Nr4a1 deletion with maternal overnutrition affects diabetic onset in offspring

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Type 2 diabetes is a metabolic disorder that results in β-cells dysfunction and leads to impaired glucose homeostasis. High rates of proliferation and differentiation of pancreatic β-cells occurs mostly during neonatal development. Proliferation decreases with age and only small amounts of β-cell growth are seen after adolescence. Therefore, initial development of β-cells is crucial to ensure proper pancreatic development. It has been suggested that early lifestyle factors, such as diet, play a significant role in the maturation of β-cells and the onset type 2 diabetes. Specifically researchers have studied how the maternal diet can affect early onset of diabetes in offspring. Researchers have seen early diabetic onset in offspring as a consequence of maternal malnutrition and overnutrition. The proposed mechanisms responsible for early diabetes onset may be associated with altered β-cell development and β-cell mitochondrial dysfunction. Previous research has shown orphan nuclear receptor Nr4a1 is involved in β-cells development and knockout causes a 50% decrease in β-cells mass. The purpose of this study is to determine if the Nr4a1 orphan nuclear receptor is necessary for development of pancreatic β-cell mass and to determine how maternal overnutrition affects this mechanism.

Introduction

Type 1 and Type 2 diabetes affects more than 9% of the American population and the incidences of diabetes continue to increase at a startling rate. Specifically, the overall incidences of type 2 diabetes are increasing rapidly. Type 2 diabetes results from dysfunctional β-cells and causes glucose imbalance. Both type 1 and type 2 diabetes impede β-cell function (insulin secretion) by destroying β-cell mass. Management for type 2 diabetes focuses on insulin secretion and glucose control and β-cell function and mass are overlooked as a treatment option. Alterations in β-cell function and mass may potentially improve glycemic levels in a type 2 diabetic.

Previously type 2 diabetes has been viewed as adult onset diabetes. However, the incidences of type 2 diabetes in younger populations are of particular concern. It has been suggested that early lifestyle factors play a significant role in the maturation of β-cells and the onset type 2 diabetes. Specifically researchers have studied how the maternal diet can affect early onset of diabetes in offspring. Various studies have seen early diabetic onset in offspring as a consequence of maternal obesity and insulin resistance. Likewise, studies have shown an increase incidence of type 2 diabetes and decreased β-cell mass and function. Ongoing studies strongly suggest a correlation between maternal nutrition and the early onset of diabetes in offspring.

We have previously shown that orphan nuclear receptor Nr4a1 is sufficient to induce β-cell proliferation in primary rat islets. We have also seen knockout of Nr4a1 causes a 50% decrease in β-cell mass at 13 days of age. These results suggest Nr4a1 is necessary for initial β-cell differentiation and proliferation. Our preliminary data suggest that Nr4a1 is necessary for mitochondrial respiration and β-cell growth. Knockdown of

Nr4a1 impairs mitochondrial respiration and decreases β-cell function and growth. Our hypothesis is that the Nr4a1 orphan nuclear receptor is necessary for development of pancreatic β-cell mass by maintaining mitochondrial activity, and incidences of maternal overnutrition may negatively affect this mechanism. The specific objectives of this project are as follows:

1. To examine the mechanism by which Nr4a1 maintains and enhances β-cell mass in the β-cell specific Nr4a1 knock out mouse model.
2. To determine if maternal overfeeding affects islet mass and β-cell function in offspring with Nr4a1 deletion.

Literature Review

Type 2 diabetes is a metabolic disorder that results in β-cells dysfunction and leads to impaired glucose homeostasis. The incidence of type 2 diabetes is increasing rapidly and is currently the seventh leading cause of death in the United States. The large increase in incidence is often related to poor diet, obesity, and a more sedentary lifestyle. Several reports indicate that type 2 diabetes is becoming more prevalent in adolescents and such reports indicate that types 2 diabetes accounts for 80% of diabetes cases among Japanese children. The average age of diagnosis is estimated at 13.5 year but has been seen as early as 8 years of age (1-6).

With greater incidence of early onset type 2 diabetes, researchers have looked at the lasting effects of maternal nutrition on offspring. Researchers have found a strong correlation between maternal overnutrition and childhood obesity and diabetic onset (7,8). Researchers have also linked maternal malnutrition with decreased β-cell mass, impaired glucose-stimulated insulin secretion, increased oxidative stress and defected

mitochondriogenesis (9). Both spectrums, overnutrition versus malnutrition, explore the importance of maternal nutrition on offspring. Most data focuses on low-protein, global calorie restricted, and high fat diets. All three feeding models exposed offspring to metabolic stress and result in impaired early development of β-cells. As evidenced by Rodiguez-Trejo et al. β-cell dysfunction and glucose intolerance can develop as a result of poor maternal nutrition. Using a controlled animal study, researchers were able to show a low protein maternal diet can decrease pancreatic weight and mean size of β-cell aggregates (10). Changes to the β-cell could be in response to the various genes that are altered through maternal nutrition. Researchers found that offspring islet expression of *Hnf1a, Hnf4a, Pdx1, Isl1, Rfx6,* and *Slc2a2* were affected by a prenatal low protein diet. These genes are involved in β-cell differentiation and overexpression could lead to premature differentiation and dysfunction. Researchers hypothesize these physical changes to the rat β-cell could predispose the rat for early development of type 2 diabetes.

Other research supports the conclusion that diet alterations during neonatal development negatively impact pancreatic growth. Yokomizo et al. concludes a high-fat maternal diet induces insulin resistance and distorts β-cell function. Animal studies show a maternal high-fat diet raises blood glucose levels and decreases insulin sensitivity in offspring (11). Other studies have confirmed these results and have seen similar results with low-protein diets (10,12,13). Overall, these results show the importance of maternal diet on the development of pancreatic β-cells and the susceptibility of offspring to develop type 2 diabetes.

High rates of proliferation and differentiation of pancreatic β-cells occurs mostly during neonatal development (14,15). Proliferation decreases with age and only small

amounts of β-cell growth is seen after adolescence. Researchers found that infants, adolescents and adult offspring when exposed to overnutrition and obesity show decreased β-cell mass and function and overall results in pancreatic impairment (16-19). Therefore, initial development of β-cells is crucial to ensure proper pancreatic development.

Researchers believe the effects of maternal diet on early onset of diabetes may be linked to mitochondria changes within the β-cell. Altered mitochondrial function negatively affects glucose stimulated insulin secretion, as the mitochondria are greatly involved in this signaling pathway. Insulin secretion and ATP production are closely related and depend on each other. Mitochondrial dysfunction could lead to reduced ATP production and therefore reduced insulin secretion. In addition to the role maternal diet plans on mitochondria change, Nr4a1 plays a key role in cell cycle progression and recent studies demonstrate that Nr4a1 is essential for metabolic and mitochondrial activity. Nr4a1 expression level varies depending on metabolic fuels provided. Studies show that glucose, free fatty acids, cholesterol and LDL all upregulate Nr4a1 expression (20,21). Other research shows Nr4a1 induces glucose metabolism and many studies have shown Nr4a1 regulates glucose utilization (22,23). Together these data demonstrate the important role orphan nuclear receptor Nr4a1 plays on mitochondrial activity. Our preliminary data demonstrates that knockdown of Nr4a1 decreases β-cell proliferation rates and impedes mitochondrial respiration in the 832-13 cell line. However, the role that Nr4a1 plays in embryonic β-cell proliferation is currently unknown. Given the relationship between mitochondrial activity and insulin secretion, we hypothesize that knockdown of Nr4a1 impairs mitochondrial respiration and decreases embryonic β-cell function and growth.

In conclusion, many studies have revealed the correlation between maternal diets, mitochondrial function and early onset of diabetes in offspring. Researchers evidently show maternal nutrition plays a key role in the development and function of β-cells. Alterations to the maternal diet can alter β-cell development and can promote diabetes in offspring. It is also inevitable that that Nr4a1 expression plays a key role in mitochondrial function and that mitochondrial dysfunction plays a key role in diabetes onset. The mitochondria is intimately involved in energy metabolism and insulin secretion and therefore any disturbances would results in impaired glucose homeostasis. Overall these studies together demonstrate the importance of early lifestyle intervention in order to ensure proper pancreatic β-cell growth.

Proposed Methods

Animal Husbandry

Mating pairs of Nr4a1 floxed-Cre male ($Nr4a1^{fl/fl}$ MIP-CRE/ERTM) mice carrying one copy of the Cre transgene and virgin 2 month old conditional Nr4a1 floxed female ($Nr4a1^{fl/fl}$) mice will be randomly divided into standard chow fed (SD-TD.06416 10% kcal fat, 70% kcal carbohydrate) or high fat diet fed groups (HFD-TD.06414 60% kcal fat, 21% kcal carbohydrate). Males will be removed from the cage once the timed pregnancy is verified by vaginal plug. Females will be injected with a dose of tamoxifen (2mg/40g body weight in corn oil) at e13.5 and e14.5 (*47*). This will result in Cre mediated Nr4a1 deletion in pups carrying the Cre/ERTM transgene, while transgene deficient pups and dames will have normal Nr4a1 expression. The outcome of the Nr4a1 β-cell deficient pups and the control pups will be ascertained, with gender matched littermates used as controls. We will compare the effect of Nr4a1 β-cell deletion in female and male population at birth, with and

without maternal high fat feeding.

Neonatal Weight

Pups fed from a standard chow and a high fat chow fed dams will be weighed at birth. This will allow us to determine if Nr4a1 deletion has a total body affect prior to birth.

Circulation Blood Glucose and Insulin

Pups will be harvested at birth and blood will be collected to measure glucose, insulin and c-peptide levels of the different groups at birth (24). These tests will help determine the effect of Nr4a1 β-cell specific deletion combined with maternal overnutrition.

Islet Composition and Mass

Pancreata will be harvested at birth and will be used for histological sections. Insulin and hematoxylin (counter) staining will be used to evaluate structure and configuration. Total β-cell mass will be determined from the cross-sectional insulin staining. Immunostaining for insulin, glucagon, and somatostatin will be done in order to determine islet composition. Dams will be given BrdU in drinking water (0.8 mg/ml) for on week before birth to determine cell proliferation rate.

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