# Melatonin in type 2 diabetes mellitus and obesity

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Abstract | Despite considerable advances in the past few years, obesity and type 2 diabetes mellitus (T2DM) remain two major challenges for public health systems globally. In the past 9 years, genome-wide association studies (GWAS) have established a major role for genetic variation within the *MTNR1B* locus in regulating fasting plasma levels of glucose and in affecting the risk of T2DM. This discovery generated a major interest in the melatonergic system, in particular the melatonin $MT_2$ receptor (which is encoded by *MTNR1B*). In this Review, we discuss the effect of melatonin and its receptors on glucose homeostasis, obesity and T2DM. Preclinical and clinical post-GWAS evidence of frequent and rare variants of the *MTNR1B* locus confirmed its importance in regulating glucose homeostasis and T2DM risk with minor effects on obesity. However, these studies did not solve the question of whether melatonin is beneficial or detrimental, an issue that will be discussed in the context of the peculiarities of the melatonergic system. Melatonin receptors might have therapeutic potential as they belong to the highly druggable G protein-coupled receptor superfamily. Clarifying the precise role of melatonin and its receptors on glucose homeostasis is urgent, as melatonin is widely used for other indications, either as a prescribed medication or as a supplement without medical prescription, in many countries in Europe and in the USA.

Melatonin is a pleiotropic hormone primarily known for its regulatory role in circadian and seasonal rhythms, sleep, retinal functions and the immune system[1]. Studies published in the past 9 years established a major role for the *MTNR1B* locus, which encodes the melatonin $MT_2$ receptor (also known as melatonin receptor type 1B), in regulating fasting plasma glucose (FPG) levels and the risk of type 2 diabetes mellitus (T2DM)[2–4]. This discovery generated a lot of interest in the metabolic effects of melatonin, particularly in its role in the development of T2DM and obesity. As a member of the G protein-coupled receptor (GPCR) family, the $MT_2$ receptor has a high potential for drug development, as a target for new therapeutics. Indeed, melatonin is already widely used for several non-metabolic indications and is also available over the counter in several countries, thus highlighting the need to better understand its metabolic effects. This Review will discuss the effect of melatonin and melatonin receptor signalling on glucose homeostasis and energy metabolism at the cellular, tissue and individual organism level.

Melatonin is unique as it can mediate different types of effects. These effects include immediate effects that occur during the night and are determined by the nocturnal secretion pattern of melatonin, prospective or delayed effects that are typically primed during the night with functional consequences during the day, chronobiotic effects that rely on the direct effect of melatonin on the circadian clock and seasonal effects that depend on the duration of the night. For instance, melatonin is involved in the regulation of sleep in diurnal species (including humans), the transition of night length information to the suprachiasmatic nuclei (SCN) and other organs[5] and the temporal organization and circadian distribution of several metabolic processes associated with energy balance and seasonal control of photoperiodic functions such as reproduction[6].

This Review will mainly focus on the immediate and delayed effects, whereas chronobiotic and seasonal effects of melatonin, although likely to contribute to the metabolic effects of melatonin, will be only briefly discussed. The contribution of melatonin to the development of obesity and T2DM and the potential of targeting the melatonin system for the treatment of these diseases will be discussed. A clear distinction will be made between animal models (that is, mice and rats) and data obtained in humans. The major contribution of genetics in identifying frequent and rare variants of the *MTNR1B* locus, including the current state of the functional characterization of the genetic variants in this gene that are associated with the risk of T2DM, will be discussed. The resulting controversial findings regarding the effect of various natural variants in the *MTNR1B*

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**Key points**

- The rs10830963 single-nucleotide polymorphism (SNP) in the *MTNR1B* locus is associated with increased fasting plasma glucose levels and impaired insulin secretion, as well as increased risk of type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus.
- Obesity seems to not be associated with the rs10830963 SNP in adults but might have a role in fetal birth weight.
- The phenotype of rs10830963 risk allele carriers includes increased *MTNR1B* mRNA expression, altered melatonin secretion and possibly further effects associated with the enhancer activity of the region surrounding the rs10830963 SNP.
- Loss-of-function of rare $MT_2$ receptor variants, in particular of melatonin-induced $G_{i1}$ and $G_z$ and spontaneous β-arrestin 2 recruitment, is associated with increased risk of T2DM.
- Lifestyle recommendations are emerging for rs10830963 risk allele carriers and further clinical evidence has to be gathered to evaluate the prescription of melatonin for patients with T2DM.
- The wide use of melatonin by millions of people, both as a supplement and as a medicine, calls for a rapid assessment of the effect of melatonin on glucose homeostasis.

locus on metabolic traits related to T2DM will be critically analysed and discussed in the context of the peculiarities of the melatonin system. In the last part of the Review, lifestyle recommendations and pharmacological targeting of the melatonin system to manage the risk of T2DM will be put into perspective.

## Melatonin synthesis and signalling

### *Melatonin synthesis*

Melatonin is an old molecule in evolutionary terms that seems to have appeared 2.5–3.0 billion years ago in photosynthetic cyanobacteria, probably fulfilling the function of an antioxidant[7]. Subsequently, melatonin became the key molecule conveying the environmental light/dark information and a ligand for membrane receptors to mediate actions on a variety of additional intracellular processes. The conserved biosynthetic pathway of melatonin starts with the hydroxylation of tryptophan to 5-hydroxytryptophan, followed by decarboxylation to generate serotonin. Serotonin is then acetylated by the rate-limiting enzyme arylalkylamine *N*-acetyltransferase to give *N*-acetylserotonin, which is finally converted by the acetyl-serotonin-methyltransferase to melatonin by *O*-methylation[8]. In vertebrates, the pineal gland is the main source of melatonin production. Following its synthesis, melatonin is immediately released into the cerebrospinal fluid (CSF) and bloodstream[9,10], with the melatonin content of the CSF reaching levels 100-fold higher than that in peripheral structures[11]. Importantly, melatonin from the pineal gland is synthesized in a circadian manner, under the control of the central biological clock — the SCN — with the highest circulating levels at night and low values during the day[12]. Thus, the circadian regulation of the central biological clock by melatonin, as a feedback mechanism, is reliant on the rhythmic melatonin concentrations in the CSF, whereas its rhythmic concentrations in the blood influences the circadian regulation of peripheral tissues; both functionalities contribute to whole body synchronization as a result of the chronobiotic properties of melatonin[13]. Melatonin is rapidly metabolized in the body, with a half-life of 20–30 minutes[14].

Local melatonin synthesis has been reported in other tissues and cells in mammals, which has a minor effect on plasma or CSF levels of melatonin. For instance, melatonin is synthesized in the retina in a circadian manner and participates in the regulation of retinal physiology, including retinal light sensitivity during the night[15]. Local, non-rhythmic melatonin synthesis has also been reported in activated macrophages and lymphocytes[16,17], where melatonin is reported to participate in the inflammatory response of the organism via promotion of M2-macrophage polarization[18], enhancement of the phagocytic activity of macrophages and regulation of the production of cytokines that participate in the resolution of inflammation[17]. The gastrointestinal epithelium also produces melatonin from its precursor serotonin, which is thought to come from enterochromaffin cells[19]. In the gut, melatonin seems to participate in the regulation of intestinal motility, immune system responses, ion transportation in the lower gut and the release of peptides involved in energy balance such as peptide YY[20]. Whereas melatonin synthesis is classically considered to take place in the cytoplasm, in the past 5 years, non-rhythmic melatonin synthesis has been reported to occur in isolated mitochondria from mouse brain cells[21] and oocytes[22], as well as in plant mitochondria and chloroplasts[23]. The full meaning of this intriguing finding remains to be clarified, including the question of the effect of mitochondrial melatonin synthesis in comparison to pineal melatonin synthesis.

### *Signalling and metabolic functions*

Melatonin has been reported to bind with high affinity to $MT_1$ and $MT_2$ receptors[1]. These receptors are members of the GPCR superfamily and primarily couple to G proteins of the $G_{i/o}$ and $G_{q/11}$ subclasses[24], which trigger multiple downstream signalling pathways. Both receptors also couple to β-arrestins, the second major mediator of GPCR function, but the consequences of this coupling are unknown[25–27]. By combining genetic and proteomic approaches, the interactome of melatonin receptors was estimated at around 200 interactors for each receptor[28]. Many of these interactors are involved in the regulation of receptor function[29,30]. Of the multiple signalling pathways activated by melatonin receptors, most have been reported to be dependent on pertussis toxin-sensitive $G_{i/o}$ proteins, mediating the inhibition of the adenylyl cyclase–protein kinase A (PKA)–cAMP-responsive element binding (CREB) pathway, the soluble guanylyl cyclase (sGC)–protein kinase G (PKG) pathway and the activity of various kinases (such as phosphatidylinositol 3-kinase (PI3K), AKT, protein kinase C (PKC) and extracellular-signal-regulated kinase (ERK)) and ion channels (such as large-conductance $Ca^{2+}$-activated $K^+$ BKCa and Kir3 channel)[31]. In several other cases, activation of the $G_{q/11}$ protein–PKC–phospholipase C (PLC) pathway has been reported in diverse physiological settings[32]. Some reports suggest that $G_s$ and $G_{16}$ proteins are mediators of the melatonin signal, however, the physiological relevance of these observations remains to be demonstrated[33,34].

Melatonin receptors are expressed at central and peripheral sites, which can both contribute to the regulation of metabolic functions. The effect of central

melatonin receptors on metabolism is probably mediated through the hypothalamic SCN, which is the biological master clock. Indeed, melatonin is known to entrain the circadian rhythm of the SCN and is currently the only approved pharmacological tool to treat circadian dysfunction of the SCN in humans[35]. Studies published in the past few years indicate that the circadian system is important in regulating the daily rhythm of plasma metabolites[36] and glucose metabolism[37] and that disruption of the master clock leads to metabolic diseases such as T2DM[38] (BOX 1). Therefore, alteration of the melatonin system has a direct effect on the function of the master clock in the SCN, which could affect the development of metabolic diseases. In the SCN, melatonin inhibits the cAMP–PKA–CREB pathway[39] and activates the PKC pathway[40,41] and Kir3 channels[42,43] to acutely inhibit the neuron firing rate, to phase-shift the circadian rhythm of neuronal firing and to regulate clock gene expression[44–47] (FIG. 1).

Expression of melatonin receptors in the periphery occurs in many tissues, but at low levels. Despite the fact that numerous animal studies suggest that melatonin has a modulatory effect on peripheral tissues, a direct effect of melatonin on these tissues has only been demonstrated in a limited number of studies[47–53]. Similar to the SCN, regulation of glucose metabolism by melatonin in peripheral tissues might involve the regulation of clock genes at the local level, but the contribution of the peripheral versus central receptors and the corresponding signal transduction pathways remain unknown. A direct effect of melatonin on specific tissue functions, such as glucose uptake or insulin secretion, has been reported. For instance, in the human hepatocyte cell line HepG2, melatonin activates the function of PKCζ, AKT and glycogen synthase kinase-3β (GSK3β) to stimulate glycogen synthesis[54]; melatonin also reduces hepatic gluconeogenesis in rats[48]. Analysis of both $MT_1$-knockout and $MT_2$-knockout mice suggests an important role for melatonin signalling in the regulation of hepatic insulin-dependent glucose metabolism via augmentation of PI3K–AKT activity[55,56]. In mouse skeletal muscle cells, melatonin activates the insulin receptor substrate 1 (IRS1)–PI3K–PKCζ pathway and stimulates glucose transport[47], a finding that is in agreement with subsequent findings of reduced glucose uptake in skeletal muscle of $MT_1$-knockout mice[55]. In isolated inguinal rat adipocytes, melatonin inhibits isoproterenol-induced lipolysis and fatty acid transport through inhibition of the cAMP–PKA pathway[49,51]. In the human brown adipocyte PAZ6 cell line, acute melatonin treatment inhibits cAMP and cGMP production; long-term treatment decreases glucose transporter type 4 (GLUT4) expression and glucose uptake, thus participating in the delayed effects of melatonin[57] (FIG. 1).

An increasing number of studies have focused on the role of melatonin in pancreatic islet populations (BOX 2) and the effect of melatonin signalling on their function. In rodent β-cells, the predominant effect of melatonin is the reduction of insulin release through inhibition of the $G_i$–cAMP–PKA[50,58,59] or cGMP[52] pathways, while a marked increase in insulin release was observed upon long-term treatment of the insulinoma cell line INS-1 with melatonin[53,58,59]. In the murine pancreatic α-cell line αTC1.9, melatonin administration increased glucagon secretion, possibly via $G_{q/11}$-dependent activation of the PLC–PI3K cascade[60]. Studies on human islets suggest that melatonin increases intracellular levels of calcium and stimulates glucagon and insulin release from α-cells and β-cells, respectively[61], probably via paracrine input from α-cells to insulin-secreting β-cells. Importantly, similar to rodent cells, long-term activation of melatonin signalling (of a duration that mimics night exposure) in human islets using melatonin or the melatonin receptor agonist ramelteon increases islet sensitivity to cAMP, which results in increased insulin secretion[62]. Insulin secretion and β-cell survival are improved in response to melatonin signalling, as shown by the decreased proteotoxicity-induced cell apoptosis and oxidative stress in human islets exposed to chronic hyperglycaemia and in islets from patients with T2DM[62], as well as by the preservation of the protein levels of the histone acetyl transferase p300 (REF.[63]). These findings are in agreement with previous reports on the role of melatonin in preventing β-cell damage[58]. Melatonin administration in the human pancreatic δ-cell line QGP-1 decreases somatostatin secretion via mechanisms involving decreased cAMP concentrations[64]. By contrast, exposure of human islets to melatonin increases somatostatin release[65], which highlights an influence of melatonin in δ-cells and the need for further experimentation.

Collectively, these data show that melatonin can act through central and peripheral receptors with

**Box 1 | Circadian rhythms and regulation of metabolism**

The daily fluctuations of metabolic parameters, such as circulating nutrient levels, and endocrine factors are mediated not only by environmental (extrinsic) but also by endogenous (intrinsic) factors, which oscillate in the absence of environmental cues[202]. These circadian clock mechanisms, which influence a broad range of metabolic processes, are generated by single-cell molecular clocks comprised of CLOCK (circadian locomotor output cycles kaput) and BMAL1 (also known as aryl hydrocarbon receptor nuclear translocator-like protein 1), the main transcriptional activators, and their targets, including PER (period), CRY (cryptochrome) and Rev-Erb isoforms, which function in circadian loops. This molecular circuitry controls many physiological processes by generating circadian oscillations both at the transcriptional and post-translational level in metabolic organs such as the liver, adipose tissue, pancreas or skeletal muscle and has a key role in the regulation of energy homoeostasis[203]. Rhythmic cycles were described for insulin secretion in isolated pancreatic islets[204] and weak daily oscillations were reported for plasma concentrations of glucagon in rodents[205], which suggests that intrinsic clocks operate in pancreatic α-cells and β-cells. Targeted disruption of the pancreatic clock compromises insulin secretion and glucose tolerance in mice, as well as in isolated islets from mice or humans[206,207].

Rhythmic control of several other metabolic processes, such as glucose and lipid metabolism, has been described and the effect of clock ablation in most cases is detrimental for metabolism[208]. Melatonin participates in the process of rhythmic regulation of metabolism by synchronising peripheral tissue clocks. Direct regulation of melatonin on the core clock machinery has been described for pars tuberalis and fetal adrenal gland[209]. Due to the presence of melatonin receptors within the suprachiasmatic nucleus itself, exogenous melatonin acts as a chronobiotic and affects core clock gene expression[12]. Indirect evidence on the relationship between melatonin and circadian oscillators were obtained using $MT_1$-knockout and $MT_2$-knockout or double $MT_1$-knockout and $MT_2$-knockout mice, in which the amplitude or phasing of core clock genes were either modified or flattened in the pancreas, liver[210] and adipose tissue[211]. Circadian disruption in shift-work or sleep disorders has been linked with metabolic disorders such as obesity and diabetes as reported in meta-analyses of human studies[212,213]. Thus, understanding of the role of melatonin in the circadian clock network can open new perspectives for metabolic disorders associated with disrupted circadian rhythms.

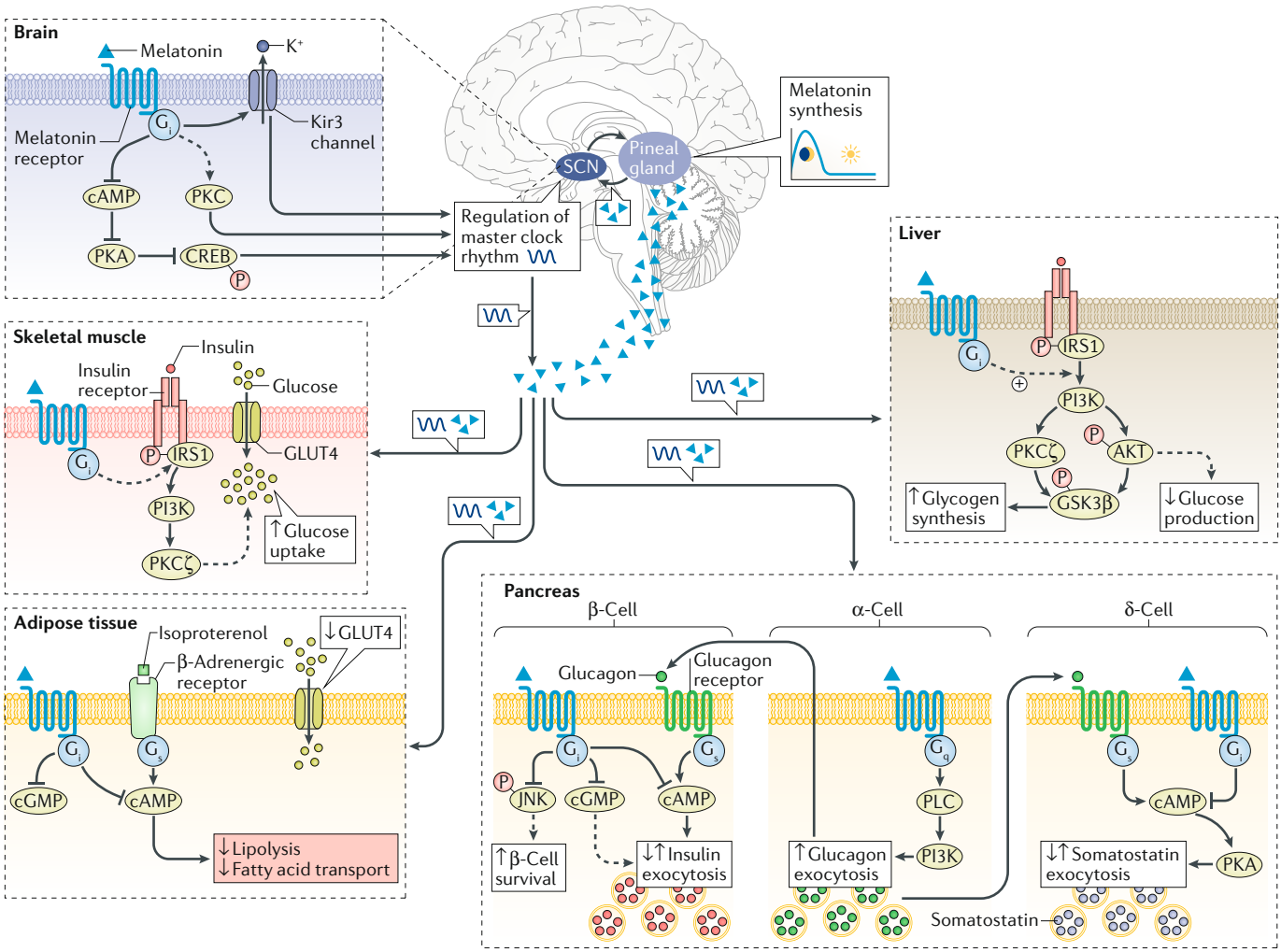


Fig. 1 | **Metabolic processes influenced by melatonin signalling in peripheral and central tissues.** Melatonin receptors are expressed at central and peripheral sites at which they contribute to the regulation of metabolic functions. Melatonin is secreted by the pineal gland in a circadian manner under the control of the master clock in the hypothalamic suprachiasmatic nucleus (SCN). Melatonin receptors in the SCN in turn inhibit the cAMP–protein kinase A (PKA)–cAMP-responsive element binding (CREB) pathway and activate the protein kinase C (PKC) pathway and Kir3 channels to regulate acute and circadian neuronal firing and clock gene expression. Melatonin in the circulation then modulates metabolic processes in the periphery either by directly acting on peripheral organs or indirectly by modulating the circadian activity of the central master clock. In mouse liver, melatonin is required for insulin-stimulated phosphatidylinositol 3-kinase (PI3K)–AKT activity, in rats it suppresses hepatic glucose production and in the human hepatocyte cell line HepG2 it activates glycogen synthesis, probably via a PKCζ–AKT–glycogen synthase kinase-3β (GSK3β) pathway. In mouse skeletal muscle, melatonin activates the insulin receptor substrate 1 (IRS1)–PI3K–PKCζ pathway to enhance the rate of glucose uptake. In inguinal rat adipocytes, melatonin inhibits the cAMP–PKA pathway and isoproterenol-induced lipolysis and fatty acid transport in some cases. In the human brown adipocyte PAZ6 cell line, melatonin acutely inhibits cGMP production and decreases glucose transporter type 4 (GLUT4) expression and glucose uptake upon long-term treatment. In rodent pancreatic β-cells, melatonin acutely reduces insulin release through inhibition of cAMP and cGMP levels, whereas it sensitizes the cAMP pathway and promotes insulin secretion upon long-term (physiological) melatonin stimulation. In human pancreatic islets, melatonin treatment increases insulin secretion and promotes β-cell survival via decreased JUN N-terminal kinase (JNK) activation. Melatonin administration in mouse pancreatic α-cells and in human pancreatic islets increases glucagon secretion, possibly by activating the $G_{q/11}$–PLC–PI3K cascade. Glucagon, in turn, can promote insulin secretion from β-cells and somatostatin secretion in δ-cells via its $G_s$-coupled glucagon receptor (GCGR). Finally, in human pancreatic δ-cells, melatonin has been reported to influence somatostatin secretion in both ways via modulation of cAMP levels. Broken lines correspond to suggested functions of melatonin receptors for which the precise pathway has to be further elucidated. The dark blue wave-formed lines illustrate circadian clock oscillations.

well-documented regulatory effects on the central circadian master clock and less well-documented effects on the function of peripheral tissues. Whereas the effect of melatonin on lipid metabolism does not seem to be predominant, its involvement in glucose homeostasis, in particular on glucose uptake, pancreatic insulin secretion and β-cell survival, has been extensively observed. Acute versus long-term stimulation paradigms seem to

Box 2 | **Overview of endocrine pancreatic cells and their functions**

The cells of the islets of Langerhans have distinct regulatory functions and exert control of glucose homeostasis by both cell intrinsic and paracrine mechanisms. Endocrine cells of the pancreas include glucagon-secreting α-cells, insulin-secreting β-cells, somatostatin-secreting δ-cells, pancreatic polypeptide-secreting γ-cells and ghrelin-producing ε-cells. Differences exist in the relative population and architecture of these cells between human and experimental models, as well as within species, which might have effect islet function and glucose homeostasis[214]. In most species studied, including humans, β-cells are predominant. β-Cells secrete insulin in response to a glucose challenge and other neurotransmitters and hormones that are released in response to food[215]. Glucagon is the main counter-regulatory hormone to insulin and is typical of the fasted state to prevent hypoglycaemia. Somatostatin suppresses the secretion of insulin, glucagon and pancreatic polypeptide[214]. Glucagon that is released locally by the α-cells within the islet is able to stimulate insulin and somatostatin secretion by a cAMP-dependent mechanism that primarily involves activation of the $G_s$-coupled glucagon receptor (GCGR), present in β-cells and δ-cells[214]. Melatonin, via its receptors, seems to have different effects on the functions of α-cells, β-cells and δ-cells, with effects on glucose metabolism that vary depending on the species used and treatment protocols[50,52,53,59–64].

be important for the functional outcome (reduced versus increased insulin secretion), which reflects the different outcomes of acute versus delayed effects of melatonin. Paracrine interactions between different pancreatic cell types have to be considered before conclusions are drawn on the effect of melatonin on insulin secretion. In addition, important species-specific differences might exist between rodent models and humans, which might have an influence on the effect of melatonin on islet function and glucose homeostasis.

## Melatonin in metabolic diseases

Melatonin regulates a variety of physiological and neuroendocrine functions in mammals, such as circadian rhythms, sleep onset and architecture, retinal function and immune function, as well as seasonal reproduction. The critical role of melatonin in the photoperiodic control of seasonal reproduction has been demonstrated by use of artificial patterns of timed melatonin infusion[66] and in pinealectomised animals[67]. These initial findings on the effects on seasonal reproduction, together with findings from the past 9 years, contributed to the increasing recognition that melatonin is an important modulator of metabolic functions and associated diseases[6,24,58,68]. Here we will discuss the effect of melatonin on the development of obesity and T2DM.

### *Melatonin in obesity*

Hibernating animals are an interesting model of body weight regulation as they undergo dramatic body weight changes during the annual cycle with massive weight gain when in preparation for hibernation, followed by weight loss during hibernation. According to the seasonal effects of melatonin, the duration of melatonin synthesis during the night reflects the variation of the day length during different seasons. Importantly, in seasonal animals such as Siberian hamsters this variation in melatonin signal triggers not only the reproductive cycle, but also a massive increase in body weight in anticipation of the reduced food intake during hibernation[69]. In this model, melatonin modulates the sympathetic nervous system through central melatonin receptors and acts directly on the adipose tissue[70]. As melatonin acts on mitochondrial gene expression in the brown adipocytes of Siberian hamster, it is plausible that the effects of melatonin are mediated via modulation of mitochondrial gene expression[71]. In rats and mice, melatonin supplementation decreases body weight gain and the amount of adipose tissue in several experimental models[6]. These effects are probably mediated by central and peripheral melatonin target tissues, which results in synchronization of circadian rhythms to the activity-feeding–rest-fasting cycle and amelioration of glucose uptake directly on adipocytes[57,72]. Even so, both $MT_1$-knockout and $MT_2$-knockout mice display insulin resistance, which suggests that the receptors are implicated in the regulation of other metabolic processes in nocturnal species rather than regulation of body weight[55,56,73].

Reports on the beneficial effects of melatonin on body weight in animal models, together with the absence of toxicity of melatonin, prompted several small-scale studies in humans (<100 participants) to evaluate the effect of melatonin administration alone or in combination with other medications in either individuals with obesity or patients with metabolic disorders (TABLE 1). In treatment protocols varying from 1 day up to 1 year, a modest reduction in body weight and plasma levels of lipids was observed in some studies, but not in others, suggesting an overall modest, if any, effect of melatonin (see TABLE 1 for references).

Genetic studies have also evaluated the influence of the melatonergic system on body weight regulation, obesity and lipid metabolism. Neither genome-wide association studies (GWAS) nor most of the more targeted studies focusing on *MTNR1*A and *MTNR1B*, which encode the $MT_1$ and $MT_2$ receptors, respectively, detected a consistent association of genes of the melatonergic system with risk of obesity[74–78]. The rs8192552 (Gly24Glu) variant, located in the coding region of the *MTNR1B* gene, was reported to be functionally defective and associated with increased BMI[79]; however, this finding was not replicated in other studies[75,80].

GWAS focusing on the role of maternal genetic variation on birth weight revealed an association of rs10830963 and rs1387153 variants, located in the *MTNR1B* locus, with increased birth weight[81]. This finding highlights the effect of the maternal genotype and the intrauterine environment on the fetus. Interestingly, carriers of the rs10830963 risk allele show extended melatonin secretion in the morning[82]. As maternal melatonin is known to cross the placenta[83] and to affect fetal growth and maturation[84], it is tempting to speculate that the modified melatonin secretion pattern of the mother might affect the fetus by prolonging the immediate effects or by altering the chronobiotic effects of melatonin.

Several studies have started to examine the effect of the rs10830963 variant, which is known to be associated with increased FPG levels and risk of T2DM[2–4] (see subsequent sections for more details), on body weight regulation in dietary weight loss intervention protocols (TABLE 2). Compared with carriers of non-risk allele, carriers of the risk allele showed a greater improvement in body adiposity and fat distribution and reduction in cholesterol levels but only when eating a low-fat diet,

Table 1 | **Human studies on the influence of melatonin or melatonin receptor agonists**

| Participants | Experimental design | Effects of melatonin alone or in combination with other drugs on metabolic parameters | Refs |
|---|---|---|---|
| Patient with obesity, BMI ≥30 kg/m$^2$ ($n$ = 30) | Melatonin (10 mg) or placebo administration 1 h before bedtime plus calorie restriction for 30 days | Reduction in BW (7%) and MDA erythrocytic concentration, increase in adiponectin and omentin 1 levels and increase in glutathione peroxidase activity | 216 |
| Postmenopausal women with overweight and increased appetite, 54–65 years ($n$ = 64) | Fluoxetine (20 mg in the morning) and melatonin (5 mg) or placebo administration in the evening for 24 weeks | Reduction in BMI (4%) in melatonin-treated group and improvement of sleep quality. Patients reported alleviation of nocturnal hunger and abdominal pain | 217 |
| Patients with MS ($n$ = 39) | Melatonin (8 mg) or placebo 1 h before bedtime for 10 weeks. After a 6-week washout, participants received the other treatment for 10 more weeks | Modest decrease in waist circumference, TG and HDL cholesterol levels, slight worsening of plasma levels of glucose, improvement in systolic BP, higher proportion of participants free from MS | 218 |
| Women with obesity, BMI ≥30 kg/m$^2$, 20–50 years ($n$ = 44) | Supplementation with a daily dose of melatonin (6 mg) or placebo in combination with low calorie diet for 40 days | Decrease of mean serum levels of TNF, IL-6, hsCRP and MDA, slight increase of mean total antioxidant capacity level | 219 |
| Patients treated with second-generation antipsychotics (20 with bipolar disorder and 24 with schizophrenia) | Melatonin (8 mg) or placebo administration for 8 weeks at 20:00 h | Attenuation of weight gain, slight decrease in TG levels and decrease in diastolic BP in the bipolar disorder group only | 220 |
| Patients with non-alcoholic fatty liver disease (27 men and 18 women) | • Group I: Essentiale forte in the dose of 3 × 1 tablet per day and tryptophan 2 × 500 mg per day<br>• Group II: Essentiale forte and melatonin 2 × 5 mg per day<br>• Group III: Essentiale forte with placebo, duration: 4 weeks | Increase in melatonin and reduction in GGTP, TG and pro-inflammatory cytokine plasma levels of IL-1, IL-6 and TNF in groups I and II | 221 |
| Patients with non-alcoholic fatty liver disease (51 men and 23 women) | • Group I: Essentiale forte in the dose of 3 × 1 tablet per day and tryptophan 2 × 500 mg per day<br>• Group II: Essentiale forte and melatonin 2 × 5 mg per day<br>• Group III: Essentiale forte with placebo, duration: 14 months | Increase in melatonin and decrease of plasma levels of GGTP, TG, LDL cholesterol, IL-1, IL-6 and TNF from groups I and II. In a few patients, melatonin and tryptophan reduced liver inflammation | 222 |
| Patients with MS who did not respond to 3-month lifestyle modification and healthy volunteers ($n$ = 60) | Melatonin (5 mg) administration in patients 2 h before bedtime for 2 months | Increase in catalase activity, decrease in TBARS, LDL cholesterol levels and BP | 223 |
| Outpatients with schizophrenia or schizoaffective disorder ($n$ = 25) | Administration of ramelteon (8 mg) or placebo 30 minutes before bedtime for 8 weeks | Decrease in total cholesterol and cholesterol to HDL ratio, small decrease in CRP and trend towards reduced fat in the abdominal and trunk areas. No improvement in glucose metabolism and inflammatory markers | 224 |
| Elderly patients with non-insulin NIDDM and healthy elderly volunteers ($n$ = 29) | Administration of melatonin (5 mg) in patients with NIDDM 1 h before bedtime for 30 days | Increase in the morning melatonin concentration and Cu-Zn superoxide dismutase activity. Reduction in MDA levels and ceruloplasmin oxidase activity. No alteration in nitrate or nitrite levels | 225 |
| Patients with T2DM poorly controlled with metformin (25 men and 21 women), 40–64 years | Administration of single daily oral doses of both melatonin (10 mg) and zinc acetate (50 mg) alone or in addition to metformin or placebo given at bedtime for 90 days. All groups were under dietary control | Melatonin and zinc reduced TC, TG and LDL cholesterol plasma levels and microalbuminuria. Combination with metformin improved the tissue responses to metformin | 99 |
| Normotensive adolescents with T1DM and healthy controls ($n$ = 17) | Administration of melatonin (10 mg) taken at bedtime daily for 7 days | Decrease of mean BP during sleep only in patients with T1DM | 226 |
| Healthy women, 24 ± 6 years, BMI: 23 ± 3.3 kg/m$^2$ ($n$ = 21) | Melatonin (5 mg) administration on two non-consecutive days at 08:45 and 20:45 h followed by placebo administration a week later | Impairment of glucose tolerance both in the morning and evening. In the morning, melatonin decreased glucose tolerance primarily by decreasing insulin release, while in the evening, by decreasing insulin sensitivity | 97 |
| Normolipidaemic postmenopausal women ($n$ = 15) | Administration of melatonin (6 mg) daily for 2 weeks | Increase of plasma TG, VLDL cholesterol, VLDL-TG, VLDL-protein, apolipoproteins C-II, C-III, E. Inhibition of lipoprotein lipase activity. No effect on plasma total cholesterol level, concentration of lipid and protein in LDL and HDL and on plasma levels of apolipoproteins A-I, A-II and B | 227 |

Table 1 (cont.) | **Human studies on the influence of melatonin or melatonin receptor agonists**

| Participants | Experimental design | Effects of melatonin alone or in combination with other drugs on metabolic parameters | Refs |
|---|---|---|---|
| Patients with T2DM and insomnia (11 men and 25 women; 46–77 years) | Administration of long-release melatonin (2 mg) or placebo 2 h before bedtime for 3 weeks. After a 1-week washout, participants received the other treatment for 3 weeks. Melatonin was then administered for 5 months | Improvements in sleep efficiency in response to short-term melatonin without affecting glucose and lipid metabolism. Reduction of $HbA_{1c}$ in response to long-term melatonin | 98 |
| Perimenopausal and postmenopausal women, 42–65 years, mean BMI: 21.6 kg/m$^2$ ($n$ = 10) | Melatonin (1 mg) at 21:00 h for 1 month | Increased serum levels of HDL cholesterol. No effect on TC, TG and LDL cholesterol | 228 |
| Postmenopausal women, 56–73 years ($n$ = 81) | Melatonin 1 or 3 mg or placebo nightly for 12 months. In addition, all participants received a daily supplementation of 800 mg calcium and 20 ug vitamin $D_3$ | Decrease in fat mass (7%) and increase in lean mass but no changes in BW and BMI. Small increase in adiponectin. No changes for leptin and insulin levels and markers of glucose homeostasis | 229 |
| Patients with T2DM ($n$ = 32) | Administration of ramelteon (8 mg) for 3 months. Half of patients continued for another 3 months | Improvement of sleep quality. No change in $HbA_{1c}$ levels. Discontinuation of ramelteon increased slightly the level of $HbA_{1c}$ | 230 |
| Postmenopausal women, 51–65 years (24 with normal BW and 30 with high BW) and 25 healthy (27–36 years) with normal BW | Melatonin (5 mg) administration at 21:00 h for 24 weeks. Examinations at 4, 8, 16, 20 and 24 weeks | Beneficial effect on the quality of sleep and reduction of BW (6%) | 231 |
| Patients with NASH ($n$ = 42) | Melatonin (2 × 5 mg per day orally) or placebo for 3 months | Decrease of AST and GGT levels, increase of melatonin. No effect on plasma TG, ALP and glucose and BMI | 232 |
| Patients with NASH ($n$ = 42) | Melatonin (2 × 5 mg per day) or placebo for 12 weeks. Follow up of a 3-month trial | Decrease of AST and GGT levels and increase of melatonin. No effect on plasma levels of TG, ALP, glucose and cholesterol | 233 |
| Elderly patients with hypertension, 77.6 ± 2.7 years ($n$ = 72) | Melatonin (5 mg) 1 h before bedtime for 1 month in addition to the diuretic indapamide (1.5 mg) | Increase in the morning melatonin levels, SOD1 and CAT activities, and decrease in the MDA level. No effect on glutathione, nitrate or nitrite and carbonyl groups and glutathione peroxidase activity | 234 |
| Postmenopausal women, BMI: 23.7 ± 0.9 kg/m$^2$, 52–61 years, 14 women were on hormone replacement therapy ($n$ = 22) | Melatonin (1 mg) or placebo at 08:00 hours on two nonconsecutive days after an overnight fast. OGTT in 13 women 45 min later. Insulin-dependent and insulin-independent glucose utilization was tested by a frequently sampled intravenous glucose tolerance test in 9 women | Reduction of glucose tolerance and insulin sensitivity | 96 |
| Patients with bipolar mood disorders, 11–17 years ($n$ = 48) | Melatonin or placebo in addition to olanzapine and lithium carbonate | Inhibition of the increase in total cholesterol levels, slow rise of systolic BP, lower but not significant increases in fasting blood levels of sugar and TG | 235 |
| Patients with T1DM of 7 ± 3.5 years duration, 16.0 ± 1.6 years ($n$ = 11) and healthy controls, 14–18 years ($n$ = 10) | Melatonin 5 mg or placebo 0.5 h before bedtime for 1 week followed by a washout week and another week that received the other treatment | Amplification of the nocturnal decline in diastolic BP | 236 |
| Patients with hypercholesterolaemia ($n$ = 16) | Melatonin (0.3 or 3 mg) or placebo at bedtime for 6 weeks | Trend toward a decreased total cholesterol and LDL with the 3 mg dosage. Three patients had significant decreases in LDL on 3 mg melatonin | 237 |
| Male patients with untreated essential hypertension, 55 ± 8 years, BMI: 26.8 ± 1.7 kg/m$^2$ (16 men) | Controlled release melatonin (2.5 mg) or placebo for 1 day or for 3 weeks 1 h before sleep | Improved sleep and reduced nocturnal BP in response to 3-week melatonin. No effect on heart rate | 238 |
| Women with treated essential hypertension ($n$ = 9) and with normal BP ($n$ = 9), 47–63 years | Slow-release melatonin (3 mg) or placebo for 3 weeks 1 h before bedtime. Participants were then crossed over to the other treatment for another 3 weeks | No influence on diurnal BP but decrease of nocturnal systolic and diastolic BP without modification in heart rate | 239 |
| Patients with overweight and histologically proven NASH ($n$ = 16) and lean healthy participants ($n$ = 15) | Melatonin (10 mg) for 28 days | Attenuation of insulin resistance (decrease in HOMA-IR and fasting insulin level), reduction in ALT, AST and GGT levels, increase in adiponectin, leptin and ghrelin plasma levels. No effect on resistin | 100 |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; BW, body weight; CAT, catalase; GGT, γ-glutamyl transpeptidase; GGTP, γ-glutamyl transferase; hsCRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; MS, metabolic syndrome; NASH, nonalcoholic steatohepatitis; NIDDM, non-insulin dependent diabetes mellitus; OGTT, oral glucose tolerance test; SOD1, superoxide dismutase; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TBARS, thiobarbituric acid reactive substrates; TC, total cholesterol; TG, triglyceride; TNF, tumour necrosis factor.

Table 2 | **Human interventional studies testing the effect of the rs10830963 SNP**

| Participants | Experimental design | Effect of rs10830963 risk allele on metabolic parameters linked to weight loss | Refs |
|---|---|---|---|
| Adults with T2DM who were overweight or obese ($n$ = 3,903) | Comparison of intensive lifestyle intervention involving weight loss and physical activity with a control intervention of diabetes mellitus support and education | Nominally significant interaction with lifestyle intervention on 1-year change (decrease) in WC was detected for rs10830963 | 85 |
| Participants with overweight or obesity ($n$ = 722) | Participants were randomly assigned to one of four diets varying in macronutrient composition during a 2-year weight-loss intervention | Association of rs10830963 with greater decrease in TC and LDL cholesterol in response to low-fat diet. Risk allele participants evidenced a smaller decrease in TC and LDL cholesterol in the high-fat diet group | 77 |
| Participants with overweight or obesity ($n$ = 722) | Participants were randomly assigned to one of four diets varying in macronutrient composition during a 2-year weight-loss intervention | Association of rs10830963 with BW, BMI and WC decrease in a low-fat diet at 6 and 24 months. Positive association of rs10830963 with BW in a high-fat diet at 6 months | 78 |
| Participants with overweight or obesity ($n$ = 722) | Participants were randomly assigned to one of four diets varying in macronutrient composition during a 2-year weight-loss intervention | Association of rs10830963 with greater increase in the energy expenditure measure respiratory quotient (measure of fuel utilization) but not with changes in resting metabolic rate in the low-fat diet compared with the high-fat diet. Significant interaction of rs10830963 with dietary fat but not protein intake on changes in energy expenditure measures | 86 |
| Participants with overweight or obesity ($n$ = 167) | Personalized nutritional intervention for weight loss, duration: 3–6 weeks | Lower weight loss in women carriers of the risk allele compared with women without the risk allele | 87 |
| Women with overweight or obesity who were habitual late eaters, 20 homozygous risk allele carriers and 20 matched homozygous non-carriers | Effect of concurrence of meal timing with elevated endogenous melatonin concentrations in glucose levels | Eating late significantly impaired glucose tolerance in rs10830963 carriers but not in non-risk carriers | 194 |
| Women with a history of GDM and/or a pre-pregnancy BMI ≥30 kg/m$^2$ ($n$ = 226) | Women at <20 weeks of gestation were randomised into an intervention group receiving counselling on diet, physical activity and weight control and a control group receiving standard antenatal care | Only non-risk carriers benefited from the intervention | 88 |

BW, body weight; GDM, gestational diabetes mellitus; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus; TC, total cholesterol; WC, waist circumference.

and not under high-fat diet conditions[77,78,85,86]. In other studies, rs10830963 risk allele carriers did not benefit from weight loss programmes, or showed only modest improvements[87,88].

Taken together, the available data do not support a major role of melatonin in body weight regulation and lipid metabolism in adults, but maternal melatonin regulation might have a notable effect on fetal birth weight. Intervention studies are still limited by the low number of participants, the variability of the applied protocols, the high number of confounding environmental parameters and the lack of information on melatonin secretion profiles in most cases.

### *Melatonin in T2DM*

Studies in rodents indicate a general beneficial role of melatonin on glucose homeostasis. Absence of melatonin in pinealectomized animals leads to a reduction in GLUT4 gene expression and protein content, glucose intolerance and peripheral and central insulin resistance, which are reversed by melatonin treatment[89,90]. In rats with streptozotocin-induced T2DM, combined treatment with melatonin and insulin promoted better glycaemic control and improved insulin sensitivity in white adipose tissue compared with the insulin or melatonin treatment alone[91]. Decreased melatonin synthesis has been reported in several animal models of T2DM[92,93] and improvement of glucose metabolism after melatonin administration was observed in the high-fat diet-fed insulin-resistant mouse model[94].

The role of melatonin in glucose homeostasis in rodents was further studied in melatonin receptor knockout mice (BOX 3). $MT_1$-knockout mice show a robust metabolic phenotype with systemic insulin resistance, which is probably the result of decreased insulin sensitivity and a pronounced inability of insulin to suppress hepatic glucose production[55,73]. $MT_2$-knockout mice also exhibit reduced hepatic insulin sensitivity but, in contrast to $MT_1$-knockout mice, show increased insulin release[56]. In humans, nocturnal melatonin levels have been reported to be lower in patients with T2DM than in controls[93,95]. Several small-scale controlled clinical studies showed that a single acute melatonin treatment worsens morning and evening glucose tolerance, both in older, healthy postmenopausal women (52 ± 6 years of age)[96] and younger healthy women (24 ± 6 years of age)[97]. By contrast, repeated administration over a 5-month period tends to have beneficial effects; $HbA_{1c}$ levels decreased, which suggests improved glycaemic control[98]. Combined administration of melatonin and zinc acetate with metformin in patients with poorly controlled T2DM improved the tissue responses to metformin[99]. Similarly, the median value of insulin resistance as defined by HOMA was statistically significantly reduced in patients with nonalcoholic steatohepatitis who were treated with melatonin for 1 month[100].

Box 3 | **Melatonin receptor knockout mice and glucose metabolism**

Only a few studies have characterized the mechanisms underlying glucose homeostasis in melatonin receptor-knockout mice. Both $MT_1$-knockout and $MT_2$-knockout mice in the melatonin proficient C3H background (a classic laboratory mouse strain known to synthesize melatonin) were associated with disturbed circadian rhythms, in relation to phase shifts and amplitude changes in the expression of the clock genes *Nr1d1* and *Dbp* in the pancreas and liver[210]. Insulin secretion was increased in islets of the $MT_1$-knockout and $MT_2$-knockout mice in the presence of melatonin compared with wild-type mice, whereas a decrease in plasma levels of insulin and an increase in blood levels of glucose in $MT_1$-knockout were observed. Another study reported impaired glucose metabolism and insulin resistance in $MT_1$-knockout but not $MT_2$-knockout mice, whereas no changes on body weight were reported[73]. Examination of tissue-specific insulin action revealed that $MT_1$-knockout mice display systemic insulin resistance, as demonstrated by a diminished insulin response in skeletal muscle, white adipose tissue and liver[55]. The main insulin signalling pathway affected by the loss of $MT_1$ is the activation of phosphatidylinositol 3-kinase (PI3K); transcripts of both catalytic and regulatory subunits of PI3K were strongly downregulated in the liver, in a similar manner to what is observed for livers from wild-type mice exposed to constant light[55]. Administration of melatonin to wild-type mice exposed to constant light restored insulin-mediated PI3K activity and insulin sensitivity, highlighting an important role of $MT_1$ during the night to secure insulin sensitivity via the regulation of the PI3K transcription and activity[55]. $MT_2$-knockout mice did not show any differences in glucose tolerance but exhibited increased insulin release and reduced hepatic insulin sensitivity compared with wild-type mice[56].

***Frequent variants of the MTNR1B locus.*** Among the candidate loci associated with increased risk of T2DM that have been identified in GWAS is the *MTNR1B* locus (reported in 2009 by three independent research consortia in cohorts of European origin). An association was described between the rs1387153 single-nucleotide polymorphism (SNP) (located 29 kb upstream of exon 1 of the *MTNR1B* gene) and increased FPG levels and T2DM risk[2]. Similar associations with stronger signals were reported for the rs10830963 SNP (located in the intronic region of the *MTNR1B* gene), which was in complete linkage disequilibrium with rs1387153 (REF.[4]). Likewise, Lyssenko and colleagues reported an association between the rs10830963 SNP and impaired insulin secretion[3], which suggests that $MT_2$ action is focused in the pancreas as a target tissue of melatonin. The effect of the rs10830963 SNP on increased FPG levels or β-cell function was replicated in other European cohorts[101–105] and by studies involving different ethnic groups, including Japanese[106,107], African American[108,109], Mexican American[110], Chinese[111–115] and Sri Lankan[115] people, whereas moderate effects were reported for Asian Indian people[116–118]. Importantly, the effect on FPG was also replicated in healthy children and adolescents of European[119,120], Asian[121] and Mexican[122] origin, as well as in children and adolescents with obesity[123–125], suggesting that the effect of the rs10830963 risk allele is apparent at early ages.

The *MTNR1B* locus was also associated with poor glycaemia over a 2–3 month period, as detected through a GWAS analysis of variants associated with increased levels of $HbA_{1c}$ (REF.[126]). Furthermore, one of the strong association signals observed for rs10830963 SNP carriers is defective early-phase insulin release, which might contribute to the increased plasma levels of glucose observed in the carriers who did not have T2DM[127–130], a finding that was replicated in two meta-analysis studies published in the past 5 years[131,132]. Moreover, the rs10830963 SNP was shown to effect the rate of progression from normal fasting glucose to impaired fasting glucose, but not the rate of progression from impaired fasting glucose to T2DM, indicating that the variant might also be important for the development of prediabetic fasting hyperglycaemia with a minor effect on the transition to the diabetic state[133]. Only a few studies have reported an association between the rs10830963 SNP and insulin resistance[101,134]. In a study published in 2018 that proposed an extended classification of patients with diabetes mellitus into six subgroups based on a cluster analysis of available metabolic, genetic and clinical data, the rs10830963 SNP was surprisingly associated most significantly ($P = 0.05$) with cluster 1 (labelled as severe autoimmune diabetes mellitus that overlaps with type 1 diabetes mellitus) and not with cluster 2 (characterized by severe insulin-deficient diabetes mellitus), as would have been expected from previous data[135].

The rs10830963 SNP has also been reported in many association studies as a risk factor for gestational diabetes mellitus (GDM) in different ethnic groups[136–142]. Interestingly, the association with GDM was restricted to those with increased pre-pregnancy BMI (≥25 kg/m$^2$), indicating that obesity might also have a role in GDM risk[142]. Considering that GDM itself is a strong risk factor for developing T2DM later in life and influences the metabolic health of the offspring[143], identification of genetic risk factors is required in determining lifestyle interventions with a preventive or therapeutic goal. Taken together, the association of the rs10830963 SNP with FPG and T2DM risk has been extensively replicated. The involvement of rs10830963 in the development of prediabetic fasting hyperglycaemia effects insulin secretion and has a minor effect on liver insulin sensitivity.

***Rare MTNR1B variants: rare but insightful.*** Many frequent variants associated with diseases are located in genomic regions with unknown function. By contrast, rare variants, which are typically discovered by targeted sequencing of the flanking or coding region of genes of interest, lead to more straightforward and testable hypotheses concerning their functional effect. Following this idea, a causal link between the *MTNR1B* locus and T2DM risk has been illustrated for the first time by a large-scale exon re-sequencing study of the *MTNR1B* gene in combination with systematic functional characterization of each corresponding $MT_2$ mutant[75]. A study that sequenced >7,600 Europeans revealed 40 non-synonymous variants (FIG. 2) of which the 36 very rare ones (minor allele frequency (MAF) below 0.1%) were significantly associated with T2DM, while the remaining four (frequent or rare ones with a MAF higher than 0.1%) did not contribute to T2DM risk. Functional characterization of the $MT_2$ mutants in terms of melatonin binding, cell surface expression and cAMP signalling revealed that four mutants completely lost their capacity to bind melatonin and an additional nine mutants did not inhibit cAMP production, despite normal cell surface expression. Statistical analysis showed that only mutants with a loss-of-function phenotype strongly associated with increased T2DM risk (OR of 5, when analyzed in aggregation)[75], establishing a firm functional link between loss of $MT_2$ receptor function and T2DM.

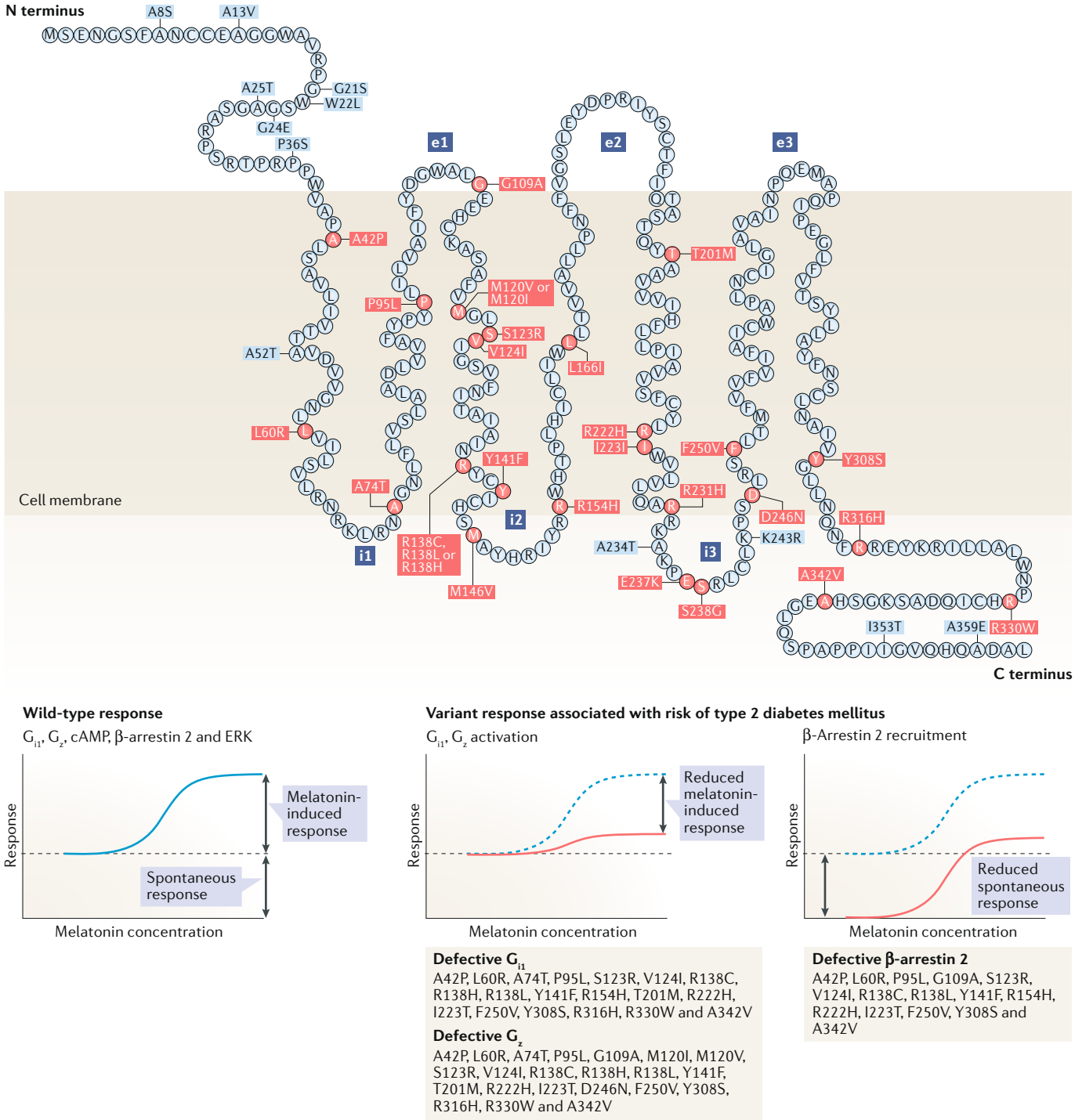


Fig. 2 | **Melatonin $MT_2$ receptor mutants and signalling defects associated with type 2 diabetes mellitus.** $MT_2$ activates the function of $G_{i1}$ and $G_z$ proteins, inhibits cAMP production, promotes extracellular-signal-regulated kinases 1 and 2 (ERK1/2) phosphorylation and recruits β-arrestin 2 in a spontaneous and melatonin-induced manner. $MT_2$ mutations are shown on the receptor sequence. Those in red result in defects in at least one of the aforementioned pathways. Defective activation of melatonin-induced $G_{i1}$ and $G_z$ proteins and spontaneous β-arrestin 2 recruitment to $MT_2$ are independently associated with the risk of type 2 diabetes mellitus. Defective $MT_2$ mutants are listed for each of these parameters. The blue lines correspond to the wild-type $MT_2$ and red lines to the variant $MT_2$.

***Signalling pathway-specific defects of rare $MT_2$ mutants.*** GPCRs such as $MT_2$ might engage multiple distinct signalling pathways. Amplifying or restoring the activation of pathways associated with the therapeutically desired effect with pathway-biased orthosteric or allosteric ligands, while excluding pathways associated with undesirable adverse effects, is an interesting therapeutic goal. Defining the functional defect of receptor mutants is thus of therapeutic relevance for the establishment of tailored medical interventions for risk allele carriers. As a first

step toward this goal we assessed the effect of all 40 $MT_2$ mutants on multiple pathways to investigate if the association between $MT_2$ loss-of-function and T2DM is linked to specific signalling defects[80] (FIG. 2). The study tested both spontaneous (ligand-independent) and melatonin-induced $MT_2$ activity for five signalling events; namely $G_{i1}$ and $G_z$ activation, inhibition of cAMP production, β-arrestin 2 recruitment and ERK1/2 activation. Only ten $MT_2$ mutants had a phenotype that was indistinguishable from the wild-type, whereas the rest showed functional defects in one or more of the tested functional parameters (TABLE 3). Detailed genetic association analysis detected three parameters that stand out in terms of significance of association with T2DM risk. These include 19 $MT_2$ mutants with defects in melatonin-induced $G_{i1}$ activation, 22 $MT_2$ mutants with defects in melatonin-induced $G_z$ activation and 15 $MT_2$ mutants with defective spontaneous (ligand-independent) β-arrestin 2 recruitment, while defects in other signalling parameters showed only a trend of association with T2DM risk. Importantly, as previously noted[75], none of the 'neutral' rare variants were associated with T2DM risk.

The reported results not only introduce $G_z$ and β-arrestin 2 recruitment as new players in $MT_2$ signalling that have a potential effect on T2DM risk, but also put spontaneous signalling of $MT_2$ at the forefront as a risk factor for T2DM. Whereas the capacity of the mutants to activate $G_z$ and $G_{i1}$ proteins correlated nicely, G protein activation correlated only poorly with β-arrestin 2 recruitment, which indicates that the influence of G protein-dependent effects on T2DM needs to be addressed independently of the effect of β-arrestin 2 recruitment. Concerning the activation of $G_z$ by $MT_2$ and the defective coupling with $G_z$ by certain mutants, it is important to note that $G_z$ expression is restricted to certain brain regions and retinal ganglion cells[144], areas where $MT_2$ is expressed[15,145], which suggests that the tissue-specific context should also be considered when evaluating the effect of receptor dysfunction. Collectively, this large-scale functional study revealed associations of T2DM risk with defects of specific melatonin signalling pathways, namely melatonin-induced $G_{i1}$ and $G_z$ and spontaneous β-arrestin 2 recruitment, opening avenues for pathway-specific personalized therapeutic interventions.

### *Controversial issues*

Even in light of all available data, the role of melatonin in glucose homeostasis remains complex and the evidence is sometimes contradictory. The most prevailing current hypothesis is based on the action of melatonin on impairing glucose homeostasis, which would be mediated by its inhibitory action on insulin secretion. A key finding for this hypothesis comes from the observation that *MTNR1B* mRNA expression levels are ~4 times higher in human pancreatic islets of carriers of the common rs10830963 variant (a risk allele) than in islets of people without this variant[3]. Accordingly, increased melatonin action is expected to increase melatonin signalling in islets and reduce insulin secretion, leading to hyperglycaemia and an increased risk of T2DM[56,146]. At first glance, this model seems to be simple and compatible with the phenotype of rs10830963 risk allele carriers, which comprises increased FPG levels and T2DM risk[3]. Consequently, limiting the action of the melatonergic system (that is, by using melatonin receptor antagonists) would be the advised treatment for patients with T2DM who have the risk allele. However, the situation is not as clear as that, as the reduced night-time melatonin levels observed in patients with T2DM[93,95] and the multiple rare $MT_2$ mutants that result in a loss-of-function phenotype and are associated with increased T2DM risk[75,80] (FIG. 2; TABLE 3) suggest rather the opposite, namely that dampening of the melatonin system leads to the development of T2DM[75,147].

These controversial statements have been discussed in several commentaries and reviews[146–149] but an explanation is still missing. Some have tried to reconcile the results by proposing an 'equilibrium hypothesis' where diversion from the equilibrium (normal situation), either by exaggerated or dampened melatonin function in frequent and rare variant carriers, respectively, becomes detrimental for glucose homeostasis[146]. Others suggested an 'age-related chronobiological hypothesis' that emphasizes the importance of the circadian system and its ageing-related deterioration in the understanding of melatonin's actions[150]. Still others defended the 'loss-of-function hypothesis' by questioning the relevance of pancreatic $MT_2$ receptors and by postulating a predominant indirect effect of melatonin on glucose metabolism through its well-established regulatory role on the central biological clock in the SCN[149]. None of these hypotheses are currently satisfying, or they need more experimental evidence to become generally accepted. Further adding to the complexity, the issue of insulin sensitization in the progression from prediabetes to T2DM should be considered to understand the effect of melatonin on insulin release in the early stages of T2DM. Revisiting some specific features of the melatonin system, including the different types of effects that melatonin has and the regulation (desensitization and sensitization) of melatonin responses, might help to understand how this hormone functions and improve comprehension of the apparent contradictions to pave the way towards a unifying model that can be agreed on by all in the field.

### *Beyond rodent models*

Mice and rats are useful models for physiological studies in mammals that offer the possibility of targeted deletion of genes of interest. However, these nocturnal rodents might be of limited use to understand the relationship between melatonin and the risk of T2DM in humans, as the night-time melatonin peak coincides with the eating phase in nocturnal rodents and with the resting and sleep phase in diurnal humans, illustrating their fundamentally different needs in regulating glucose levels during the night. Furthermore, different diurnal fluctuations of glucose tolerance and insulin sensitivity exist in rodents and humans[151]. In humans, both are highest during the early morning with a progressive decline towards sleep onset. In nocturnal rodents, glucose tolerance and insulin sensitivity are in phase opposition to humans illustrating the fundamental difference in decoding the melatonin message in diurnal and nocturnal species.

Table 3 | **Defects in functional parameters of rare $MT_2$ mutants**

| Variant name | Ligand binding | $G_{\alpha i1}$ | | $G_{\alpha z}$ | | β-Arrestin 2 | | cAMP | ERK |
|---|---|---|---|---|---|---|---|---|---|
| | | Spontaneous | Melatonin-induced[a] | Spontaneous | Melatonin-induced[a] | Spontaneous[a] | Melatonin-induced | Melatonin-induced | Melatonin-induced |
| A8S | – | – | – | – | – | – | – | – | – |
| A13V | – | – | – | – | – | – | – | – | – |
| G21S | – | – | – | – | – | – | – | – | – |
| W22L | – | – | – | – | – | – | – | – | – |
| G24E | – | – | – | – | – | – | – | – | – |
| A25T | – | PD | – | – | – | – | – | – | – |
| P36S | – | – | – | – | – | – | – | – | – |
| A42P | TD | SD | TD | TD | TD | SD | TD | TD | TD |
| A52T | – | – | – | – | – | – | – | – | – |
| L60R | TD | SD | TD | TD | TD | SD | TD | TD | TD |
| A74T | – | – | PD | – | PD | – | PD | – | PD |
| P95L | TD | SD | TD | TD | TD | SD | TD | TD | TD |
| G109A | – | – | – | – | PD | PD | PD | PD | – |
| M120I | – | PD | – | – | PD | – | – | – | PD |
| M120V | – | SD | – | SD | PD | – | PD | PD | – |
| S123R | – | SD | TD | SD | TD | SD | SD | TD | TD |
| V124I | – | PD | PD | – | PD | PD | SD | – | SD |
| R138C | – | SD | TD | TD | TD | SD | SD | TD | TD |
| R138H | – | PD | TD | TD | TD | – | TD | TD | TD |
| R138L | – | SD | TD | SD | TD | SD | TD | TD | TD |
| Y141F | – | PD | PD | – | PD | SD | – | – | – |
| M146V | – | – | – | – | – | – | – | – | PD |
| R154H | – | – | PD | – | – | PD | – | – | PD |
| L166I | – | – | – | – | – | – | PD | – | — |
| T201M | – | SD | PD | SD | PD | – | PD | – | SD |
| R222H | – | – | SD | SD | PD | SD | SD | PD | TD |
| I223T | – | PD | SD | SD | SD | SD | SD | PD | TD |
| R231H | – | – | – | – | – | – | PD | – | – |
| A234T | – | – | – | – | – | – | – | – | – |
| E237K | – | – | – | – | – | – | PD | – | – |
| S238G | – | – | – | – | – | – | PD | – | – |
| K243R | – | – | – | – | – | – | PD | – | – |
| D246N | – | – | – | – | PD | – | TD | – | – |
| F250V | – | SD | SD | SD | SD | SD | TD | TD | TD |
| Y308S | TD | SD | TD | SD | TD | SD | TD | TD | TD |
| R316H | – | SD | SD | SD | SD | – | TD | SD | PD |
| R330W | – | – | PD | – | PD | – | – | PD | – |
| A342V | – | – | PD | – | PD | PD | PD | – | – |
| I353T | – | – | – | – | – | – | – | – | – |
| A359E | – | – | – | – | – | – | – | – | – |

Partially defective (PD) defines 10–50% defects for spontaneous activity, and 10–50% defects in efficacy ($E_{max}$) or defects in potency ($EC_{50}$) for melatonin-induced activity. Severely defective (SD) defines 50–90% defects for spontaneous activity, and 50–90% defects in $E_{max}$ or defects in both $E_{max}$ and $EC_{50}$ parameters for melatonin-induced activity. Totally defective (TD) defines 90–100% defects for spontaneous activity, and lack of melatonin-induced activation. ERK, extracellular-signal-regulated kinase. [a]Defect associated with risk of type 2 diabetes mellitus.

### *Melatonin regulates multiple functions*

Melatonin is a pleiotropic hormone that regulates multiple physiological functions, a feature that is expected for a molecule that is considered to be the signal informing multiple tissues that night is beginning. Surprisingly, when it comes to explaining the action of melatonin on glucose homeostasis, the inhibitory action of melatonin on insulin secretion in pancreatic β-cells is often put at the forefront. This emphasis is mainly based on the well-known coupling of melatonin receptors to the $G_i$–cAMP pathway[1,24] and genetic association studies involving the rs10830963 SNP (see previous section). However, most of the functional studies on insulin release have been obtained in rodents and several similar studies in human β-cell lines and islets suggest rather the opposite, that is, a stimulatory role for melatonin on insulin secretion. This discrepancy might be explained by differences in the stimulation protocol (short-term versus long-term stimulation)[53,62], as the cAMP system is known to become sensitized upon long-term melatonin stimulation[152]. Another explanation might rely on an indirect, stimulatory effect of melatonin on glucagon secretion by pancreatic α-cells through a $G_{q/11}$–PLC-dependent pathway and subsequent insulin secretion by β-cells through $G_s$-coupled glucagon receptors[60,61,153] (FIG. 1). Stimulation of glucagon secretion by melatonin might be further potentiated by food intake during the biological night as glucagon upregulation presents one of the main modifications observed under conditions of circadian misalignment in humans[154].

The opposing effects of melatonin on insulin secretion through $G_{i/o}$ and $G_{q/11}$ proteins are also an integral part of the 'equilibrium hypothesis', which seeks to reconcile the controversial findings on common and rare $MT_2$ variants. However, in our opinion the biological relevance of this $G_{i/o}$ to $G_{q/11}$ balance remains to be demonstrated. As a matter of fact, melatonin modulates many other cellular functions in pancreatic islets and other cell types[31], such as cell survival[62,63,155], cell proliferation[156,157] and release of $Ca^{2+}$ (REF.[153]) and cytochrome *c*[21], that might be as relevant for glucose homeostasis as the direct regulation of insulin secretion (FIG. 1).

### *Beyond the $G_{i/o}$–cAMP pathway*

Much attention has also been given to the inhibitory effect of melatonin on the $G_{i/o}$–cAMP pathway, which is the most extensively studied signalling pathway of melatonin receptors but is certainly not the only signalling pathway for these receptors. Further downstream effectors of $G_{i/o}$ activation, such as ion channels, kinases (PI3K, AKT and ERK1/2) and sGC have been reported (see previous section), and are of potential relevance for the regulation of glucose homeostasis by melatonin[31]. In addition to $G_i$ activation, melatonin receptors activate the $G_{q/11}$–PLC pathway[153] and recruit β-arrestins (FIG. 1). Further interesting new insights are coming from the signalling profiles of rare $MT_2$ receptor variants that have been associated with increased risk of T2DM[80]. Indeed, in addition to $G_{i/o}$ protein activation, defective $G_z$ protein activation and β-arrestin 2 recruitment are associated with increased risk of T2DM[80] (FIG. 2). These findings provide two new lines of investigation that have to be considered to fully understand the association of $MT_2$ receptor function with T2DM risk, possibly not only in pancreatic β-cells but also in the brain[144,158].

### *Low levels of melatonin receptor expression*

Melatonin receptor expression is widespread but at low to very low levels (~1 fmol/mg of protein or lower)[28,31]. Unfortunately, after extensive validation we and many other laboratories have concluded that commercially available antibodies against melatonin receptors are not reliable enough to detect endogenous proteins in rodents[159] and, apart from some exceptions, are even unable to detect the recombinant receptors[160]. Considerable expression of melatonin receptors is only observed at three sites — the retina, the hypothalamic SCN and the hypophysal pars tuberalis[24]. At most other sites, melatonin receptor expression remains controversial and most of the evidence for its existence relies on functional studies.

However, absence of detection of receptor expression does not mean that these receptors are not functionally relevant, as demonstrated for the expression of the $MT_2$ receptor, which is barely detectable by in situ hybridization and 2-[$^{125}$I]-iodomelatonin binding in the SCN but has an important role in the regulation of the biological master clock at this site[161]. Not surprisingly, expression of melatonin receptors in peripheral tissues related to glucose metabolism in humans is similarly problematic and controversial. Expression in insulin-sensitive tissues in humans has only been reported in brown and white adipose tissue[57] and pancreas at the mRNA level[2]. Since the discovery of the common rs10830963 variant that is associated with T2DM risk and the reported increase in *MTNR1B* mRNA levels in carriers of the risk allele[3], expression of melatonin receptors in human pancreatic islets was intensely studied using various techniques, such as expression quantitative trait loci (eQTL) studies[56,162,163] and single-cell RNA-sequencing[164,165]. All studies agree that melatonin receptor mRNA levels are low, in some instances even absent or only detected in a subset of cells. In conclusion, in our opinion, unfortunately $MT_2$ expression studies are unlikely to provide convincing supportive evidence for the functional relevance of $MT_2$ in the human pancreas due to the expression of very low mRNA levels and the lack of satisfactory tools (that is, antibodies) to detect the $MT_2$ protein.

### *Increased $MT_2$ mRNA expression*

Increased $MT_2$ expression could be a relevant feature of rs10830963 risk allele carriers that might explain their T2DM phenotype; however, currently available data do not support this conclusion. Whether the observed difference in *MTNR1B* mRNA levels also translates into increased $MT_2$ expression levels remains unknown. In addition, an increase in receptor number does not necessarily result in an increased receptor signalling capacity due to spare receptors or chronic receptor desensitization, as shown for $MT_2$ in in vitro experiments[80,166]. Beyond the questions of whether variation of $MT_2$ expression contributes to the T2DM phenotype of rs10830963 risk allele carriers, other modifications cannot be excluded and have to be taken into account,

as illustrated by the significantly longer (~10%) duration of melatonin secretion that has been reported in rs10830963 risk allele carriers compared with non-carriers[82]. Intriguingly, increased *MTNR1B* mRNA levels have not been reported in pancreatic islets of donors with T2DM versus normoglycaemic donors, which indicates that increased *MTNR1B* mRNA expression is not a general trait of patients with T2DM[167].

### *Melatonin target tissues beyond β-cells*

Many studies focused their attention on the pancreas as the main melatonin target tissue involved in glucose homeostasis; however, the involvement of several other peripheral tissues, and in particular the brain, should also be considered (FIG. 1). In this context, it is of interest to consider the increased *MTNR1B* mRNA expression observed in rs10830963 risk allele carriers, an effect that was proposed to be specific for pancreatic β-cells[3]. Molecular studies provided the first mechanistic hypothesis by proposing that the region surrounding the rs10830963 SNP is targeted by the forkhead box protein A2 (FOXA2) transcription factor and exhibits enhancer activity[168]. This enhancer activity was proposed to be increased specifically in human islets by the binding of the transcription factor neurogenic differentiation 1 (NeuroD1) to the rs10830963 sequence of risk allele carriers. Although of interest, these data are not conclusive in their current state. First, it is difficult to demonstrate β-cell specificity as NeuroD1 is also expressed in the brain and digestive tract, two tissues known to express $MT_2$ receptors[169]. Second, the specificity of the enhancer effect for the transcription of the *MTNR1B* gene is not demonstrated, and enhancers are well-known to target loci located hundreds of kb away within large-scale topologically associating domains, implying that the transcription of other genes could be modulated by this enhancer.

The idea of modified gene expression of other genes in rs10830963 risk allele carriers is supported by the observed modification of the melatonin secretion profile in risk allele carriers[82]. This interesting observation reinforces the potential importance of central effects of melatonin in relation to glucose homeostasis regulation, as melatonin is principally secreted by the pineal gland (FIG. 1). As melatonin synthesis is under the control of the biological master clock in the SCN, this finding suggests that the central circadian clock might be modified in risk allele carriers. Importantly, parameters related to the sleep–wake cycle seem to be unaltered in carriers of the risk allele[82]. Taken together, the nature of the most relevant melatonin target tissue to explain the effects of melatonin on glucose homeostasis in humans remains unknown based on currently available data. In light of the widespread modulatory role of melatonin, the involvement of central and peripheral tissues would not be surprising.

### *Timing in the melatonergic system*

The importance of appropriate timing is another distinctive feature of the melatonergic system, which is reflected by the delayed, circadian and seasonal effects of melatonin. This notion is first of all based on the circadian secretion profile of melatonin, with high levels being secreted during the night-time. It is important to point out that sampling of melatonin in human plasma is not always compatible with routine blood withdrawal practice, which typically occurs in the morning between 08:00 h and 11:00 h, when melatonin levels are low and have no predictive value for night-time levels. Possible alternatives are the measurement of melatonin in saliva during the night or its metabolite 6-sulfatoxy-melatonin in the first morning urine, which allows a good estimation of the melatonin levels during the previous night. The importance of timing is not only true for the production of melatonin but also for the sensitivity of the body to melatonin, which is not constant over the 24-hour cycle[170,171]. The lengthened melatonin secretion profile in the morning in rs10830963 risk allele carriers is interesting in this respect[82]. Based on the nocturnal melatonin secretion profile, the action of melatonin (that is, regulation of glucose homeostasis) would be expected to occur in the same time frame — at night-time. However, melatonin is also known to have delayed effects, as illustrated by the sensitization of the cAMP system following the long-term presence of melatonin[152] or the modulation of GLUT4 expression upon long-term melatonin treatment[6,57].

Two studies published in 2018 further support the notion that the effect of melatonin receptor activation might also be visible during the daytime, at a time when the regulation of plasma concentrations of insulin is most important in humans. First, nocturnal activation of the $MT_1$ receptor was shown to modulate insulin sensitivity during the day in mice via the regulation of the transcription of PI3K[55]. Second, the association between loss of spontaneous $MT_2$ receptor activity with increased T2DM risk, as shown for carriers of rare $MT_2$ mutations, is not dependent on the presence of melatonin[80]. These observations change our understanding of the time windows at which nocturnal melatonin might modulate insulin sensitivity, which warrants further attention. In conclusion, the notion of timing is an important element of the melatonergic system that has to be considered and translated into clinical practice.

### *$MT_1$ in dysregulated glucose homeostasis*

Since the discovery of the association of common and rare SNPs in the *MTNR1B* locus with the risk of T2DM, research efforts have focused on the $MT_2$ receptor as the primary melatonin target relevant for glucose homeostasis. In turn, only minor attention has been given to the potential role of the $MT_1$ receptor. Studies in rodent cells and rodent models suggest that $MT_1$ and $MT_2$ receptors are at least equally important and an extensive characterization of $MT_1$-knockout mice that was published in 2017 provided supportive evidence for the importance of this receptor in the regulation of insulin sensitivity[55].

Expression of $MT_1$ is more abundant than that of $MT_2$ and both have similar expression patterns at central and peripheral sites[31]. A notable difference between $MT_1$ and $MT_2$ receptors is their regulation. Whereas $MT_2$ has been proposed to desensitize the cAMP response upon melatonin stimulation[172], activation of $MT_1$ has been associated with super-sensitization of the cAMP response during the subsequent period of withdrawal[173].

The sensitization of the cAMP system is probably involved in the stimulatory effect of melatonin on insulin secretion upon long-term treatment of INS-1 cells and human pancreatic islets with melatonin[53,62]. Intriguingly, no association has been reported so far between the *MTNR1A* gene (which encodes the $MT_1$ receptor) and T2DM or obesity. The associations of rare *MTNR1A* variants with these diseases and traits are currently unknown. Overlapping expression patterns, together with redundant signalling functions, is intriguing in light of the apparently different contributions of both receptors in the regulation of glucose homeostasis in mice[174]. Apart from differences in receptor regulation, different subcellular localization of $MT_1$ and $MT_2$ might be another possible explanation, which has emerged following the demonstration of the mitochondrial localization of $MT_1$ receptors[21,175]. Furthermore, differences in the molecular microenvironment, such as differences in the capacity of heterodimer formation between $MT_1$ and $MT_2$ (REFS[176,177]), should be also considered, in our opinion.

Taken together, research on melatonin and glucose homeostasis made considerable progress in the past 5 years. To resolve the contradictory findings outlined here, a broader vision in terms of potential signalling pathways, physiological actions and target tissues, together with a better integration of the rhythmic-behaviour of the melatonergic system, has to be considered. The latest results in this area provide some interesting new lines of investigation to be followed.

### *Strategies to manage risk of T2DM*

GPCRs are popular drug targets, as ~30% of the currently marketed drugs act on GPCRs[178,179]. Currently marketed drugs targeting melatonin receptors are indicated for insomnia (ramelteon), insomnia in the elderly (slow-release melatonin preparation), non-24-h sleep-wake disorder in totally blind individuals (tasimelteon) and major depressive disorders (agomelatine)[180]. All these compounds are nonselective agonists of the $MT_1$ and $MT_2$ receptors. Agomelatine belongs to a new class of melatonin receptor ligands as it displays two complementary activities by targeting not only melatonin receptors but also serotonin $5\text{-}HT_{2C}$ receptors[181]. Other multi-target-directed ligands are currently under evaluation. For instance, piromelatine (a melatonin receptor and $5\text{-}HT_{1A}$ and $5\text{-}HT_{1D}$ receptor agonist) has already completed a phase II clinical trial for insomnia and is in a phase IIb clinical trial to treat mild Alzheimer disease[182,183]. Metabolic diseases, including T2DM, are currently not among the clinical indications for melatonin receptor ligands. In the course of controlled clinical trials designed for other indications (such as insomnia and depression), altered glucose homeostasis, insulin secretion or T2DM risk were not reported as measured secondary outcomes. Several small-scale controlled clinical studies have been carried out and will be discussed in the following paragraphs.

Apart from medication, lifestyle recommendations are an essential component of the repertoire of therapeutic options to combat the epidemics of obesity and T2DM. Proper adjustment of the individual circadian rhythm to the environment is part of these recommendations and the hormone melatonin, as a reliable output and input signal of the central circadian system, is an obvious parameter to be considered. Personalization of lifestyle recommendations is becoming more and more possible as a result of the increasing number of genetic variants that have been associated with altered metabolic traits and T2DM risk. Based on the gene variants present in a patient, specific recommendations can be made. A confounding parameter to be taken into account when providing therapeutic recommendations, and also diagnosis, is the notable rate of melatonin self-medication, as the number of countries allowing commercialization of melatonin without any medical prescription is quickly increasing, including the USA and several European countries[184,185].

### *Pharmacological targeting*

The availability of melatonin depends on the country. In several countries, such as the USA, melatonin is considered a dietary supplement and can be purchased in drug stores without any prescription. In other countries, such as the UK, melatonin can only be obtained with a prescription. Melatonin is popular for the promotion of improved sleep initiation and for fast adjustment in situations of circadian misalignment (such as jet-lag when travelling over several time zones), but also as a prophylactic anti-ageing treatment and as a preventive treatment for neurodegenerative diseases and cancer[58]. The rationale behind melatonin supplementation is based on the observation that the amplitude and thus the strength of the natural melatonin rhythm tends to decline with age[58]. This decline is even further accelerated in people with neurodegenerative diseases such as Alzheimer disease, Parkinson disease and Huntington disease[1]. An estimated 3.1 million adults in the USA (1.3% of US adults) use exogenous melatonin[184], 1.4 million packages are sold in France per year[186] and the average yearly cost of melatonin prescription for insomnia and anxiety is more than £22 million in the UK[187]. These facts, in conjunction with the increased numbers of night shift workers (in the UK, the number of people who work night shifts increased by 275,000 (9%) between 2011 and 2016)[188] and the high numbers of individuals with late-night eating behaviour syndrome (the prevalence of night-eating syndrome is estimated to be 1.1–1.5% in the general population[189]) means that the understanding of human melatonin receptor function and its role in glucose homeostasis is of primary importance for public health care.

Should treatment with melatonin or melatonin receptor ligands be considered in patients with T2DM? Solely based on the finding that patients with T2DM have lower night-time melatonin levels than participants without T2DM[93,95], melatonin replacement could be advised. As detailed in previous sections, preclinical data on the use of melatonin in these patients are conflicting, ranging from improved glycaemic control to impaired glucose tolerance depending on the protocol applied (for example, chronic versus acute administration)[190]. Clearly, further studies are necessary before considering melatonin administration in patients with T2DM. In this context, it is worth mentioning that not

only is the right drug dosage important for melatonin but also the time of administration and the duration of the effect. Melatonin is generally taken once a day, 1–2 hours before an individual's bedtime to mimic, at least partially, the natural night-time melatonin peak. As the amplitude of the melatonin rhythm varies between different individuals, and given that this parameter is rarely determined before starting melatonin self-medication, achievement of supra-physiological levels has to be assumed in many cases[82]. This high level of uncontrolled melatonin supplementation has to be taken into account when evaluating the individual risk of developing T2DM in those who are obese and are at high risk of developing T2DM.

Another aspect relevant to pharmacological intervention in patients with T2DM who are carriers of the rs10830963 risk allele might come from their increased response to approved T2DM drugs compared with non-carriers[191]. An increased insulin response to glucagon-like peptide 1 (GLP1) has been reported in carriers of the rs10830963 risk allele, which suggests that these patients might benefit from treatment with GLP1 agonists[191]. Furthermore, the deleterious effect of the rs10830963 SNP on β-cell function seems to not only persist over time but to worsen with time in individuals with impaired glucose tolerance[3,192], which underscores the benefits of intervening pharmacologically at an early stage, before glycaemic deterioration occurs.

Altogether, there is currently not enough clinical evidence to consider prescribing melatonin to patients with T2DM in general. The identification of frequent variants within the *MTNR1B* locus has opened the possibility of personalized medication, as will be discussed in the next section. Independent of the genetic background, the high level of self-medication, with several million people taking melatonin on a daily basis, has to be surveyed closely by health authorities to minimize the potential risks until more consistent clinical data on melatonin treatment in humans are available.

### *Personalized health care*

Classification of the population according to their genotype is of high clinical and public health relevance as health-care recommendations for associated risks (for example, T2DM and GDM) can be refined in a genotype-specific manner. Among the multiple variants identified at the *MTNR1B* locus, the rs10830963 variant is of particular interest because of its high prevalence (MAF ~30%) and robust association with T2DM risk[3]. On the basis of the assumption that increased $MT_2$ receptor function is causally linked to the increased T2DM risk seen in rs10830963 risk allele carriers, it would be expected that melatonin treatment would worsen the T2DM phenotype. This effect has been observed upon acute melatonin treatment in the morning[96], and both morning and evening hours[97], but not upon long-term melatonin administration at bedtime[98,100], which improved glycaemic control. However, the status of the rs10830963 locus was not defined in these studies. When separating rs10830963 risk from non-risk carriers, acute morning melatonin administration worsened glucose tolerance in risk allele carriers, while no difference on glucose tolerance was observed in the evening between the two genotypes[193]. Longer administration of evening melatonin over a 3-month period resulted in decreased first-phase insulin release and an increase in glucose concentrations in both risk and non-risk carriers, with more pronounced affects in the risk carriers[56]. In regard to the baseline glucose levels in the risk allele carriers, no impairment was observed after 3 months of melatonin administration and hence further insights in the regulation of endogenous melatonin levels might be more informative.

Taken together, rs10830963 risk allele carriers seem to be particularly glucose intolerant in the morning when their endogenous melatonin levels are still elevated, but their risk of glucose intolerance in the evening is similar to that of carriers of non-risk alleles. Consistently, the increased risk of T2DM for the risk allele carriers was more pronounced in early risers than in late risers, further indicating that the decreased glucose tolerance in the morning might be casually linked to the elevated endogenous melatonin levels[82]. Another study focused on the evening hours by evaluating the influence of the rs10830963 risk allele on glucose tolerance in the context of an early versus a late dinner with the idea that the late dinner falls under conditions when melatonin levels start to rise. On the basis of the assumption that elevated melatonin levels are detrimental for glucose tolerance, glucose tolerance should be worse at late dinners. This effect was observed in the general population and in homozygous rs10830963 risk allele carriers but surprisingly not in homozygous non-risk carriers[194]. The origin of the difference between the two phenotypes is not clear. However, rs10830963 risk allele carriers should thus avoid late dinners. Similarly, concurrence of meals and melatonin treatment should be avoided in risk allele carriers who take melatonin or synthetic melatonin receptor agonists for medical or prophylactic reasons, which is typically in the evening 1–2 hours before going to bed.

Clinical trials testing the effect of risk alleles on lifestyle interventions might provide further insights. Several intervention studies have evaluated the effect of the rs10830963 SNP on weight loss and improvement of plasma lipid parameters[77,78,85–88]. Unfortunately, these studies mainly monitored obesity-related parameters for which no clear association with the rs10830963 SNP exists, as discussed previously. Carriers of the rs10830963 risk allele are particularly susceptible to putative desynchronizing environmental agents, such as noise pollution, as a positive correlation between noise and changes in $HbA_{1c}$ levels over 8 years has been observed[195]. A possible explanation for this observation is the increased susceptibility of risk carriers for morning glucose intolerance due to their extended melatonin secretion in the morning hours, which reflects the increased risk of T2DM in risk allele carriers who are early risers[82].

Unfortunately, controlled studies of clinical melatonin treatment and interventional studies are not available for carriers of rare *MTNR1B* variants with a loss-of-function phenotype (which is associated with an increased risk of T2DM). Such studies would be

extremely informative to solve the controversy of the association of loss-of function or gain-of-function hypotheses with T2DM risk but are limited by the low prevalence (MAF <0.1%)[75] of these very rare variants.

In conclusion, many insights have been obtained from melatonin treatment studies of rs10830963 risk allele carriers, who seem to be particularly glucose intolerant in the morning when their endogenous levels are still elevated. Refining these studies in terms of treatment protocols in addition to clinical trials to clarify the effect of risk alleles on lifestyle interventions will certainly provide further valuable insights in the future.

## Perspectives

The characterization of the phenotype of carriers of the rs10830963 risk allele turned out to be particularly fruitful; however, several important questions related to this SNP remain to be solved. In rs10830963 risk allele carriers, no statistically significant association was reported with several circadian traits, except for a reported association with changes in the melatonin secretion profile[82]. As melatonin is the most reliable output signal of the central circadian rhythm-generating clock, the observed changes in offset and duration of melatonin synthesis are probably due to modifications in the central circadian machinery. Future studies will have to clarify this point. An important question to be addressed is whether normalizing the melatonin secretion profile will also normalize glucose metabolism of the risk allele carriers. Notably, several therapeutic options are available to test this hypothesis. For example, as melatonin synthesis is inhibited by light, a light therapy early in the morning can be applied under controlled laboratory conditions. Furthermore, β-blockers could be used early in the morning to block β-adrenergic receptors that regulate melatonin synthesis in the pineal gland[196]. Alternatively, the effect of melatonin could be inhibited by treating risk allele carriers with the melatonin receptor-specific antagonist S20928 (REF.[197]). Delaying the time of breakfast for risk allele carriers might be another measure to decrease their risk of T2DM, which can be tested easily.

Further genetic studies are also likely to provide valuable insights in the future, in particular, in defining the effect of the other players in the melatonin system, notably the two genes involved in the melatonin synthesis pathway (*AANAT* and *ASMT*) and *MTNR1A*. Loss-of-function mutants have been detected for *ASMT* in people with autism spectrum disorders[198,199]. Unfortunately, their metabolic phenotype has not been reported. A similar situation exists for $MT_1$, for which several loss-of-function mutations have been identified, but no data on the metabolic phenotype is available[200]. For the *MTNR1A* gene, copy number variants might also be relevant as a bioinformatic study revealed that this gene is ranked fourth among >100 clinically relevant genes encoding GPCRs in terms of the number of observed gene duplications[187]. Given that $MT_1$ and $MT_2$ receptors show overlapping expression profiles, often with higher expression levels for $MT_1$, together with the high redundancy between these two receptors in terms of cellular functions, the absence of frequent gene variants for $MT_1$ that are associated with the risk of T2DM remains a mystery that needs to be solved to better understand the distinctive features of the $MT_2$ receptor.

In light of the evidence presented in this Review, the use of melatonin both as a supplement and as a medicine needs to be carefully re-evaluated as it might significantly affect glucose homeostasis. Improved selectivity of future medication (for example, by signalling pathway-biased ligands) might be of interest in this context. The identification of an association of loss-of-function of specific melatonin signalling pathways with T2DM risk[80] and the description of the first biased ligands for melatonin receptors are promising steps in this direction[175]. Combination therapies are also likely to gain more importance in the future, an idea that fits well with the notion that melatonin is a 'modulator' rather than a 'driver' for many physiological functions[91,99]. The capacity of melatonin receptors to form oligomeric complexes composed of $MT_1$ and $MT_2$ receptors or between melatonin receptors and other GPCRs, such as the $5\text{-}HT_{2C}$ receptor, warrants more attention in the future as it opens new options for multi-target-directed ligands.

Finally, along with the 'new' receptor-dependent functions of melatonin, the 'old' function as an antioxidant should not be ignored, as this is a function that emerged a long time before melatonin receptors appeared during evolution[7]. Several reports suggest that melatonin might have important protective receptor-dependent and receptor-independent effects on mitochondrial functions[159,201] that could be relevant for the metabolic effects of melatonin, an aspect that should be studied in more detail in the future.

## Conclusions

Since the discovery of the association of frequent SNPs within the *MTNR1B* locus with the risk of T2DM in 2009, much progress has been made in the understanding of the interplay between melatonin and glucose homeostasis in humans. The effect of the risk allele rs10830963 SNP probably starts early during the development of prediabetic fasting hyperglycaemia by affecting insulin secretion. Obesity, an important risk factor for T2DM development, seems to not be associated with the rs10830963 SNP in adults but might have a role in fetal birth weight through effects on maternal melatonin secretion.

Controlled clinical studies, particularly those including carriers of the rs10830963 SNP, provided important information on the effect of melatonin treatment on glucose homeostasis and started to unravel the underlying molecular mechanisms of the association, which includes increased *MTNR1B* mRNA levels, a modified melatonin secretion profile and possibly other effects due to the enhancer activity of the region surrounding the rs10830963 SNP. The relative contribution of these different effects to the association of this SNP with T2DM remains to be established. Importantly, personalized lifestyle recommendations are starting to emerge based on interventional studies. Rare mutations in the *MTNR1B* gene provided further important insights, revealing that loss-of-function, and not gain-of-function

of particular signalling functions of the $MT_2$ receptor is associated with the risk of T2DM. These in vitro data will have to be validated in patient tissues and in vivo. Studies of defects in a subset of $MT_2$ signalling functions will eventually open the avenues for pathway-specific personalized therapeutic interventions.

Based on the existing data, it is yet to be established whether melatonin treatment for patients with T2DM causes beneficial or adverse effects. However, the high level of self-medication, with several million people taking melatonin on a daily basis, warrants a rapid answer to this question. To fully consolidate the role of the melatonergic system in glucose homeostasis in humans, the integration of the unique features of the melatonergic system with a broader vision in terms of the other components of the melatonergic system could result in opportunities for new therapeutic approaches in the treatment of T2DM, including multi-target-directed ligands.

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