections of 1 of 3 low doses of streptozotocin (STZ; 30, 55 or 75 mg/kg body weight) for 3 consecutive days. We measured fed plasma glucose levels before and 4 weeks after the STZ injections, after which we isolated islets for measurement of glucose-stimulated insulin secretion (GSIS) and insulin content. For the 30 and 55 mg/kg STZ doses, our data suggest that 30 mg/kg but not 55 mg/kg STZ results in the maintenance of both normal blood glucose (Figure 3A) and GSIS (Figure 3B, 3D). In contrast, insulin content is significantly reduced in mice treated with 30 mg/kg STZ (Figure 3C), suggesting there is some loss of β cells. Similarly, insulin content is reduced in mice treated with 55 mg/kg STZ (Figure 3C), resulting in levels significantly lower than that observed in islets from 30 mg/kg STZ-treated mice (Figure 3C). The 75 mg/kg STZ dose resulted in marked islet toxicity, with significant loss of β cells, as demonstrated with insulin immunostaining (Figure 3E); thus, very few islets were available for GSIS and insulin content measures.

From these data, we have determined that the 30 mg/kg STZ dose will be sufficient to examine the cholesterol-induced toxicity on residual β-cells in early stages of diabetes before the manifestation of frank hyperglycemia.

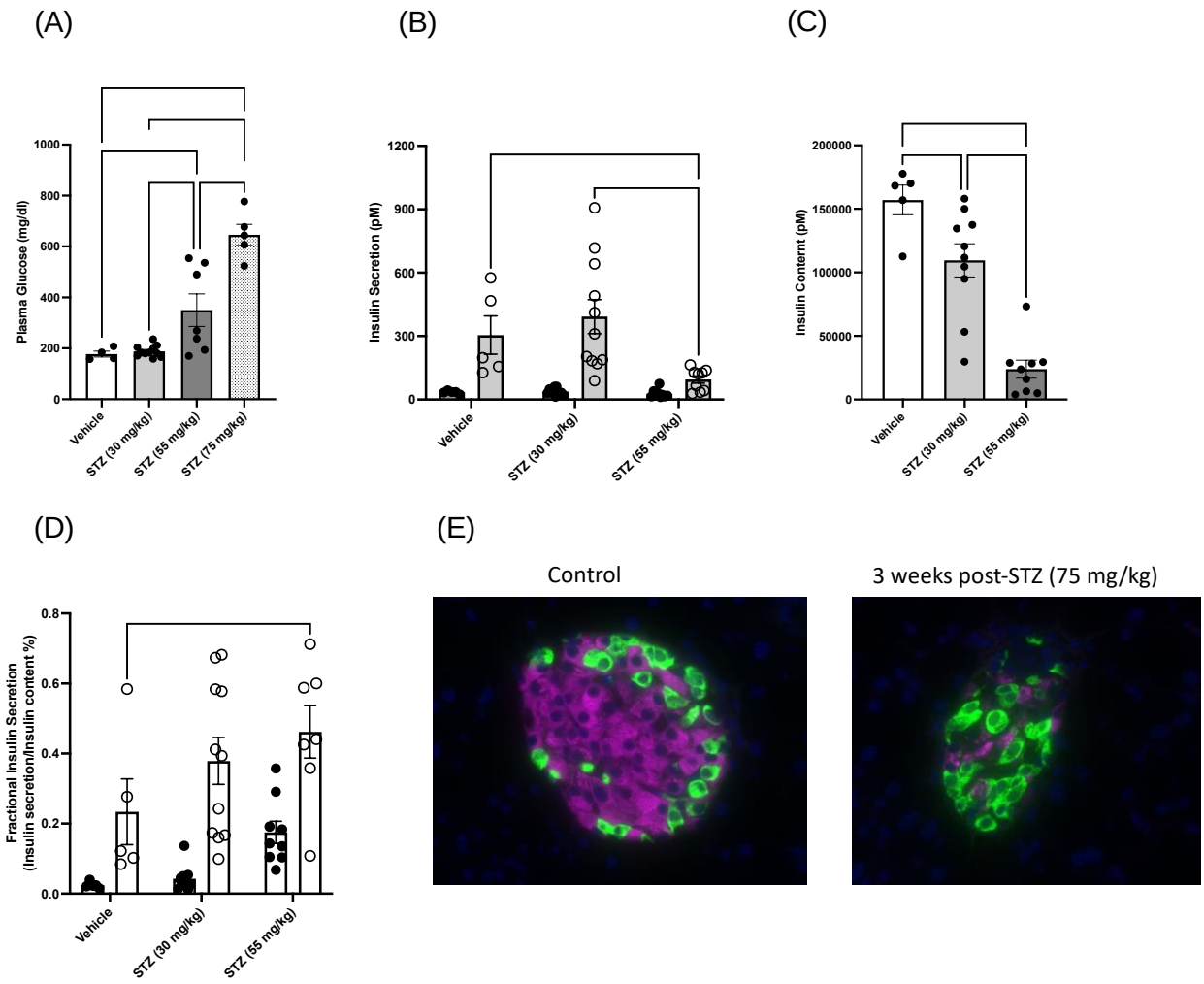

Figure 3: (A) Fed plasma glucose levels, (B) insulin secretion in response to 2.8 mM (solid circle) and 20 mM glucose (open circle), (C) insulin content and (D) fractional insulin secretion in response to 2.8 mM (solid circle) and 20 mM glucose (open circle) of islets from mice treated with vehicle, 30 mg/kg or 55 mg/kg STZ. (E) Insulin (pink) and glucagon (green) immunostaining of islets from mice treated with 75 mg/kg STZ.

4) ***Ex vivo* treatment of islets from STZ-treated mice with cholesterol:** Having established a suitable early-stage T1D model, we sought to determine the effect of *ex vivo* cholesterol treatment of islets on Star mRNA expression. Isolated islets from 30 mg/kg STZ- or vehicle-treated mice were treated with vehicle or 0.5 mM cholesterol for 24 h, after which qPCR was performed to measure Star mRNA levels. Previously, we observed that increased cholesterol accumulation in islets increased StAR expression and was associated with mitochondrial dysfunction (unpublished data). Our preliminary data now suggest that in the absence of cholesterol, there is a trend for increased Star mRNA expression in islets from STZ-treated mice compared to islets from vehicle-treated mice (Figure 4, grey bars). In addition, in the presence of cholesterol, Star mRNA expression appears to be further elevated in islets from both STZ- and vehicle-treated mice (Figure 4, green bars). We are now in the process of measuring GSIS and insulin content in cholesterol- and vehicle-treated islets.

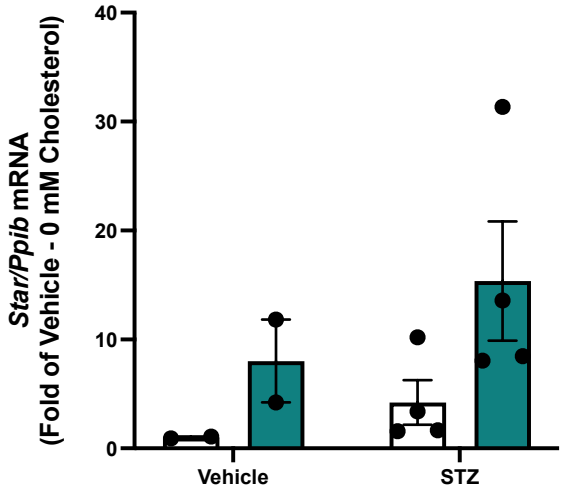

Figure 4: Expression of Star mRNA in vehicle- (grey bars) or 0.5 mM cholesterol-treated (green bars) islets from mice injected with vehicle or 30 mg/kg STZ.

Next Steps: We will treat β-cell specific StAR knock-out mice with low-dose STZ and expose isolated islets from these mice to cholesterol *ex vivo* in order to evaluate the role of StAR in mediating cholesterol-induced β-cell dysfunction and/or loss.

5) **Overexpression of CYP27A1 in β cells.** We designed a lentiviral vector (Lt.Cyp27a1) to overexpress CYP27A1 driven by an insulin (Ins2) promoter in β cells (Figure 5A). To determine whether it can be used to upregulate the expression of CYP27a1 in isolated islets, we performed a dose-response study in which C57BL/6J islets were transduced with increasing doses of Lt.Cyp27a1 for 20 h and then cultured for an additional 24 h in 11.1 mM glucose. Our preliminary data suggest that we can successfully transduce islets with Lt.Cyp27a1 resulting in the upregulation of Cyp27a1 mRNA in a dose-dependent manner (Figure 5B).

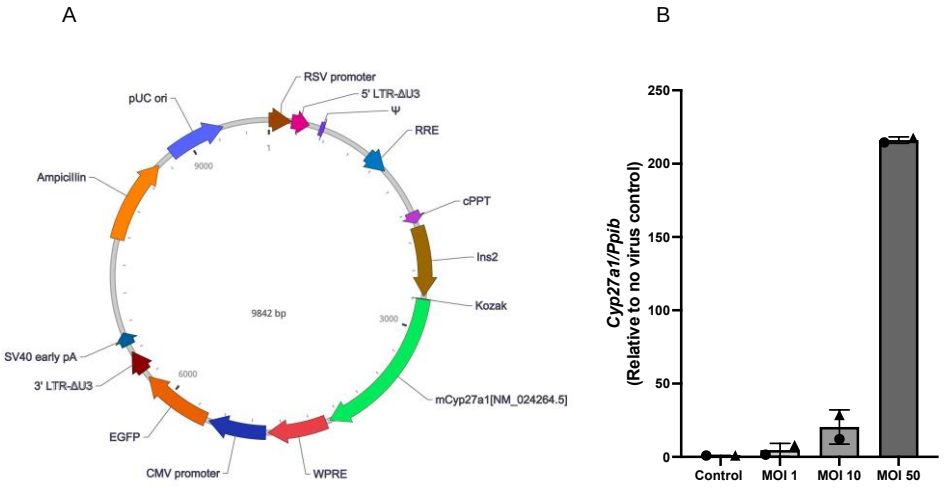

Figure 5: (A) Lentiviral vector designed to overexpress CYP27a1 in β cells. (B) Expression of Cyp27a1 mRNA with different multiplicity of infections (MOIs).

Next Steps: We will use Lt.Cyp27a1 to overexpress CYP27a1 in islets from STZ- or vehicle-treated mice and then treat them with cholesterol to evaluate whether CYP27A1 overexpression can rescue StAR-mediated mitochondrial dysfunction and improve β-cell function.

Concluding Remarks: We thank the Diabetes Research Connection for their support of our project. In addition, we are tremendously grateful to all the donors for their generous contributions to our research. We hope our studies will show that it is possible to protect residual β cells in T1D from dysfunction and ultimately will have a beneficial effect on the quality of life of individuals with T1D.