Chapter 5
Conclusions

Chapter 5. Conclusions

Diabetes mellitus (DM) is a leading cause of mortality worldwide, characterized by hyperglycemia resulting from defective insulin secretion/increased cellular insulin resistance. Autoimmune-mediated destruction of pancreatic β-cells causes type 1 diabetes (T1D). On the contrary, type 2 diabetes (T2D) is manifested by insulin resistance in peripheral tissues due to the impaired insulin signalling (Kahn, 2003). Obesity-induced insulin resistance and secretion defects are the major risk factors for T2D (Kahn, 2003). Glucotoxicity and lipotoxicity trigger nitro-oxidative stress, activating pro-inflammatory cytokines such as leptin, TNF-α, and resistin, resulting in insulin resistance. β-cell failure in T2D is preceded by insulin resistance, hyperinsulinemia, and chronic hyperglycemia (Saiem Al-Dahr and Jiffri, 2010). Defects in the insulin signaling pathway within peripheral tissues, such as the liver, skeletal muscle, and adipose tissue, give rise to insulin resistance. The progressive decline in β-cell function and mass is a critical factor in the ontogenesis of T1D and T2D. Thus, it is imperative to devise therapeutic strategies that could effectively prevent or reverse the progressive decline of β-cell function and mass.

The study was sought to explore the potential beneficial effects of melatonin (M), γ-Aminobutyric acid (GABA), and the combination (M+G) treatment in T1D and T2D rodent models. Further, the study aimed to investigate *LEP* and *LEPR* genetic variants and their transcript levels in PBMCs, protein levels in plasma, and genotype-phenotype correlation with various metabolic parameters and T2D susceptibility in Gujarat population.

The results of *in vivo* studies on T1D mouse model suggest that monotherapies are as effective as combination therapy, which alleviate glucose homeostasis in T1D mouse models by promoting β-cell regeneration and reducing β-cell loss.

Further, the results of *in vivo* studies on T2D mouse model indicate that that monotherapies as effective as combination therapy in ameliorating HFD-induced T2D symptoms by enhancing glucose and lipid metabolism, insulin sensitivity, mitochondrial biogenesis, and restoring β-cell mass in peripheral tissues such as the liver, skeletal muscle, and adipose tissue.

The findings on genetic association study revealed that the genetic variant of *LEPR rs1137101 A/G*, along with elevated leptin and decreased sOb-R protein levels, could pose a risk towards T2D susceptibility in Gujarat population (Fig. 5.1).

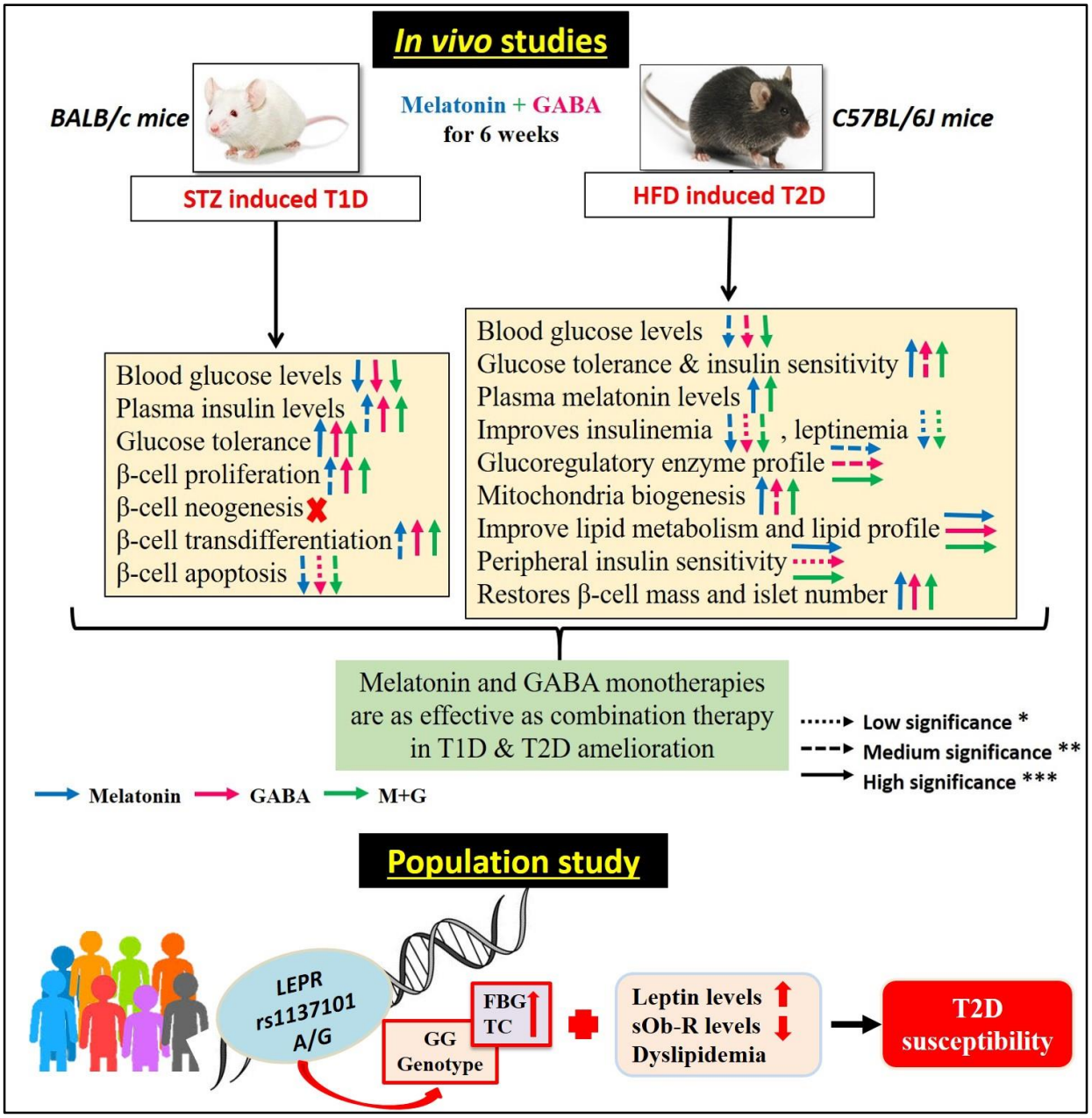

Figure 5.1. Summary. The *in vivo* studies revealed that monotherapies are as effective as combination therapy in ameliorating glycemic dysregulation by increasing insulin levels, glucose tolerance, β-cell regeneration, and reducing β-cell apoptosis in T1D mouse model. Moreover, the monotherapies and combination therapy revealed their potential to ameliorate T2D manifestations by improving glucose and lipid metabolism, increasing insulin sensitivity in the peripheral tissues, elevating mitochondrial biogenesis, and restoring β-cell mass. Further, the genetic association studies showed that the genetic variant of *LEPR rs1137101 A/G*, along with elevated leptin and decreased sOb-R protein levels, could pose a risk towards T2D susceptibility in Gujarat population.

[Abbreviations: T1D: Type 1 diabetes; T2D: Type 2 diabetes; FBG: Fasting blood glucose; TC: Total cholesterol; STZ: Streptozotocin; HFD: High-fat diet; sOb-R: Soluble leptin receptor]

Further, regenerative therapies that were effective in rodents were generally not so in humans, e.g., Glucagon-like peptide 1 receptor (GLP-1R) agonists (Hadjiyanni et al., 2008; Mittermayer et al., 2017). However, these effects of melatonin and GABA were first observed in mice (Tian et al., 2004, 2014, Kanter et al., 2006), and it also appears valid in humans, as demonstrated by our group and others (*in vitro* and xenotransplanted human islets in mice) (Tian et al., 2013; Purwana et al., 2014; Patel et al., 2022), suggesting the potential applications for treating diabetic patients. We assume that combination therapy sub-additively ameliorates T1D and T2D diabetic manifestations, as monotherapies are found as effective as combination therapy. Toews and Bylund (2005) reported that if the sum of the responses to the two drugs individually is greater than the maximal response possible by the system, then sub-additivity occurs without any true interaction between the drugs. Further, we believe that the reasons for getting a sub-additive effect could be: i) the selected dose of melatonin and/or GABA might be the maximum effective doses, or ii) there is no true interaction between melatonin and GABA in the system.

The limitation of our study is that we have not looked into the mechanistic aspect of the melatonin or GABA's mode of action. Unlike GABA, most of the pathways by which melatonin exerts its pleiotropic effects are well known (Karamitri and Jockers, 2019; Cecon et al., 2018). However, we have tried to summarize here, the already known and possible hypothesized mode of action of melatonin and GABA based on the previous studies which substantiate our findings. The possible mode of action of melatonin and GABA and their combinatorial effects on the amelioration of diabetes manifestations in the peripheral tissues (e.g., pancreas, liver, skeletal muscle, and adipose tissue) are shown in Fig. 5.2.

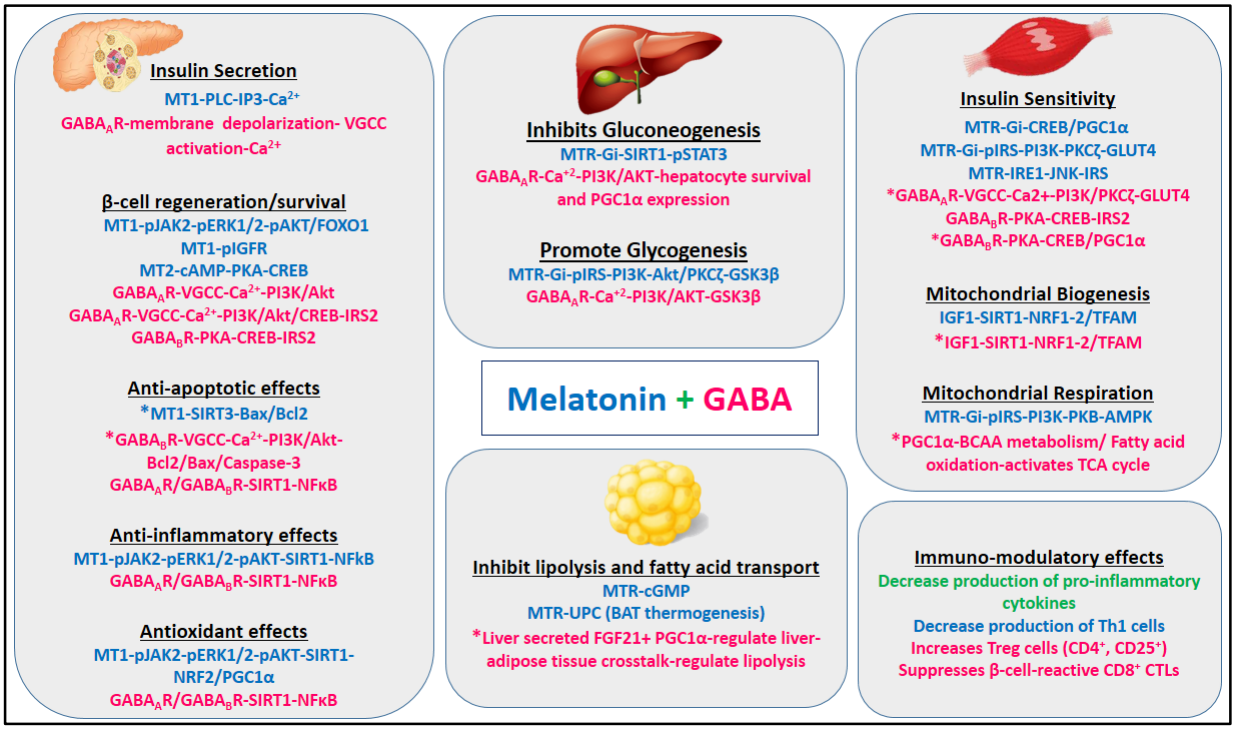

Figure 5.2. Possible mode of action of melatonin, GABA and their combinations in the amelioration of diabetes manifestations in the peripheral tissues (pancreas, liver, skeletal muscle and adipose tissue). Melatonin mediates its action (Cecon et al., 2018; Karamitri and Jockers, 2019) on insulin secretion (MT1-IP3-Ca^{2+}), β-cell regeneration and survival (cAMP-PKA-CREB; pJAK2-pERK1/2-pAKT/FOXO1), insulin sensitivity (MTR-Gi-CREB/PGC1α; pIRS1-GLUT4; IRE1-JNK-IRS1), glycogenesis (MTR-Gi-pIRS1-GSK3β), inhibits gluconeogenesis (MTR-Gi-SIRT1-pSTAT3), mitochondrial biogenesis (IGF1-SIRT1-NRF1/2-TFAM), mitochondrial respiration (MTR-Gi-pIRS-PI3K-PKB-AMPK), inhibits lipolysis (MT-cGMP), and 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. Melatonin decreases the production of Th1 cells. GABA mediates its action (Purwana et al., 2014; Prud'homme et al., 2014; Ye et al., 2017; Wang et al., 2017; Hatazawa et al., 2014, 2018; Befroy et al., 2007; Lira et al., 2010) on insulin secretion ($GABA_AR$ -VGCC activation-Ca^{2+}), insulin sensitivity ($GABA_BR$-PKA-CREB-IRS2; *$GABA_BR$-PKA-CREB/PGC1α; *$GABA_AR$-VGCC-Ca2+-PI3K/PKCζ-GLUT4) β-cell regeneration and survival ($GABA_AR$-VGCC activation-Ca^{2+}-pI3K/Akt; VGCC-Ca^{2+}-pI3K/Akt /CREB-IRS2; $GABA_BR$-PKA-CREB-IRS2), exerts anti-apoptotic effects ($GABA_AR$/$GABA_BR$-SIRT1-NFκB; *$GABA_BR$-VGCC-Ca^{2+}-pI3K/Akt-Bcl2/Bax/Caspase-3), anti-inflammatory effects ($GABA_AR$/$GABA_BR$-SIRT1-NFκB), glycogenesis ($GABA_AR$-Ca^{+2}-PI3K/AKT-GSK3β), inhibits gluconeogenesis (*$GABA_AR$-Ca^{+2}-PI3K/AKT-hepatocyte survival and PGC1α), inhibits lipolysis (Liver secreted $FGF21^+$ PGC1α-regulate liver-adipose tissue crosstalk-regulate lipolysis), mitochondrial biogenesis (*IGF1-SIRT1-NRF1-2/TFAM), mitochondrial respiration (*PGC1α-BCAA metabolism/ Fatty acid oxidation-activates TCA cycle). GABA suppresses β-cell-reactive $CD8^+$ CTLs and increases Treg cells ($CD4^+$, $CD25^+$). Both melatonin and GABA exert anti-inflammatory effects by reducing the production of pro-inflammatory cytokines.

[Abbreviations: AMPK: AMP-activated protein kinase; $GABA_AR$: GABA receptor type A; $GABA_BR$: GABA receptor type B; MTR: Melatonin receptor; MT1: Melatonin receptor 1; MT2: Melatonin receptor 2; BAX: BCL2 associated X protein; BCL2: B-cell lymphoma 2; cGMP: Cyclic guanosine monophosphate; Caspase-3: cysteine–aspartic acid protease; CREB: -response element binding protein; FOXO1: Forkhead box protein O1; GLUT4: Glucose transporter type 4; IGF1: Insulin-like growth factor 1; IP3: Inositol trisphosphate; IRE1: Inositol-requiring enzyme 1; IRS2: Insulin receptor substrate 2; NFκB: Nuclear Factor kappa-light-chain-enhancer of activated B cells; NRF1/2: Nuclear respiratory factor 1 and 2; 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; 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; pSTAT3: Phospho-Signal transducer and activator of transcription 3; SIRT1/3: Sirtuin 1/3; GSK3B: Glycogen Synthase Kinase 3 Beta; JNK: c-Jun N-terminal kinase; TFAM: transcription factor A, mitochondrial; BCAA: Branched-chain amino acids; VGCC: Voltage-gated calcium channels; TCA: The tricarboxylic acid; Th1: T helper type 1; Tregs: Regulatory T cells; CTLs: cytotoxic T cell.] *Hypothesized pathway.

Melatonin and GABA function in the peripheral tissues (pancreas, liver, skeletal muscle, and adipose tissue) *via* distinct signaling pathways to improve insulin sensitivity and secretion, alleviate glycolipid metabolism, promote β-cell regeneration and survival, and enhance mitochondrial biogenesis and respiration. Furthermore, they exert anti-apoptotic, antioxidant, and anti-inflammatory actions, thereby increasing β-cell survival (Fig. 5.2). However, melatonin and GABA, in combination, only act parallelly in immunomodulation. This could be the possible reason for getting a sub-additive effect. Thus, monotherapies are as effective as combination therapy in ameliorating T1D and T2D manifestations.

References:

Al-Dahr MH, Jiffri EH. Increased adipose tissue expression of tumor necrosis factor-alpha and insulin resistance in obese subjects with type II diabetes. World Journal of Medical Sciences. 2010;5:30-5.

Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, et al. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes. 2007;56(5):1376-81.

Cecon E, Oishi A, Jockers R. Melatonin receptors: molecular pharmacology and signalling in the context of system bias. British journal of pharmacology. 2018;175(16):3263-80.

Hadjiyanni I, Baggio LL, Poussier P, Drucker DJ. Exendin-4 modulates diabetes onset in nonobese diabetic mice. Endocrinology. 2008;149(3):1338-49.

Hatazawa Y, Qian K, Gong DW, Kamei Y. PGC-1α regulates alanine metabolism in muscle cells. PloS one. 2018;13(1):e0190904.

Hatazawa Y, Tadaishi M, Nagaike Y, Morita A, Ogawa Y, Ezaki O, et al. PGC-1α-mediated branched-chain amino acid metabolism in the skeletal muscle. PloS one. 2014;9(3):e91006.

Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia. 2003;46(1):3-19.

Kanter M, Uysal H, Karaca T, Sagmanligil HO. Depression of glucose levels and partial restoration of pancreatic β-cell damage by melatonin in streptozotocin-induced diabetic rats. Archives of toxicology. 2006;80(6):362-9.

Karamitri A, Jockers R. Melatonin in type 2 diabetes mellitus and obesity. Nature Reviews Endocrinology. 2019;15(2):105-25.

Lira VA, Benton CR, Yan Z, Bonen A. PGC-1α regulation by exercise training and its influences on muscle function and insulin sensitivity. American Journal of Physiology-Endocrinology and Metabolism. 2010;299(2):E145-61.

Mittermayer F, Caveney E, De Oliveira C, Fleming GA, Gourgiotis L, Puri M, et al. Addressing unmet medical needs in type 1 diabetes: a review of drugs under development. Current Diabetes Reviews. 2017;13(3):300.

Patel R, Parmar N, Rathwa N, Palit SP, Li Y, Garcia-Ocaña A, et al. A novel therapeutic combination of sitagliptin and melatonin regenerates pancreatic β-cells in mouse and human islets. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2022;1869 (8):119263.

Prud'homme GJ, Glinka Y, Udovyk O, Hasilo C, Paraskevas S, Wang Q. GABA protects pancreatic beta cells against apoptosis by increasing SIRT1 expression and activity. Biochemical and biophysical research communications. 2014;452(3):649-54.

Purwana I, Zheng J, Li X, Deurloo M, Son DO, Zhang Z, et al. GABA promotes human β-cell proliferation and modulates glucose homeostasis. Diabetes. 2014;63(12):4197-205.

Tian J, Dang H, Chen Z, Guan A, Jin Y, Atkinson MA, et al. γ-Aminobutyric acid regulates both the survival and replication of human β-cells. Diabetes. 2013;62(11):3760-5.

Tian J, Dang H, Nguyen AV, Chen Z, Kaufman DL. Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-cell

autoimmunity and promoting β-cell replication in newly diabetic NOD mice. Diabetes. 2014;63(9):3128-34.

Tian J, Lu Y, Zhang H, Chau CH, Dang HN, Kaufman DL. γ-Aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. The Journal of Immunology. 2004;173(8):5298-304.

Toews ML, Bylund DB. Pharmacologic principles for combination therapy. Proceedings of the American Thoracic Society. 2005;2(4):282-9.

Wang S, Xiang YY, Zhu J, Yi F, Li J, Liu C, et al. Protective roles of hepatic GABA signaling in acute liver injury of rats. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2017;312(3):G208-18.

Ye DW, Rong XL, Xu AM, Guo J. Liver-adipose tissue crosstalk: A key player in the pathogenesis of glucolipid metabolic disease. Chinese journal of integrative medicine. 2017;23(6):410-4.