Chapter 7
Conclusions

Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia, insulin resistance, pancreatic β-cell failure, reduced incretin effect and disturbed circadian rhythm. DM is classified into two main categories: type 1 (a juvenile autoimmune disease lacking insulin production causing hyperglycemia) and type 2 (a metabolic disorder resulting in insulin resistance leading to hyperglycemia). Glucotoxicity (high glucose) along with lipotoxicity (high free fatty acids) induces nitro-oxidative stress which activates pro-inflammatory cytokines eventually leading to insulin resistance. Insulin resistance is an underlying cause that precedes β-cell failure in type 2 diabetes (T2D) (Saiem Al-Dahr and Jiffri, 2010). Insulin resistance arises due to defects in the insulin signalling mechanism in the peripheral tissues such as liver, skeletal muscle and adipose tissue. Progressive deterioration of β-cell function and mass is a crucial 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.

Our studies aimed to focus on the association of *TNF-α* and *MTNR1B* polymorphisms with T2D, plasma TNF-α, FFA, melatonin levels, and their genotype-phenotype correlation with T2D in Gujarat population. Further, the therapeutic potential of melatonin (M); dipeptidyl peptidase-IV (DPP-IV) inhibitor, sitagliptin (S); and the combination (S+M) treatment was evaluated in T1D and T2D *in vitro* and *in vivo* experimental mouse models, and humanised euglycemic mouse model of islet transplantation.

Our results on population studies suggest that genetic variants of *TNF-α* & *MTNR1B* along with reduced levels of melatonin, elevated TNF-α and FFA levels alter the metabolic profile which could be a potent risk factor towards T2D in Gujarat population.

Our *in vitro* studies suggest that pancreatic β-cells under glucotoxic and gluco-lipotoxic stress conditions when subjected to S, M, and S+M treatment, the combination treatment could additively induce β-cell proliferation.

Our *in vivo* studies on T1D mouse models suggest that combination therapy brings about glucose homeostasis in both young and old T1D mouse models by inducing β-cell regeneration and reducing β-cell loss.

Our *in vivo* studies on T2D mouse model also suggest that combination therapy ameliorates HFD-induced T2D manifestations by improving glucose and lipid metabolism, increasing β-

cell mass, insulin and leptin sensitivity in the peripheral tissues, and elevating mitochondrial biogenesis.

Combination therapy also significantly increases human β-cell proliferation in humanized euglycemic mouse model of islet transplantation.

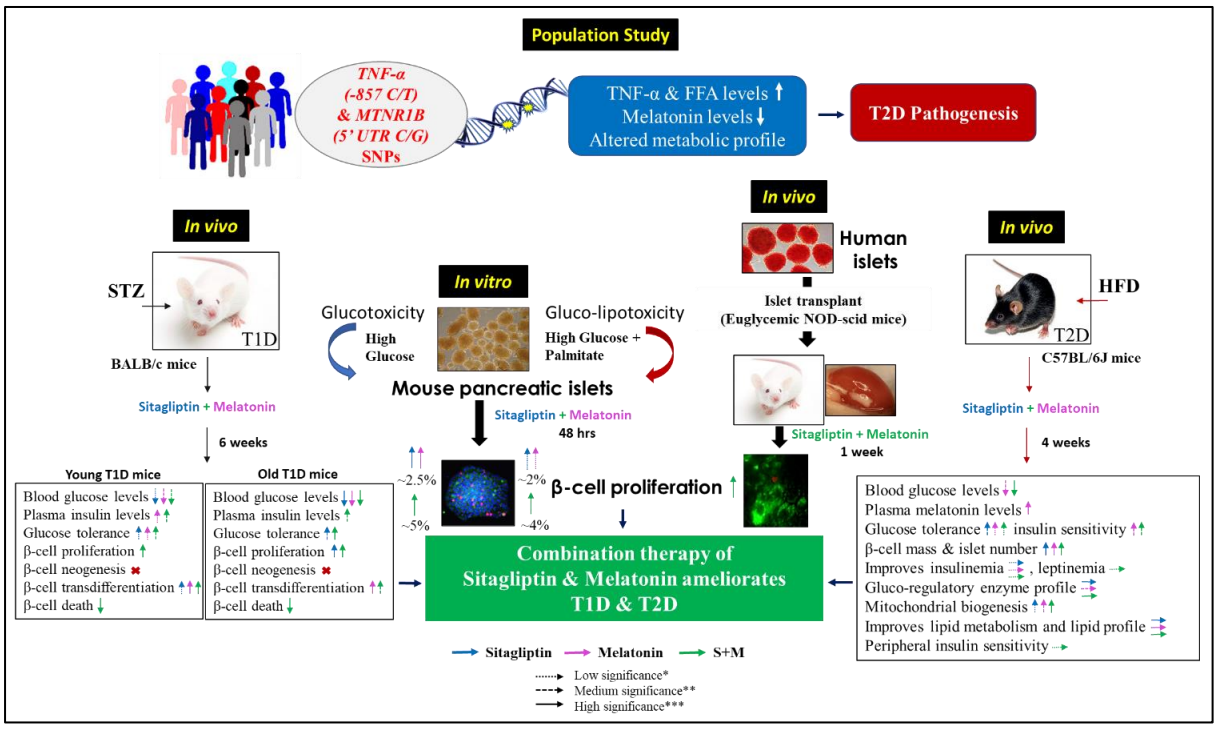

Figure 7.1 Summary. Our population studies showed that polymorphisms in *TNF-α* and *MTNR1B* are associated with elevated plasma TNF-α levels, FFA levels, reduced melatonin levels and altered metabolic profile, thus contributing towards the risk of T2D. Our *in vitro* studies revealed that melatonin and sitagliptin in combination showed an additive effect in inducing pancreatic β-cell proliferation upon exposure to glucotoxicity and gluco-lipotoxicity. Further, the combination treatment showed greater therapeutic effect as compared to monotherapies in ameliorating glycemic dysregulation by increasing insulin levels, glucose tolerance, β-cell regeneration and reducing β-cell apoptosis in T1D young and old mouse models. Also, the combination therapy exhibited 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. The combination therapy also increases human β-cell proliferation in euglycemic human islet transplant model in mice.

Sitagliptin and melatonin act via different signalling pathways in the peripheral tissues (pancreas, liver, skeletal muscle and adipose tissue) to increase insulin sensitivity and insulin secretion, modulate glyco-lipid metabolism, β-cell regeneration and survival, mitochondrial biogenesis and respiration. Besides, they also mediate anti-apoptotic, antioxidant and anti-inflammatory effects thereby increasing β-cell and islet graft survival (Fig. 7.2). Sitagliptin and melatonin in combination act parallelly in immuno-modulation, insulin secretion and

pancreatic β-cell survival. Thus, combination therapy has a greater therapeutic potential than monotherapies in ameliorating diabetes manifestations.

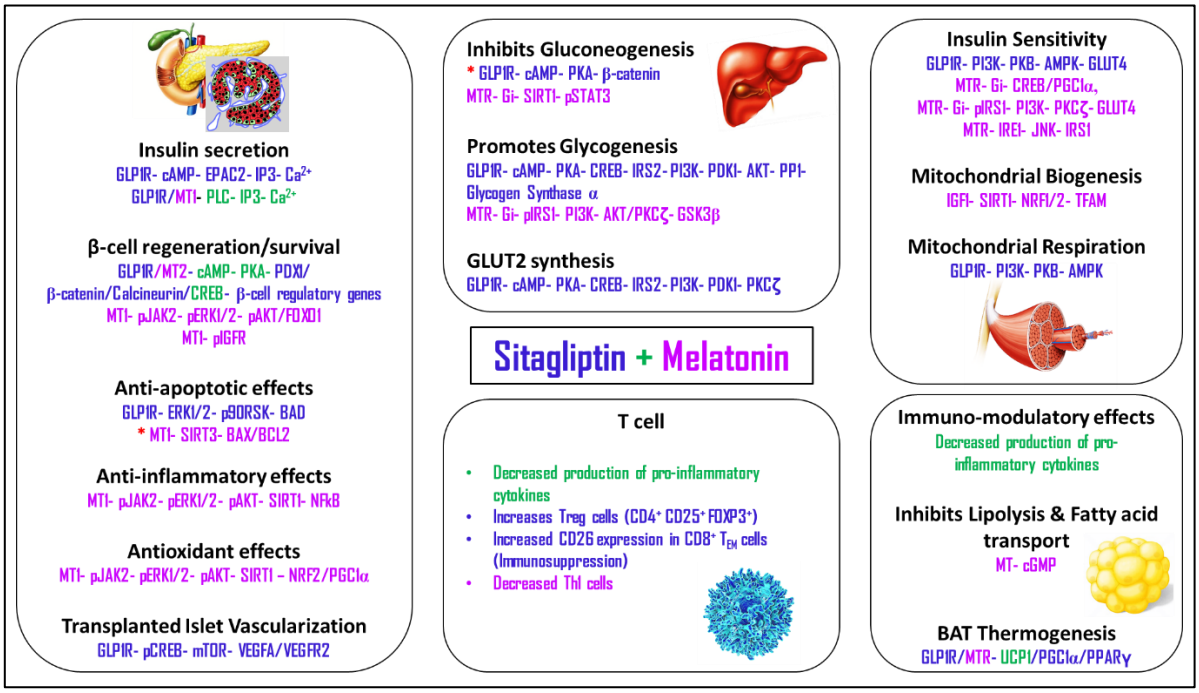

Figure 7.2 Possible mode of action of sitagliptin (independent/via GLP-1), melatonin and their combination in the amelioration of diabetes manifestations in peripheral tissues (pancreas, liver, skeletal muscle and adipose tissue). Sitagliptin mediates its action partly via increasing GLP-1 levels and through GLP-1R (Rowlands et al., 2018) on insulin secretion (cAMP/PLC-IP3-Ca^{2+}), β-cell regeneration and survival (cAMP-PKA-CREB), insulin sensitivity (PI3K-AMPK-GLU4), glycogenesis (cAMP-AKT-Glycogen synthase α), GLUT2 synthesis (cAMP-CREB-IRS2-PKCζ), inhibiting gluconeogenesis (cAMP-PKA- β catenin), BAT thermogenesis (UCP1/PGC1α/PPARγ; Shimasaki et al., 2013), mitochondrial respiration (PI3K-AKT-AMPK), and transplanted islet vascularization (pCREB-VEGFR; Samikannu et al., 2013). It also mediates anti-apoptotic (ERK1/2-p90RSK-BAD) and direct anti-inflammatory effects by increasing Treg cells, $CD8^+$ T_{EM} cells (Alonso et al., 2015). Melatonin mediates its action (Cecon et al., 2018; Karamitri and Jockers, 2019) on insulin secretion (MT1-IP3-Ca^{2+}), β-cell regeneration and survival (cAMP-PKA-CREB), insulin sensitivity (MTR-Gi-CREB/PGC1α/pIRS1-GLUT4/IRE1-JNK-IRS1), glycogenesis (MTR-Gi-pIRS1-GSK3β), inhibiting gluconeogenesis (MTR-Gi-SIRT1-pSTAT3), mitochondrial biogenesis (IGF1-SIRT1-NRF1/2-TFAM), inhibiting lipolysis (MT-cGMP), BAT thermogenesis (MTR-UCP). It also mediates antioxidant effects (MT1-pAKT-NRF2/PGC1α), anti-apoptotic effects (*MT1-SIRT1-BAX/BCL2), and anti-inflammatory effects by reducing Th1 cells and MT1-pJAK2-pERK1/2-pAKT-SIRT1-NFκB pathway. The combination therapy could mediate its action via same signalling pathway on insulin secretion, β-cell regeneration and survival, BAT thermogenesis, and anti-inflammatory effects by reducing the production of pro-inflammatory cytokines.

AMPK: AMP-activated protein kinase; BAD: BCL2 associated agonist of cell death; BAX: BCL2 associated X protein; BCL2: B-cell lymphoma 2; cAMP: Cyclic adenosine monophosphate; cGMP: Cyclic guanosine monophosphate; CREB: cAMP-response element binding protein; FOXO1: Forkhead box protein O1; GLP1R: Glucagon like peptide 1 receptor; GLUT4: Glucose transporter type 4; IGF1: Insulin-like growth factor 1; IP3: Inositol trisphosphate; IRE1: Inositol-requiring enzyme 1; IRS2: Insulin receptor substrate 2; MT1: Melatonin receptor 1; MT2: Melatonin receptor 2; MTR: Melatonin receptor; mTOR: Mammalian target of rapamycin;

NFκB: Nuclear Factor kappa-light-chain-enhancer of activated B cells; NRF1/2: Nuclear respiratory factor 1 and 2; PDK1: Pyruvate dehydrogenase kinase 1; PDX1: Pancreatic and duodenal homeobox 1; PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PI3K: Phosphoinositide 3-kinase; PKA: Protein kinase A; PKB/AKT: Protein kinase B; PKCζ: Protein kinase C zeta; PLC: Phospholipase C; PP1: Protein phosphatase 1; PPARγ: Peroxisome proliferator-activated receptor gamma; pERK1/2: Phospho-Extracellular signal-regulated protein kinases 1 and 2; pIGFR: Phospho-Insulin-like growth factor 1 receptor; pIRS1: Phospho-Insulin receptor substrate 1; pJAK2: Phospho-Janus kinase 2; p90RSK: p90 ribosomal S6 kinase; pSTAT3: Phospho-Signal transducer and activator of transcription 3; SIRT1: Sirtuin 1; $T_{EM:}$ T effector memory; TFAM: Mitochondrial transcription factor A; Th1: T helper type 1; UCP1: Uncoupling protein 1; VEGFA: Vascular endothelial growth factor A; VEGFR2: Vascular endothelial growth factor receptor 2. *Hypothesized pathway.

As mentioned in Fig. 7.2, DPP-IV could mediate its action partly via GLP-1. Gut is the primary source of GLP-1 which augments glucose-stimulated insulin secretion. Chambers et al., (2017) showed that GLP-1 is also produced in the α-cells of pancreatic islets, and is responsible for its incretin effect in mice. This raises the possibility that intra-islet GLP-1 also regulates β-cell function. Our studies support this finding as we have observed β-cell proliferation *in vitro* in mouse islets. Interestingly, DPP-IV being an adipokine may also mediate its action directly via modulating chemokines and cytokines which are crucial for β-cell survival. For instance, saxagliptin, a DPP-IV inhibitor, induces β-cell proliferation *in vitro* and *in vivo* via stromal cell-derived factor-1α (SDF-1α)- a chemokine and its receptor (CXCR4) (Li et al., 2017). Thus, we assume that sitagliptin may be working in a similar manner, though its mode of action needs to be explored.

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