Synopsis of the Ph.D. thesis on

Studies on Genotype-Phenotype Correlation in Type II Diabetics and Evaluation of Melatonin and DPP-IV Inhibitor on Experimental Diabetic Models

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Introduction

Diabetes Mellitus (DM) is an endocrine disorder comprising of insulin resistance, pancreatic β-cell failure, abnormally high blood glucose levels, and a reduced incretin effect. DM is classified into two main categories: type 1 (an autoimmune disease in juvenile with a lack of insulin production causing hyperglycemia) and type 2 (a metabolic disorder resulting from the body's inability to respond to the physiological levels of insulin or relative deficiency of insulin causing hyperglycemia and thereby insulin resistance). Glucotoxicity (high glucose levels) along with lipotoxicity (high free fatty acids) induces nitro-oxidative stress and pro-inflammatory signals, in turn activating pro-inflammatory cytokines eventually leading to insulin resistance. Insulin resistance is a fundamental defect that precedes β-cell failure in type 2 diabetes (T2D) (Saiem Al-Dahr and Jiffri, 2010). Insulin resistance is majorly caused due to defect in the insulin signaling mechanism in the peripheral tissues (liver, skeletal muscle, and adipose tissue). Progressive deterioration of β-cell function and mass is a central parameter in the development of T1D and T2D. Therefore, it is pertinent to develop novel therapeutic approaches that could stop or even reverse deterioration of β-cell function and mass.

Tumor Necrosis Factor-Alpha (TNF-α):

TNF-α, a pro-inflammatory cytokine expressed in adipose tissue of obese animals and human subjects is implicated in the induction of insulin resistance in obesity and T2D (Cao *et al*., 2008). TNF-α suppresses insulin induced tyrosine phosphorylation of insulin receptor and its substrates affecting insulin sensitivity (Hotamisligi *et al*., 1995). Asian Indians have a higher percentage of body fat and lower muscle mass for a given BMI compared with Caucasians / African Americans, and also there is a high tendency for deposition of ectopic fat (Misra *et al*., 2013). Such a body composition of Indians is partly responsible for predisposition to obesity and insulin resistance (Bhardwaj *et al*., 2011). It is well-known that T2D is a multifactorial and polygenic disorder. Several single nucleotide polymorphisms (SNPs) in *TNFA* promoter (-238 G/A, -308 G/A, -857 C/T, and -863 C/A) were found to be involved in the pathogenesis of T2D in different ethnicities (Banerjee *et al*., 2014). These SNPs were found to be associated with increased *TNFA* transcript levels (Kroeger *et al*., 1997), BMI, and plasma lipids. In addition, -238G/A and -308G/A polymorphisms of *TNFA* were associated with altered circulating free fatty acid levels, and insulin resistance in obese subjects with T2D (Fontaine-Bisson *et al*., 2007). Hence, we aimed to explore the role of TNF-α towards T2D susceptibility in Gujarat population.

Melatonin:

Changing lifestyle trends such as a tendency to nocturnality and intake of high calorie diet cause disturbance of the sleep/wake cycle along with other circadian rhythms. Melatonin, a tryptophan derived small indolic molecule is mainly secreted by the pineal gland locally and several other tissues including pancreas (Reiter *et al*., 1991; Stefulj *et al*., 2001). Melatonin was investigated mostly for its role in sleep and circadian regulation, including acute effects as well as circadian phase-shifting effects (Jung-Hynes *et al*., 2010). However, in recent years its role in glucose homeostasis, T2D risk and its treatment has received increasing attention. This is partly because of the identification of T2D risk variants in *MTNR1B* (Melatonin Receptor 1B), and due to the adverse impact of circadian disruption on glucose metabolism (Mulder *et al*., 2009). Melatonin, a hormone of darkness also has antioxidant (Galano *et al*., 2011) and anti-inflammatory properties (Chahbouni *et al*., 2010). There might be an association between melatonin and T2D based on the findings that insulin secretion is inversely proportional to plasma melatonin concentration (Peschke *et al*., 2013). Melatonin mediates its action via two receptors: MT1 (MTNR1A) and MT2 (MTNR1B), which are also expressed in pancreatic islets (Ramracheya *et al*., 2008). In rodent ß-cells, the predominant effect of melatonin is in the reduction of insulin release through inhibition of the Gi–cAMP–PKA or cGMP pathways. Moreover, studies on human islets suggest that melatonin increases intracellular calcium levels via IP3 signaling and stimulates glucagon and insulin release from α-cells and ß-cells, respectively (Ramracheya *et al*., 2008). Insulin secretion and ß-cell survival are reported to be improved in response to melatonin signalling, by decreasing proteotoxicity- induced cell apoptosis and oxidative stress in human islets exposed to chronic hyperglycemia and in islets from patients with T2D (Costes *et al*., 2015). Studies on rodents have suggested that melatonin administration reduces body fat and HbA1c levels, increases GLUT4 expression and insulin sensitivity in peripheral tissues in diet induced obese T2D mouse model (Karamitri and Jockers, 2019). According to GWAS study and meta-analysis, the rs4753426 and rs10830963 SNPs in the *MTNR1B* locus are associated with increased fasting plasma glucose levels and impaired insulin secretion, as well as increased risk of T2D (Prokopenko *et al*., 2008; Bouatia-Naji *et al*., 2009; Zhan *et al*., 2015). The phenotype of rs10830963 risk allele carriers includes increased *MTNR1B* transcript levels, altered melatonin secretion and possibly further effects associated with the enhancer activity of the region surrounding the rs10830963 SNP (Gaulton *et al*., 2015). Hence, the present study was aimed to explore the association of *MTNR1B* SNPs and melatonin levels with T2D susceptibility in Gujarat population and the potential beneficial effects of melatonin administration in different diabetic models.

DPP-IV (Dipeptidyl peptidase-IV)

During the past two decades, intensive investigation of the incretin hormone glucagon-like peptide-1 (GLP-1) led to the development of two categories of novel therapeutic agents for T2D: GLP-1 agonists and DPP-IV inhibitors (Lee and Jun, 2014; Holst and Madsbad, 2016). DPP-IV inhibitors such as sitagliptin prevent the degradation of GLP-1 and another incretin GIP, and hence elevate endogenous incretin levels. GLP-1 and GIP produced by intestinal L and K cells respectively augment glucose stimulated insulin secretion (GSIS) by pancreatic β-cells and inhibit glucagon secretion from α-cells. In addition, GLP-1 as well as GLP-1 based drugs were found to promote beta cell expansion in various mouse models (Takeda *et al*., 2012; Drucker, 2013). Although GLP-1-based drugs were found to ameliorate T2D (Buteau *et al*., 2003), they showed marginal therapeutic effects in T1D human subjects and rodent models, possibly due to their restricted immune regulatory effects (Hadjiyanni *et al*., 2008). Further, sitagliptin was the first oral DPP- IV inhibitor approved by the FDA for the treatment of T2D in October 2006. In long-term clinical studies, sitagliptin demonstrated its pharmacodynamic efficacy in reducing HbA1c levels, fasting blood glucose levels and improving postprandial glucose levels (Dhillon, 2010). Sitagliptin was also reported to have a protective effect on islet graft size by reducing CD4 T-cells homing into the islets after islet transplantation (Kim, *et al*., 2008; Kim *et al*., 2009) and also led to β-cell regeneration and reduced insulin resistance in diabetic rodents (Poucher et al., 2012; Shen *et al*., 2018).

Hypothesis

From the above, we have hypothesized that *TNFA* and *MTNR1B* polymorphisms along with altered melatonin levels could be associated with T2D risk in Gujarat population by altering the metabolic parameters, plasma TNF-α and free fatty acid (FFA) levels. We have also hypothesized that melatonin (M) and DPP-IV inhibitor-sitagliptin (S) alone, and in combination (S+M) treatment could ameliorate diabetic manifestations in different experimental diabetic models by promoting β-cell regeneration, glucose homeostasis and insulin sensitivity in a synergistic/ additive manner.

Significance of the Study

The proposed study would help in understanding the role of *TNFA* and *MTNR1B* polymorphisms towards T2D risk in Gujarat population. Association studies of SNPs present in these genes would help to identify risk haplotypes for disease susceptibility for developing prognostic markers and personalized treatment strategies for T2D. Further, it will provide a clear understanding of the involvement of melatonin along with DPP-IV inhibitor in inducing

pancreatic β-cell regeneration. This information eventually can be of use in translational research for development of targeted drug therapy.

Proposed Objectives:

1. **To investigate genotype-phenotype correlation of *TNFA* and *MTNR1B* polymorphisms with T2D susceptibility in Gujarat population.**
   (a) To assess *TNFA* polymorphisms: promoter -238 G/A(rs361525), -308 G/A(rs1800629), -857 C/T(rs1799724) and -863C/A (rs1800630).
   (b) To assess *MTNR1B* polymorphisms: promoter -1193 C/T (rs4753426), 5' UTR G/C (rs10830962) and intron C/G (rs10830963).
   (c) To monitor plasma TNF-α, FFA, melatonin levels, and *TNFA* transcript levels in PBMCs.

2. **To study the *in-vitro* effect of melatonin, sitagliptin and combination treatment on β-cell proliferation in mouse pancreatic islets**
   (a) To study dose and time dependent effect of melatonin, sitagliptin, and combination treatment on mouse pancreatic β-cell proliferation.
   (b) To study effect of melatonin, sitagliptin, and combination treatment on mouse pancreatic β-cell proliferation under glucotoxic stress.
   (c) To study effect of melatonin, sitagliptin, and combination treatment on mouse pancreatic β-cell proliferation under gluco-lipotoxic stress.

3. **To study the effect of melatonin, sitagliptin and combination treatment on pancreatic β-cell proliferation in chemically induced diabetic mice.**
   (a) To establish streptozotocin induced T1D mouse model.
   (b) To evaluate glucose tolerance.
   (c) To study β-cell regeneration by β-cell proliferation, neogenesis and trans-differentiation.
   (d) To assess β-cell apoptosis.

4. **To study the effect of melatonin, sitagliptin and combination treatment on High Fat Diet (HFD) induced diabetic manifestations.**
   (a) To establish HFD induced T2D mouse model.
   (b) To evaluate glucose tolerance and insulin sensitivity.
   (c) To estimate of plasma levels of insulin, leptin and melatonin, and lipid profile.
   (d) To study transcript levels of glucoregulatory enzymes in liver.
   (e) To study expression of proteins involved in insulin signaling pathway in skeletal muscle.
   (f) To assess pancreatic β-cell mass.

5. **To study the effect of melatonin, sitagliptin and combination treatment in improving islet graft function & proliferation.**
   a) To evaluate glucose tolerance
   b) To evaluate β-cell proliferation

Results:

<u>Objective 1</u>: To investigate genotype-phenotype correlation of *TNFA* and *MTNR1B* polymorphisms with T2D susceptibility in Gujarat population.

To assess genotype-phenotype correlation of *TNFA* and *MTNR1B* polymorphisms with T2D susceptibility, plasma and PBMCs were separated from 3 ml venous blood drawn from ethnically matched 478 diabetic patients and 502 age-matched non-diabetic individuals. Genomic DNA and RNA were isolated from PBMCs. PCR-RFLP was used for genotyping of *TNFA* and *MTNR1B* polymorphisms and qPCR to estimate *TNFA* transcript levels. TNF-α, FFA, and melatonin concentrations were estimated from plasma samples by ELISA. Plasma lipid profile and FBG levels were assessed by commercially available kits and Accu-Chek glucometer, respectively. T2D subjects recruited for the study displayed FBG levels>125 mg/dL. This study was conducted according to the declaration of Helsinki and was approved by the Institutional Ethical Committee for Human Research (IECHR), Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India (FS/IECHR/2013/1). *p* values less than 0.05 were considered significant for all the association analysis. The statistical power of detection of the association of *TNFA* and *MTNR1B* polymorphisms with the disease at the 0.0125 and 0.017 level of significance (Bonferroni's correction) respectively was determined by using the G* Power software. Genetic association studies of *TNFA* revealed the involvement of *TNFA* -857 C/T in T2D patients (p<0.0001), ii) 2.072 fold increase in *TNFA* transcript levels in PBMCs of patients and, increased plasma TNF-α (p=0.0122) particularly in obese patients (p=0.0405), increased plasma FFA (p=0.0215) and, iii) association of *TNFA* -238 G/A with body mass index (BMI) (p=0.0270) and, -857 C/T with FBG (p=0.0122) and triglycerides (TG) (p=0.0015). Correlation analysis suggests that TNF-α concentrations are positively correlated with BMI (r=0.3, p=0.04) and negatively correlated with HDL (r=-0.39, p=0.001) while the FFA concentrations are positively correlated with BMI (r=0.35, p=0.0004). Our results on *MTNR1B* association studies suggest that none of the *MTNR1B* polymorphisms were associated with T2D in our population (p>0.05), however, *MTNR1B* intron C/G GG genotype was significantly associated with elevated FBG levels (p=0.0029). Further, melatonin levels were significantly reduced in T2D patients as compared to controls (p<0.001). It can be concluded that reduced leves of melatonin, elevated TNF-α and FFA levels along with the genetic variants of *TNFA* & *MTNR1B* alter metabolic profile which could be a potent risk factor towards T2D in Gujarat population.

<u>Objective 2</u>: To study the *in-vitro* effect of melatonin, sitagliptin and combination treatment on β-cell proliferation in mouse pancreatic islets.

An *in-vitro* system was designed to assess the preliminary therapeutic effects of melatonin, sitagliptin and combination treatment on pancreatic β-cells under glucotoxic and gluco-lipotoxic stress. C57BL/6J male mice islets were used for the study. Pancreatic islets were isolated by collagenase digestion. Islets were dispersed by 0.05% Trypsin treatment. Cells were exposed to basal glucose (5.5mM), high glucose (25mM) and palmitate (250µM) for 48 hrs to induce glucotoxicity and gluco-lipotoxicity, respectively. Cells were fixed in 4% formaldehyde and β-cell proliferation (Ki67/Insulin co-positive) was assessed by immunocytochemistry (ICC) using confocal microscopy. For the dose and time dependent study, three different doses of sitagliptin (1µM, 10µM and 100µM) and melatonin (1nM, 10nm, 100nM) were used for 24 or 48 hrs. Dose and time dependent studies in C57BL/6J mice pancreatic islets suggest that the maximum effective dose (MaxED) and minimum effective dose (MinED) to induce β-cell proliferation were 100µM - 48 hrs sitagliptin; 10nM melatonin - 24 hrs and 10uM sitagliptin - 48 hrs; 1nM melatonin - 48 hrs, respectively. MaxED showed no additive or synergistic effect in the combination (S+M) treated group. However, MinED dose showed additive effect of sitagliptin and melatonin on β-cell proliferation. Further, MinED dose was selected for glucotoxic and gluco-lipotoxic studies. Under glucotoxic stress, the monotherapies and combination treated islets showed a significant rise in β-cell proliferation ($p<0.001$). In addition, under gluco-lipotoxic stress, a significant increase in β-cell proliferation was observed in the monotherapies ($p<0.05$) and combination ($p<0.001$) treated islets but relatively lower as compared to the islets under glucotoxic stress. According to our observation, the number and size of the cells under gluco-lipotoxic stress were decreased as compared to the cells under glucotoxic stress. We conclude that pancreatic β-cells under glucotoxic and gluco-lipotoxic stress conditions when subjected to the monotherapies and combination therapy, the combination treatment could additively induce β-cell proliferation.

<u>Objective 3:</u> To study the effect of melatonin, sitagliptin and combination treatment on pancreatic β-cell proliferation in chemically induced diabetic mice.

After observing an additive effect of sitagliptin and melatonin on β-cell proliferation *in-vitro*, we wanted to study its effect in streptozotocin (STZ)-induced T1D mouse model. To address whether the age-related decline in melatonin and β-cell proliferation affects β-cell regeneration capacity, two different age groups were selected for the study i.e., 8 weeks young and 30 weeks old BALB/c male mice. 8 weeks young and 30 weeks old (n=30 each) male BALB/c mice were randomly divided into five groups: 1) Non-diabetic Control 2) Diabetic Control [DC] 3)

Sitagliptin [S] treated 4) Melatonin [M] treated and 5) Sitagliptin + Melatonin [S+M] treated. Mice were administered with streptozotocin (50 mg/Kg BW, i.p. for 5 consecutive days to induce T1D. T1D was confirmed with two consecutive readings of non-fasting blood glucose levels greater than 350 mg/dL. The mice were treated with sitagliptin (5g/Kg diet) and/ melatonin (0.5 mg/Kg BW, i.p.) daily for 6 weeks along with BrdU (100 mg/Kg BW, i.p.). Body Weight (BW) and FBG were measured once a week throughout the experiment. Intraperitoneal Glucose Tolerance Test (IPGTT) was performed after 6 weeks of treatment. Thereafter, mice were sacrificed, and the pancreas were harvested for immunohistochemical (IHC) studies for β-cell regeneration (β-cell proliferation, neogenesis and trans-differentiation) and apoptosis. Our results in young T1D mice suggest that the monotherapies and the combination treatment significantly reduced FBG levels (S, $p<0.05$; M, $p<0.01$; S+M, $p<0.01$) by increasing insulin levels (M, $p<0.05$; S+M, $p<0.001$) with a concomitant increase in glucose tolerance (S, $p<0.05$; M; $p<0.05$; S+M, $p<0.01$, respectively) as compared to DC group. IHC analysis revealed that the monotherapies and combination therapy induced β-cell proliferation (S, $p<0.01$; M, $p<0.001$, S+M, $p<0.001$), neogenesis (S, $p<0.001$; M, $p<0.01$, S+M, $p<0.001$), trans-differentiation (S, $p<0.05$; M, $p>0.05$; S+M, $p<0.05$), and reduced apoptosis ($TUNEL^+$ cells: S, M, and S+M; $p<0.001$, AIF (apoptosis inducing factor) translocation: S, M, and S+M; $p<0.001$). Further, our results in old T1D mice suggest that combination therapy significantly increase insulin levels ($p<0.01$), and glucose tolerance (S, $p<0.01$; M, $p>0.05$; S+M, $p<0.001$) with a concomitant reduction in blood glucose levels (S, M, S+M; $p<0.001$) as compared to DC. However, there was no significant difference observed between initial and final blood glucose levels in the M group ($p>0.05$). IHC analysis revealed that the combination therapy had an additive effect in promoting β-cell proliferation (S, $p<0.01$; M, $p<0.05$, S+M, $p<0.001$), neogenesis (S, $p<0.01$; M, $p<0.05$, S+M, $p<0.001$) and trans-differentiation (S, M, S+M; $p<0.001$). Although melatonin treatment was not as effective as sitagliptin treatment in β-cell proliferation and neogenesis, significant additive effect was observed in S+M group in old diabetic mouse model. Moreover, all the treated groups showed significant decrease in apoptosis ($TUNEL^+$ cells: $p<0.001$; AIF translocation: $p<0.001$) as compared to the DC group. To conclude, while sitagliptin is potent in inducing β-cell neogenesis and trans-differentiation, melatonin promotes β-cell proliferation in young and old T1D mice. Interestingly, combination therapy (S+M) additively brings about glucose homeostasis in T1D mouse model by inducing β-cell regeneration and decreasing β-cell loss.

<u>Objective 4:</u> To study the effect of melatonin, sitagliptin and combination treatment on High Fat Diet (HFD) induced diabetic manifestations.

As the combination treatment helped in combating β-cell loss in T1D mouse model, it was interesting to study whether this combination could also improve T2D manifestations. To investigate the effects of S+M in T2D, an insulin resistant diet induced T2D model (C57BL/6J male mice) was established. 32 animals were fed with high fat diet (HFD) and 8 animals were fed standard laboratory normal chow diet (NCD) for 25 weeks. After the confirmation of T2D by measuring two consecutive readings of FBG>200mg/dL, the treatment of sitagliptin (5g/Kg in diet) and melatonin (10mg/Kg BW, i.p.) and the combination (S+M) was administered for four weeks. BW and FBG were measured once weekly. IPGTT and intra-peritoneal insulin tolerance test (IPITT) were measured after four weeks of treatment. 1.5 ml blood was collected, and tissues (pancreas and insulin sensitive peripheral tissues) were harvested to assess various parameters. Our results suggest that combination therapy (p<0.001) was superior to the monotherapies (S, p<0.01; M, p<0.01) in reducing FBG levels, increasing glucose tolerance (S, p<0.01; M, p<0.05; S+M p<0.001) and insulin sensitivity (S, p>0.05; M, p>0.01; S+M, p<0.001). The BW of HFD group were significantly increased as compared to NCD group (p<0.001). The monotherapies and combination therapy did not show a significant change in the final BW as compared to their initial BW (p>0.05). Moreover, assessment of food and water intake revealed that there was a significant reduction in food (HFD, S, M, S+M; p<0.001) and water intake (HFD, p<0.001; S, p<0.01; S+M; p<0.001) after 4 weeks of the treatment as compared to NCD group. However, the water intake was significantly higher in M group as compared to HFD group (p<0.05). In addition, plasma lipid profile (TG, TC, HDL-c, and LDC-c) were restored in the monotherapies and combination treated groups as compared to HFD group (TG and TC, p<0.05; HDL-c and LDL-c, p<0.01). Assessment of plasma insulin, leptin and melatonin levels revealed that there was hyperinsulinemia and hyperleptinemia in HFD group as compared to NCD (p<0.01), and the levels were restored in the treatment groups (insulin: p<0.05; leptin: S+M, p<0.05). There was no change observed in melatonin levels, however, it was significantly increased in the M group (p<0.05). Further, transcript levels and specific activity of glucoregulatory enzymes from liver were monitored. Glucokinase (GCK) transcript levels and activity were significantly increased in S (p<0.001) and S+M (p<0.01) as compared to HFD group. The transcript levels and activity of Fructose 1-6, bisphosphatase (FBPase) and Phosphoenol pyruvate carboxykinase (PEPCK) were significantly increased in HFD group (p<0.05 and p<0.01, respectively) and significantly reduced in all the treatment groups (p<0.05 and p<0.01, respectively) indicating reduced gluconeogenesis. The glycogen synthase transcript levels and the glycogen content were significantly increased in all the treatment groups as compared to HFD (p<0.001) indicating an increase in glycogenesis. Also,

the transcript levels and activity of glycogen phosphorylase were reduced in all three treatment groups as compared to HFD (S, M, $p<0.01$; S+M, $p<0.001$) indicating reduced glycogenolysis. The transcript levels of GLUT2 was significantly reduced (S, S+M, $p<0.001$) and Glucose -6-phosphatase (G6Pase) was significantly increased in respective treatment groups (M, $p<0.001$) indicating increased glucose uptake. In addition, transcript levels of *SIRT1* and *PGC1α* which are involved in mitochondrial biogenesis pathway showed significant increase in all the treatment groups as compared to HFD ($p<0.001$). Furthermore, protein expression of IR1β in skeletal muscle was significantly reduced in HFD ($p<0.05$) but was significantly increased in S+M treated group ($p<0.05$). Insulin sensitivity will be further evaluated by western blot analysis of pIRS/IRS, pAkt/Akt and GLUT4 proteins involved in insulin signaling pathway from the skeletal muscle and the results will be shown in the thesis. There was no change in islet number ($p>0.05$) however, β-cell mass was significantly increased in S+M group ($p<0.001$). From the above results, we conclude that the combination therapy is able to ameliorate HFD-induced T2D manifestations by improving glucose and lipid metabolism, increasing β-cell mass, increasing insulin and leptin sensitivity in the peripheral tissues, and elevating mitochondrial biogenesis.

<u>Objective 5:</u> To study the effect of melatonin, sitagliptin and combination treatment in improving islet graft function & proliferation.

This experiment will be completed, and the results and discussion will be shown in the thesis.

Conclusion: Our population studies suggest that the elevated TNF-α and FFA levels, the reduced levels of melatonin, along with the genetic variants of *TNFA* & *MTNR1B* alter metabolic profile which could be a potent risk factor towards T2D in Gujarat population. Our *in-vitro* studies suggest that the combination treatment of melatonin and sitagliptin on mouse pancreatic islets could additively induce β-cell proliferation under glucotoxic and gluco-lipotoxic stress as compared to monotherapies. In addition, our *in-vivo* studies suggest that the combination therapy can ameliorate T1D and T2D in mouse models by inducing β-cell regeneration, glucose regulation and increasing insulin sensitivity. In conclusion, we suggest that melatonin promotes β-cell proliferation whereas sitagliptin promotes β-cell neogenesis and in concert both the drugs enhance β-cell regeneration. The results are summarized in Figure 1.

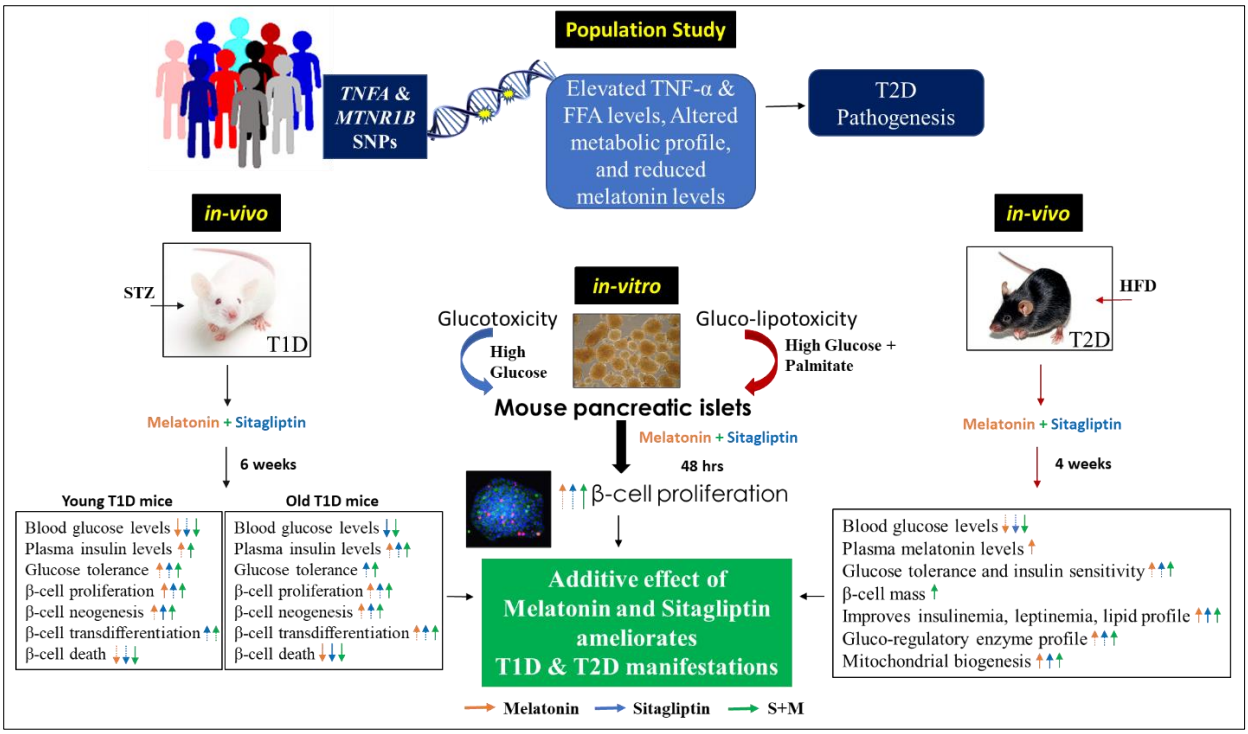

Figure 1: Our population study revealed that polymorphisms in *TNFA* and *MTNR1B* are associated with elevated TNF-α levels, FFA levels, altered metabolic profile and reduced plasma melatonin levels, thus contributing towards the risk of T2D. Our *in-vitro* study revealed that melatonin and sitagliptin in combination shows an additive effect in inducing pancreatic β-cell proliferation upon an exposure to glucotoxic and gluco-lipotoxic stress. Further, the combination treatment showed additive effect as compared to monotherapies in ameliorating glycemic dysregulation by increasing insulin levels, glucose tolerance and β-cell regeneration, and reducing β-cell apoptosis in T1D mouse model. In addition, the combination therapy also showed its potential in ameliorating T2D manifestations by improving glucose and lipid metabolism, increasing β-cell mass, increasing insulin and leptin sensitivity in the peripheral tissues, and elevating mitochondrial biogenesis.

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Achievements:

1. Awarded DST International Travel Support for poster presentation at American Diabetes Association (ADA) 79th Scientific Sessions, USA, 2019
2. Awarded Council of Scientific & Industrial Research - Senior Research Fellowship (SRF), 2018.

Publications:

1. **Patel R**, Palit SP, Rathwa N, Ramachandran AV, Begum R (2019). Genetic variants of Tumor Necrosis Factor-α and its levels: A Correlation with Dyslipidemia and Type 2 Diabetes Susceptibility. *Clin. Nutr*. 38:1414-1422. (IF:6.40)
2. **Patel R**, Rathwa N, Palit SP, Ramachandran AV, Begum R (2018). Association of melatonin & MTNR1B variants with type 2 diabetes in Gujarat population. *Biomed. Pharmacother*. 103:429-434. (IF:3.74)
3. **Patel R**, Dwivedi M, Mansuri MS, Laddha NC, Thakker A, Ramachandran AV, Begum R (2016). Association of neuropeptide-Y (NPY) and interleukin-1beta (IL1B), genotype-phenotype correlation and plasma lipids with Type-II diabetes. *PloS One*. 11:e0164437. (IF:3.057)
4. Rathwa N, **Patel R**, Palit SP, Parmar N, Rana S, Ansari MI, Ramachandran AV, Begum R. (2020). β-Cell Replenishment: Possible Curative Approaches for Diabetes Mellitus. *Nutr Metab Cardiovasc Dis*. 30:1870-1881. (IF: 3.70)

5. Rathwa N, Parmar N, Palit SP, **Patel R**, Ramachandran AV, Begum R**.** (2020) Intron specific polymorphic site of vaspin gene along with vaspin circulatory levels can influence pathophysiology of type 2 diabetes. *Life Sci*. 243:117285. (IF: 3.64)
6. Palit SP, **Patel R**, Jadeja SD, Rathwa N, Mahajan A, Ramachandran AV, Dhar MK, Sharma S, Begum R. (2020) A genetic analysis identifies a haplotype at adiponectin locus: Association with obesity and type 2 diabetes. *Sci Rep*. 10:2904. (IF:3.99)
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8. Rathwa N, **Patel R**, Palit SP, Ramachandran AV, Begum R (2019). Genetic variants of resistin and its plasma levels: Association with obesity and dyslipidemia related to Type 2 Diabetes susceptibility. *Genomics*.111:980-985. (IF: 6.20)
9. Pramanik S, Rathwa N, **Patel R**, Ramachandran AV, Begum R (2018). Treatment Avenues for Type 2 diabetes and Current perspectives on Adipokines. *Curr Diabetes Rev*. 14:201-221.

Manuscripts under preparation:

1. A novel drug combination of melatonin and DPP-IV inhibitor promotes β-cell regeneration and insulin sensitivity.
2. Melatonin as a diabetic therapeutant: A boon or a bane?

Oral/ Poster presentations:

1. **Patel R,** Palit SP, Rathwa N, Parmar N, Dhimmar H, Pancholi DA, Ramachandran AV, Begum R. Melatonin and DPP-IV inhibitor. An untrodden path towards regenerating β-cells. Poster presentation delivered at NextGen Genomics, Biology, Biochemistry and Technologies (NGBT) Conference (Sep 30th to 2nd Oct 2019) at Taj Lands End, Mumbai India **(*Received Best Poster Award*)**.
2. **Patel R**, Pramanik S, Rathwa NN, Parmar NR, Dhimmar H, Pancholi DA, Ramachandran AV, Begum R. Melatonin and DPP-IV inhibitor: A novel combinatorial approach for β-cell regeneration. Poster presentation delivered at American Diabetes Association 79th Scientific Sessions (7-11 June 2019) at Moscone Center, San Francisco-94103, California, USA **(*Received DST International Travel Award for poster presentation*)**.
3. **Patel R**, Rathwa N, Palit SP, Parmar N, Dhimmar H, Ansarullah, Vasu V, Ramachandran AV, Begum R. β-cell regenerative potential of melatonin and DPP- IV inhibitor in amelioration of T1D. Oral presentation delivered at International Conference on Reproduction, Endocrinology and Development (19-21 January 2019) at School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India.
4. **Patel R**, Rathwa N, Palit SP, Parmar N, Ansarullah, Ramachandran AV, Begum R. Replenishing β-cells with Melatonin & DPP-IV inhibitor: An in-vivo study. Poster presentation delivered at International Conference on 'Proteins, miRNA and Exosomes In Health and Diseases' held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11 -13 December 2018.
5. **Patel R**, Rathwa N, Palit SP, Parmar N, Dhimmar H, Ansarullah, Ramachandran AV, Begum R. Assessment of Therapeutic Potential of Melatonin And DPP-IV Inhibitor On β-

Cell Regeneration in Diabetic Mouse Model. Oral presentation delivered at International Conference on Reproductive Physiology and Comparative Endocrinology (ICRPCE) & The 36th meeting of SRBCE, 20 – 22 January 2018 held at BITS Pilani, KK Birla Goa Campus, Goa, India.

6. **Patel R**, Ramachandran AV, Begum R. Evaluation of Melatonin, MTNR1B and TNFA in Type II Diabetics. Poster presentation delivered at International Conference on Reproductive Biology and Comparative Endocrinology & The 35th Annual Meeting of The Society for Reproductive Biology and Comparative Endocrinology, 9-11 February 2017 held at Department of Animal Biology, University of Hyderabad, Hyderabad, India.
7. **Patel R**, Dwivedi M, Mansuri MS, Ansarullah, Laddha NC, Ramachandran AV, Begum R. Involvement of Neuropeptide-Y and Interlukin-1 beta in pathogenesis of Type 2 Diabetes: A Genotype-Phenotype Correlation Study. Poster presentation delivered at Two-day National Symposium on Omics…to Structural Basis of Diseases, 30 Sept. and 1 Oct 2016 held at The M. S. University of Baroda, Vadodara, Gujarat, India.
8. **Patel R**, Mansuri MS, Ansarullah, Ramachandran AV, Pathak J, Begum R. Involvement of Melatonin & Melatonin Receptor 1B in the pathogenesis of Type 2 Diabetes in Gujarat population: A Genotype-Phenotype correlation study. Poster presentation delivered at International conference on bioactive chemicals for reproduction and human health, 26-28 Feb 2015 held at Devangere University, Devangere, India.
9. **Patel R**, Mansuri M, Parasrampuria M, Bendre A, Ansarullah, Ramachandran AV, Begum R. Role of Melatonin Receptor 1B (MTNR1B) in Type 2 Diabetes: Case Study in Gujarat Population. Poster presentation delivered at Three-day national symposium on Emerging Trends in Biochemical Sciences, Dec 2014 at the Department of Biochemistry, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India (***Received Second Prize***).
10. Palit SP, **Patel R,** Rathwa N, Parmar N, Dalvi N, Ramachandran AV, Begum R. L-glutamine and Pitavastatin: resuscitating the dying β-cells. Poster presentation delivered at NextGen Genomics, Biology, Biochemistry and Technologies (NGBT) Conference (Sep 30th to 2nd Oct 2019) at Taj Lands End, Mumbai India.
11. Rathwa N, **Patel R,** Palit SP, Parmar N, Ramachandran AV, Begum R. GABA in combination with CR as possible therapeutic approach for ameliorating insulin resistance and favoring β-cell regeneration in Type 2 Diabetes. Poster presentation delivered at NextGen Genomics, Biology, Biochemistry and Technologies (NGBT) Conference (Sep 30th to 2nd Oct 2019) at Taj Lands End, Mumbai India.
12. Parmar N, **Patel R,** Palit SP, Rathwa N, Shetty S, Patel N, Ramachandran AV, Begum R. Evaluation of genetic variants of Leptin and Leptin Receptor as risk factors for T2D in Gujarat population. Poster presentation delivered at NextGen Genomics, Biology, Biochemistry and Technologies (NGBT) Conference (Sep 30th to 2nd Oct 2019) at Taj Lands End, Mumbai India **(*Received Best Poster Award*)**.
13. Rathwa NN, **Patel R**, Pramanik S, Parmar NR, Ramachandran AV, Begum R. Calorie restriction in combination with GABA ameliorates type 2 diabetes. Poster presentation delivered at American Diabetes Association 79th Scientific Sessions (7-11 June 2019) at Moscone Center, San Francisco-94103, California, USA.
14. Palit SP, **Patel R**, Rathwa N, Dalvi N, Ramachandran AV, Begum R. L-glutamine and Pitavastatin: a therapeutic approach to revive the insulin gold mine. Poster presentation

delivered at ICRED- 2019, 37th Annual Conference of the International Conference on Reproductive Biology and Comparative Endocrinology (19-21 January 2019) at School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India **(*Received Best Poster Award*)**.

15. Rathwa N, Parmar N, Palit SP, **Patel R**, Dhimmar H, Ramachandran AV, Begum R. Genetic Variants of Omentin-1 and Vaspin: Association with Type 2 Diabetes Susceptibility. Poster presentation delivered at International Conference on Reproduction, Endocrinology and Development (19-21 January 2018) School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India.
16. Rathwa N, **Patel R**, Palit SP, Parmar N, Ansarullah, Bhaskaran RS, Ramachandran AV, Begum R. Therapeutic potential of γ-aminobutyric acid and calorie restriction in type 2 diabetic mouse model. Poster presentation delivered at International Conference on Reproduction, Endocrinology and Development (19-21 January 2018) School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India.
17. Pramanik S, **Patel R**, Rathwa N, Ramachandran AV, Begum R. Haplotype at adiponectin locus and its remarkable association with type 2 diabetes. Poster presentation delivered at International Conference on 'Proteins, miRNA and Exosomes in Health and Diseases' held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11th - 13th December 2018 (***Received Second Prize***).
18. Rathwa N, Palit SP, **Patel R**, Dhimmar H, Ramachandran AV, Begum R. Genetic Variants of Omentin-1 and its levels: Association with Type 2 Diabetes Susceptibility in Gujarat population. Poster presentation delivered at International Conference on 'Proteins, miRNA and Exosomes in Health and Diseases' held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11-13 December 2018.
19. Rathwa N, Parmar N, Palit SP, **Patel R**, Ramachandran AV, Begum R. Association of Vaspin levels and its Genetic Variants with Type 2 Diabetes Susceptibility. Poster presentation delivered at International Conference on 'Proteins, miRNA and Exosomes in Health and Diseases' held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11-13 December 2018.
20. Rathwa N, Palit SP, **Patel R**, Dhimmar H, Bhati H, Parmar N, Ramachandran AV, Begum R. Genetic Variants of Omentin-1 And Vaspin: Association with Obesity and Dyslipidemia Related to Type 2 Diabetes Susceptibility. Poster presentation delivered at International Conference on Reproductive Physiology and Comparative Endocrinology (ICRPCE) & The 36th meeting of SRBCE, 20 – 22 January 2018 held at BITS Pilani, KK Birla Goa Campus, Goa, India.
21. Pramanik S, **Patel R**, Rathwa N, Patel N, Rana S, Ramachandran AV, Begum R. "Adiponectin: a watchdog in inflammation induced metabolic disorder" Poster presentation delivered at "Immunocon-2017. 44th Annual Conference of the Indian Immunology Society (IIS)", 14-16 December 2017 held at Institute of Science, Nirma University, Ahmedabad, Gujarat, India (***Received Best Poster Award***).
22. Palit SP, Rathwa N, **Patel R**, Rana S, Patel N, Ramachandran AV, Begum R Association of Adiponectin and Resistin genetic variants with Type 2 Diabetes. Poster presentation delivered at Two-day National Symposium on Omics…to Structural Basis of Diseases, 30 Sept. and 1 Oct. 2016 held at The M. S. University of Baroda, Vadodara, Gujarat, India.

Participation in Workshop:

- Participated in Wellcome Trust/ DBT India sponsored workshop on "Science Communication" at the Department of Microbiology and Biotechnology Centre, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India on 11th March 2016.

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