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Lauritzen ES, Kampmann U, Pedersen MGB, et al. Three months of melatonin treatment reduces insulin sensitivity in patients with type 2 diabetes-A randomized placebo-controlled crossover trial. J Pineal Res. 2022;73(1):e12809.

What We Know, Think We Know, or Are Starting to Know

If we were to play a game of word association and I said "melatonin", I' d bet the house you would instantly say "sleep". And you would be quite correct; the rise in melatonin in the evening is the signal for the body to shift from the "daytime" phase of waking, activity, and feeding, to the "night-time" phase of sleeping, rest, and fasting [1,2] [*see **Geek Box**, below, for more detail].

Although melatonin is primarily known for its role in regulating the sleep-wake cycle, melatonin in fact participates in a range of biological functions by acting through melatonin receptors in different tissues throughout the body [3]. A genetic variant in melatonin receptor 1b [*MTR1B*] is associated with higher fasting glucose, impaired responses to glucose tolerance tests, and higher type-2 diabetes [T2D] risk [4–6].

It is important to note that there may be *endogenous* melatonin, i.e., the melatonin produced and secreted in the body, and *exogenous* melatonin, i.e., melatonin taken in supplement form. Some prior research has found that insulin sensitivity and glucose tolerance were impaired when endogenous melatonin was elevated during the night [7], while other research has shown the supplemental melatonin may also result in insulin resistance, particularly in individuals with *MTR1B* genetic variants [5].

However, the effects of both supplemental melatonin and the most common MTR1B genetic variant in individuals with T2D is not well researched, and the present study investigated this question.

*Geek Box: Melatonin and the Circadian System

In humans [and all mammals], the suprachiasmatic nucleus [SCN], located in the hypothalamus, is the source from which circadian rhythms are generated. "Circadian" means "around the day", and circadian rhythms are rhythms in our biological processes that are generated in the body and can synchronise to the 24 h day. We require circadian rhythms to synchronise to the light-dark cycle in order to appropriately time when, for example, we have a drive to sleep and drive to wake up, and for when the processes involved in metabolism of food are optimal.

Melatonin is produced and secreted from the pineal gland, but the rhythm in melatonin is generated by the SCN. The precise timing of the melatonin rhythm in a 24 h period is highly sensitive to time cues from light, specifically the absence of short wavelength blue light [picture the colour of the sky], and low colour intensities, influence the timing of melatonin release.

As a result, the melatonin rhythm is maintained at basal, near undetectable levels during the light period of the day, before rising and peaking during the biological night or dark phase. However, with modern environments creating artificial light exposure at night, and with humans in industrialised countries spending a majority of the light period in enclosed buildings, these factors can influence the timing and strength of the melatonin rhythm.

Melatonin is crucial to the overall regulation of the circadian system and our ability to regulate to the 24 h day/night cycle, however, the metabolic interactions and effects of melatonin are starting to be better understood, and could provide an explanation for the associations between food intake during the night and metabolic disease risk.

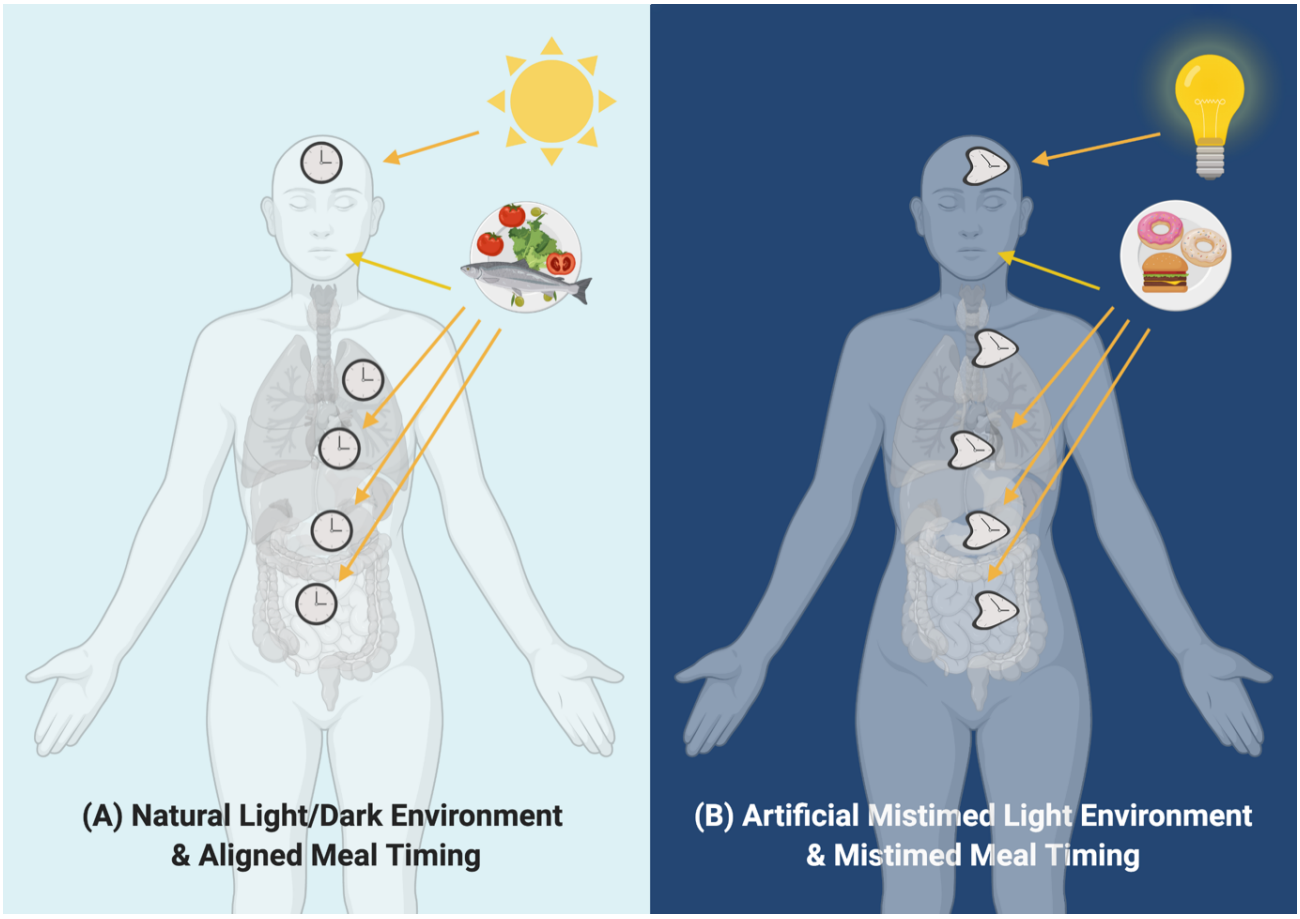


Figure *from [8] illustrating the relationship between external environmental time-cues (known by the German word, "Zeitgebers") and synchronisation of the circadian system.* ***(A; Left)*** *Indicates alignment of light cues and meal timing to the solar day resulting in a synchronised circadian system, however;* ***(B; Right)*** *indicates that mistimed environmental time-cues, including artificial light and food timing during the biological night, contribute to desynchrony of the circadian system. Emerging research suggests a role for endogenous melatonin in potentially mediating some of the adverse metabolic effects of food intake during the later evening and night.*

The Study

This was a randomised, double-blind, placebo-controlled, crossover [participants underwent both arms of the study] intervention trial. Participants were required to have a diagnosis of T2D, and were Danish men aged between 40 to 70yrs.

The intervention was 10mg of supplemental melatonin per day, compared to a placebo, with both consumed before bedtime. Participants were randomised to the order of treatment, i.e., melatonin for 12-weeks before crossing over to the placebo for 12-weeks [melatonin>placebo] or the inverse sequence [placebo>melatonin]. Whichever order participants undertook, both study arms were separated by a 4-week washout period.

Participants underwent genotype testing to determine *MTNR1B* status, and to stratify participants according to their *MTNR1B* genotype. There were two main genetic variants in the study, which for simplicity we will refer to as "Risk-type" and "Normal-type". Before and after each arm of the study, participants attended the investigators' research centre for metabolic testing, including an intravenous glucose tolerance test and insulin sensitivity test and measurements of plasma melatonin.

The primary outcomes were changes in insulin sensitivity and insulin secretion, and whether these outcomes differed according to *MTNR1B* genotype. Secondary outcomes included glucose uptake, fatty acid metabolism, and energy expenditure.

Results: 17 participants completed the study. The average age was 65yrs, bodyweight was 95kg, and BMI was 29kg/m2.

- ***Insulin Sensitivity*:** After 12-weeks of melatonin supplementation, insulin sensitivity decreased by 12% compared to the placebo [more on this finding under ***Key Characteristic***, below]. However, there was no interaction between Risk-type or Normal-type genetic variants and insulin sensitivity.

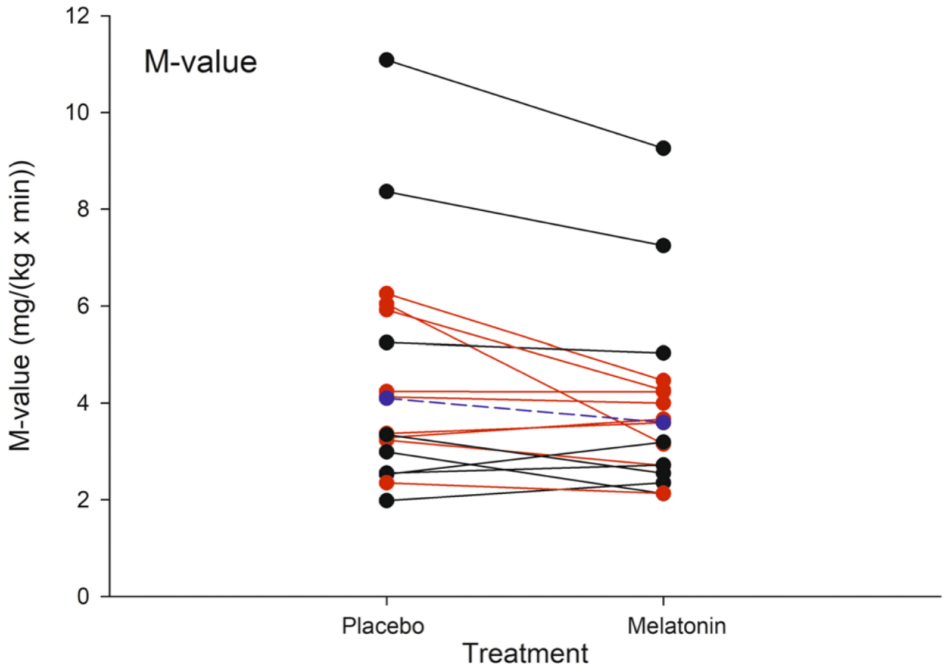


Figure *from the paper illustrating the change in insulin sensitivity ["M-value", where "M" stands for "metabolizable glucose", left Y-axis] during the placebo or melatonin supplement treatment. Each line joined by circles represents a single participant; the black lines represent participants with the "Normal-type" genetic variants, while the red lines represent participants with the "Risk-type" genetic variants. One of the great things about these before-after graphs is that there is nowhere for the data to hide; you can really see where the differences in the data occurred. For example, you can see that the where the line decreases from placebo [**left**] to melatonin [**right**], this indicates a decrease in insulin sensitivity. If you look at the trajectory of the lines, you can see that some participants had large decreases in insulin sensitivity, while some had no change.*

- ***Plasma Insulin*:** Insulin levels from 2-3 h post intravenous glucose tolerance test were 24% higher after melatonin supplementation compared to the placebo, however, there was no interaction with the melatonin receptor genotypes and insulin levels.
- ***Endogenous Melatonin Levels*:** Measured melatonin levels were significantly higher after supplementation compared to placebo. However, the increase in melatonin was 3.8-times higher in the Risk-types compared to the Normal-types, indicating a significant interaction between melatonin receptor genotype and melatonin supplementation. There was no correlation between plasma endogenous melatonin levels and the increase in insulin resistance [more under ***Interesting Finding***, below].

The Critical Breakdown

Pros: Randomisation method was appropriate [computer generated], and the allocation of participants was concealed until the end of the study, thus both investigators and participants were blinded to their allocation. Compliance, which was assessed by counting returned pills for both supplement and placebo, appeared to be quite high and similar rates of 96% and 99% for melatonin and placebo, respectively. Gold standard assessments of insulin sensitivity were undertaken using advanced measurement methods [more under Key Characteristic, below]. Medication usage was not altered during the study, so the outcomes were not influenced by any changes to diabetes drugs or doses. This was the first trial to focus on melatonin supplementation in participants with T2D.

Cons: The sample size estimated 18 participants would be required, and 17 completed the trial; thus, the trial is likely underpowered to detect more robust statistical differences. The authors, to their credit, do draw attention to this and highlight that the lack of interaction effect of melatonin receptor genotype may be due to type-2 error, aka "false negatives". The study population was entirely White/Caucasian and male, and thus the findings may not generalise to women and other ethnic groups, given that there may be both sex and ethnic differences in melatonin [9,10]. The authors refer to pre-specifying analyses, but there is no mention of the trial's registration.

Key Characteristic

The study measured insulin sensitivity using what is called the "hyperinsulinemic-euglycemic clamp" [HEC], which is the gold standard method of measuring insulin sensitivity in humans. To put the findings in some context, let's understand what the HEC does.

The HEC is a means of assessing whole-body metabolism of glucose into peripheral tissues, and the sensitivity of tissues to insulin, during a steady state of elevated glucose levels. To perform a HEC, both glucose and insulin are infused into the blood [usually through a catheter in the forearm] at the same time to create conditions of normal plasma glucose ranges [hence "euglycemic"], but elevated insulin levels [hence "hyperinsulinemic"].

Under these conditions, the rate of glucose infusion matches the rate of glucose uptake by tissues, which itself reflects the sensitivity of these tissues to insulin. This is referred to as the "M-value", for "metabolisable glucose", and this is *lower* in individuals with T2D compared to healthy individuals [11].

To properly quantify insulin sensitivity, the calculations for HEC must factor in body weight; there are several ways to do this, either using total body weight [kg], body surface area, or fat-free mass [FFM] [11]. The calculation is obtained per minute of the glucose-insulin infusion. The present study used total body weight, so the data is expressed as milligrams [mg] of glucose metabolism per kg of bodyweight, by minutes of infusion: mg(kg x min).

So, what exactly is the reference range when the data is expressed as mg(kg x min)? Previous research suggests that a measure of <4.9mg(kg x min) indicates insulin resistance [11].

In the present study, the M-value decreased to 3.6mg/(kg x min) during melatonin supplementation compared to 4.1mg/(kg x min) during placebo.

Thus, in the participants already with T2D in the present study, although the 12% change is modest, it indicates a worsening of insulin sensitivity from melatonin supplementation.

Interesting Finding

There was no correlation between the decreases in insulin sensitivity and measured levels of plasma melatonin. Based on previous research, we could expect an interaction, i.e., elevated plasma melatonin levels would correlate with decreased insulin sensitivity.

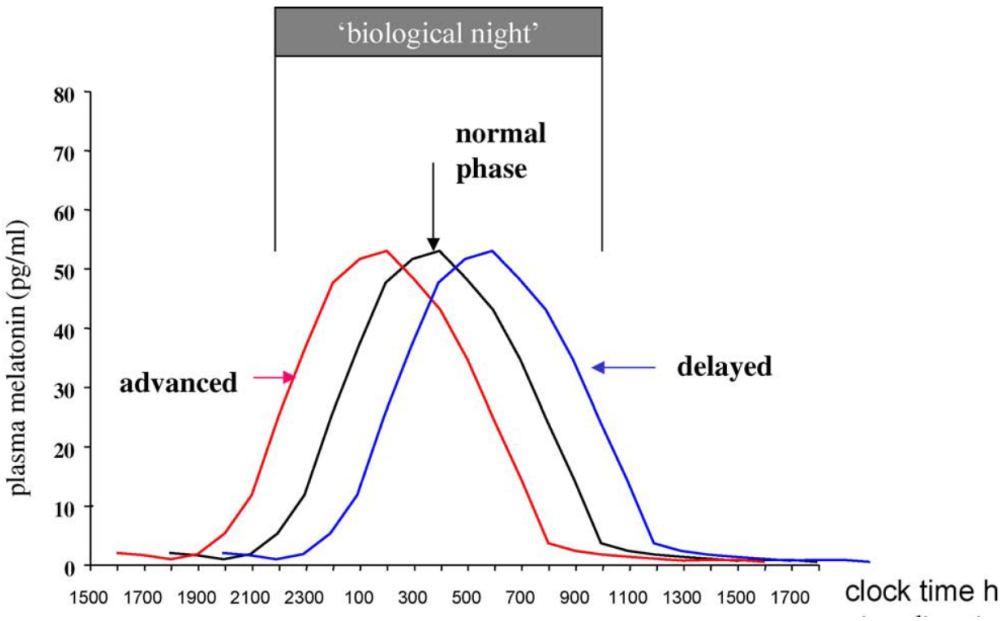
For example, Eckel *et al*. [7] examined the effects of 5 h sleep [delaying bedtime by 2 h and waking 2 h earlier than habitual] on intravenous and oral glucose tolerance tests administered 1 h after waking. Plasma endogenous melatonin levels remained elevated for a number of hours, reflecting wakefulness during the biological night, and impaired insulin sensitivity was inversely correlated with melatonin levels, such that the longer melatonin remained elevated after waking in the short sleep condition, the more insulin sensitivity was impaired [7].

What might explain the lack of correlation between plasma melatonin and insulin in the present study? The authors do note that it may be due to the small sample size, however, there is another potential explanation. Pay attention to the **figure** below [1], which we will use to illustrate this point.

Recall that the circadian rhythm in melatonin falls during the day and rises during the night, however, the precise timing of this rhythm may differ; this is known as the "phase", and is illustrated by the **advanced** [**red line**], **normal phase** [**black line**], and **delayed** lines. You can see the shape of these curves are the same; it is their alignment with the "clock time" [i.e., 24 h time] that differs.

Now, we now from the methods that the testing was conducted in the morning from 08:30 h onwards, and plasma melatonin levels were significantly higher after supplementation. In fact, melatonin levels were similar to the levels in the study by Eckel *et al*. [7]. However, the study by Eckel *et al*. [7] included a full assessment of the melatonin phase, and the correlations with reduced insulin sensitivity were observed for each hour after waking that melatonin was elevated above daytime levels.

Further, the Eckel *et al*. [7] study included sleep restriction, which itself deteriorates insulin sensitivity [12], and it may be that both sleep curtailment *and* elevated melatonin interact to more negatively impact glucose tolerance.



Relevance

There is still a long way to go until we have a more comprehensive understanding of the role of melatonin in metabolic health. Circadian studies of diet are difficult and expensive to conduct. For example, in order to fully measure circadian phase, hourly blood samples are required over a ~10-12 h period in controlled light conditions [13]. This ideally needs a laboratory setting, which in turn tends to mean small sample sizes.

Nevertheless, the potential interaction between diet and melatonin does warrant more research. For example, several recent cross-sectional studies have indicated a relationship between the timing of the nocturnal rise in melatonin ["melatonin onset"], as an approximate start of the biological night, and adiposity [14–16].

In one study, participants with a midpoint of energy intake [defined as the clock time at which 50% of total daily energy was reached] in close proximity to melatonin onset were associated with significantly higher body fat percentage [16]. The same group also reported that, compared to lean participants, participants with higher body fat percentage consumed a greater proportion of total daily energy at a later melatonin phase [15].

Importantly, both studies found no associations between temporal distribution of energy and adiposity when analysed only in relation to 24 h clock time, and these relationships were only evident when analysed in relation to circadian phase, i.e., in relation to evening melatonin onset [15,16].

It may also be that whether melatonin is measured in the evening or morning may be a factor. A recent intervention tested the effects of oral glucose tolerance tests consumed in the evening either 4 h or 1 h before bedtime in 845 participants with and without the *MTNR1B* gene; they found that the later glucose tolerance test resulted in impaired insulin sensitivity, which was worse in *MTNR1B* carriers [17]. The fact that the present study did not detect an effect of genotype is likely due to its small sample size, and potentially the fact that melatonin was assessed in the biological morning.

It should also be noted that in the present study, the decrease in insulin sensitivity occurred independent of factors influencing glucose uptake into cells, so the mechanistic understanding of the effects of melatonin on insulin sensitivity needs to develop.

Finally, it is important to distinguish between from the effects of the melatonin supplement observed over 12-weeks, as this was *exogenous* melatonin consumed chronically, whereas the lack of correlation with insulin resistance was based on measured *endogenous* melatonin after acute 2 h tests following the treatment periods. Currently, interventions have shown decreased insulin sensitivity correlated with *endogenous* plasma melatonin [7,17], and effects of *exogenous* supplemental melatonin [5].

Application to Practice

The supplemental dose used in the present study was 10mg per day; this is very high considering that the minimum effective dose for supplemental melatonin is 0.5mg [1]. However, given the popularity of the use of melatonin as a sleep supplement, there is a word of potential caution that has emerged from this line of research; do not go overboard if melatonin is available in your jurisdiction.

We have an evidence-base developing that suggests eating later into the night, and in close proximity to sleep, has deleterious effects on metabolic health. What role melatonin plays in this picture remains to be fully determined. Nevertheless, supplemental [exogenous] melatonin is not the only consideration here; endogenously produced melatonin also appears to be playing a role. It is prudent advice to try to avoid large intakes of energy later into the night.

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