https://doi.org/10.46389/rjd-2025-1890
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2025; volume 32, issue 2, pages 216–221

Original Article

Protective effect of melatonin on hyperglycemia-induced consequences in rats with insulin resistance

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Received: 3 March 2025 / Accepted: 3 June 2024

Abstract

The aim of the study was to determine the effect of melatonin on the content of glucose, insulin, total lipids, total cholesterol, high-density lipoproteins (HDL) and pyruvate on the activity of pyruvate kinase (PK) in the blood of rats with insulin resistance (IR). 18-month-old outbred white male rats were divided into three groups: 1) control (intact); 2) rats with IR (daily subcutaneous administration of dexamethasone solution, dose of 0.125 mg/kg for 13 days); 3) IR rats that received metformin (intragastrically at a dose of 200 mg/kg per day); 4) IR rats were orally administered melatonin (dose of 10 mg/kg). Fasting blood glucose was measured using a glucometer, and serum insulin was measured using an immunochemiluminescent analyzer on day 14. The IR index was calculated using the homeostasis model assessment (HOMA) index for IR. Parameters were determined in the blood using standard methods. Statistical processing of the results was performed using IBM SPSS Statistics 21, and according to our data, glucose and pyruvate levels in IR rats increased by 170% and 173%, respectively, which was accompanied by an increase in insulin levels and the HOMA-IR index by 5.8 times and 14 times respectively compared to controls. Daily treatment with metformin and melatonin reduced fasting glucose levels by 34% and 58%, respectively, compared with control. Metformin administration reduced insulin levels and HOMA-IR by 65% and 77%, and melatonin administration reduced these values by 78% and 90%, respectively, compared with control. Melatonin administration in IR rats resulted in the normalization of all investigated indexes.

Keywords: melatonin, rats, blood, insulin resistance

Introduction

Metabolic syndrome is one of the serious problems of human existence in the modern world. Technological progress, with all its advantages, has a downside. This includes the need to intensify the rhythm of life, frequent moving, and working at the computer at night, which is typical for most people. More and more people in conditions of competition are at risk of chronic stress and disruption of the circadian clock. Signs of this are the suppression of melatonin production and increased cortisol concentration in the body [1]. Such processes lead to the development of metabolic syndrome. This means the development of insulin resistance (IR), which leads to the appearance of hypertension, lipid metabolism disorders, and fatty liver [2].

As noted above, the basis of the development of disorders is the suppression of melatonin production. As is known, this hormone is a synchronizer of the interaction of various hormones. In relation to cortisol, it is in physiological opposition. That is, with a decrease in melatonin levels, cortisol production increases. In addition, melatonin has powerful antioxidant properties [3]. This hormone is able to influence the expression of genes of the glutathione antioxidant defense system, activating it. Therefore, with a lack of the latter, the body's antioxidant defense decreases. This leads to the development of oxidative stress. Under such conditions,

the risk of endothelial dysfunction increases and IR appears. In turn, an increased cortisol level contributes to hyperglycemia, leading to even greater oxidative stress [2] and the release of free fatty acids into the bloodstream [4]. Such events eventually lead to the formation of cardiovascular pathology.

Metformin has traditionally been used in the treatment of IR. This drug has proven itself as a hypoglycemic agent that can prevent complications, including hypertension. However, despite its advantages, it has disadvantages. With prolonged use, there is a risk of acidosis. In this case, patients should be in intensive care. However, such a negative effect can occur mainly in the group of patients with impaired renal function, with an increased ability to accumulate metformin in the body. Given its high safety profile, careful use of metformin has continued since the 1980s in view of the growing need to combat IR [5].

Taking into account recent studies, melatonin has a number of advantages over metformin. In particular, modulating its receptors increases the activity of superoxide dismutase and other antioxidant defense enzymes involved in the subsequent stages of neutralizing free radicals. Another significant advantage is the counteraction of the Warburg effect and the protection of mitochondria. Such an effect allows the activation of more energetically advantageous aerobic pathways. All this allows us to realize a greater opportunity for the protection of cells, in particular, the vascular endothelium. In addition, it is known that impaired interaction with the MT2 receptor leads to the development of type 2 diabetes.

There is an opposition in the relationships between insulin and melatonin. Increased insulin inhibits the formation of melatonin, and conversely, the appearance of IR with age can be overcome by the influence of melatonin. Considering that melatonin has an immunoregulatory effect, it is advisable to neutralize the effect of immunosuppression in conditions of prolonged elevated cortisol and, therefore, IR [6]. With aging, melatonin production decreases and shifts to later hours, while cortisol production increases and peaks in the early hours of the night. Maintaining the circadian rhythm with melatonin may provide a new strategy for treating pathologies associated with internal order disorders. The clinical benefit of reducing cortisol levels in the first half of the night may lie not only in improving sleep but also in better control of blood pressure, metabolism and mood [7].

Taking into account the growing need to prevent the development of complications from sleep-wake disorders and the consequences of chronic stress, we investigated the effect of melatonin on some indicators of carbohydrate and lipid metabolism in the blood of rats with experimental type 2 diabetes mellitus induced by dexamethasone—IR.

The study aimed to determine the effect of melatonin on the content of glucose, insulin, total lipids, total cholesterol, high-density lipoproteins and pyruvate and on the activity of pyruvate kinase (PK) in the blood of rats with IR.

Material and methods

The study was conducted in accordance with the Rules for the Use of Experimental Animals (1977) and the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE). Sexually mature 18-month-old outbred white male rats were divided into three groups: 1) control (intact); 2) IR rats (dexamethasone solution at a dose of 0.125 mg/kg for 13 days daily); 3) IR rats that received metformin (SANDOZ, Poland) at a dose of 200 mg/kg per day; 4) IR rats were orally administered melatonin (Sigma, USA) at a dose of 10 mg/kg (Mok, J.X., *et al*., 2019) daily. To establish the hypoglycemic effect of melatonin compared with metformin, fasting blood glucose was measured from the tail vein using a glucometer (One Touch Ultra Easy, LifeScan, USA) and serum insulin was measured using an immunochemiluminescent analyzer (Snibe Co., Ltd, China using the Maglumi test kit, China) on day 14. The IR index was calculated using the homeostasis model assessment (HOMA) index for IR according to the formula: HOMA-IR = blood glucose (mg/dL) × fasting insulin (μU/mL) ÷ 405 [4]. Rats were sacrificed on the 14th day of the experiment. The activity of PK and content of pyruvate, total lipids and cholesterol, and high-density lipoproteins (HDL) ("Reagent" and "Filisit-diagnostics" Research and Production Enterprise, Ukraine) were determined in the blood using standard methods [8].

Statistical processing of the results was performed using IBM SPSS Statistics 21. The significance of the difference between the indicators was assessed using the parametric Student's t-test (with a normal distribution) and the nonparametric Mann-Whitney U-test (with a non-normal distribution). Differences were considered statistically significant at $p \leq 0.05$.

Table 1: Influence of melatonin on fasting glucose, insulin levels and HOMA-IR in the blood of rats with dexamethasone diabetes (n=6, x±Sx).

Groups/parameters	Fasting plasma glucose, mg/dL	Serum insulin level, μU/ml	HOMA-IR
Control	100±20	2.7±0.15	0.67±0.03
IR	270±35[a]	15.7±0.20[a]	9.42±0.24[a]
IR+Metformin	180±28[a, b]	5.4±0.10[a, b]	2.16±0.04[a, b]
IR+Melatonin	115±26[b]	3.5±0.06[b]	0.89±0.03[b]

Note: Changes are reliable (p≤0.05); [a] – concerning control; [b] – concerning IR.

Results

According to our data, fasting glucose levels in IR rats increased by 170%, which was accompanied by an increase in insulin levels and the HOMA-IR index by 5.8 times and 14 times, respectively, compared to controls (Table 1).

Daily treatment with metformin and melatonin reduced fasting glucose levels by 34% and 58%, respectively, compared with control. Metformin administration reduced insulin levels and HOMA-IR by 65% and 77%, and melatonin administration reduced these values by 78% and 90%, respectively, compared with control.

Dexamethasone caused an increase in total lipid levels by 70% (Figure 1) and total cholesterol by 95% (Figure 2) in the blood of rats compared to the intact group, against a background of a decrease in HDL capacity by 50% (Figure 3).

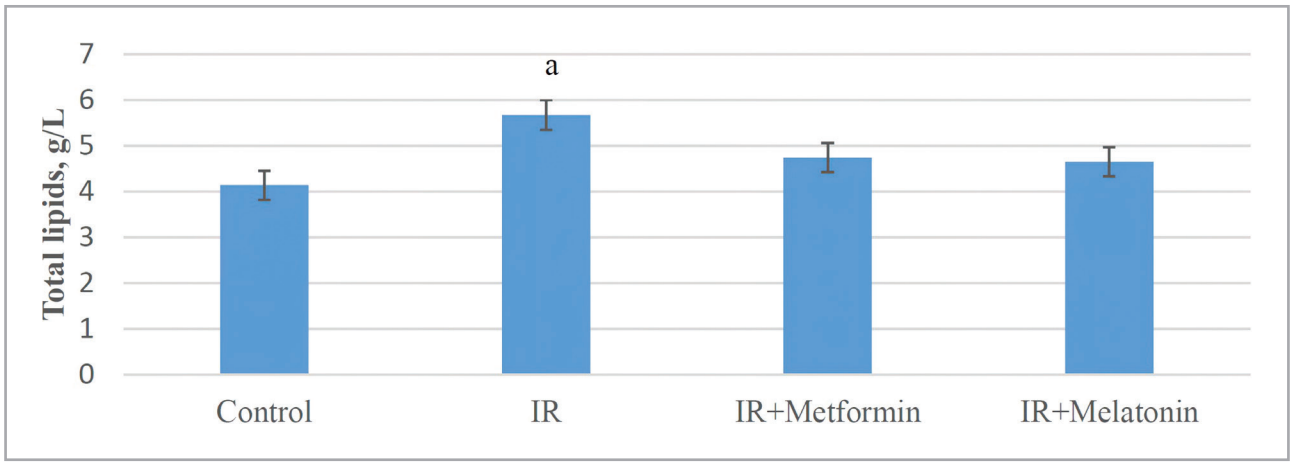

Figure 1: Total lipids, g/L (n=6, x±Sx). Changes are reliable (p≤0.05); a – concerning control; b – concerning IR.

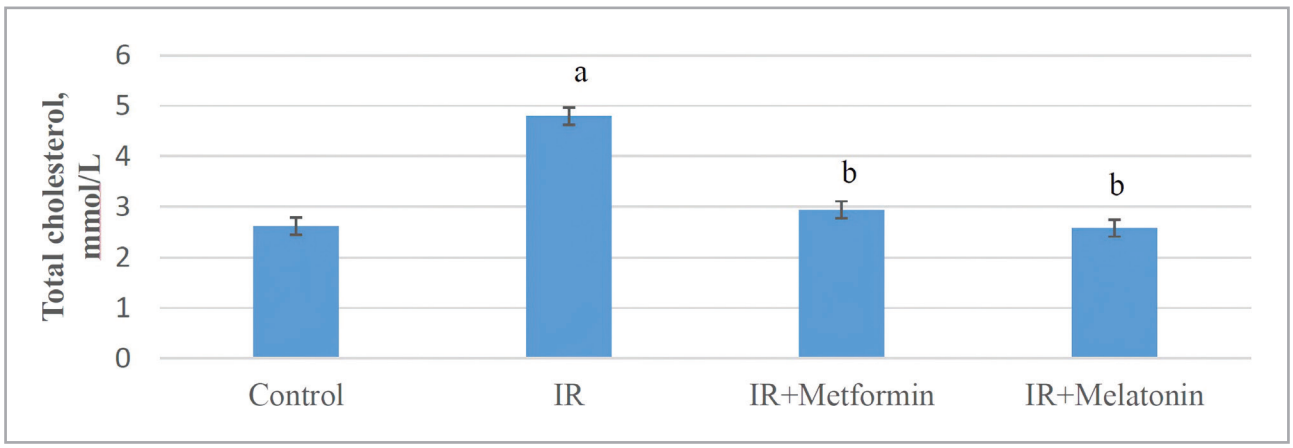

Figure 2: Total cholesterol, mmol/L (n=6, x±Sx). Changes are reliable (p≤0.05); a – concerning control; b – concerning IR.

https://doi.org/10.46389/rjd-2025-1890

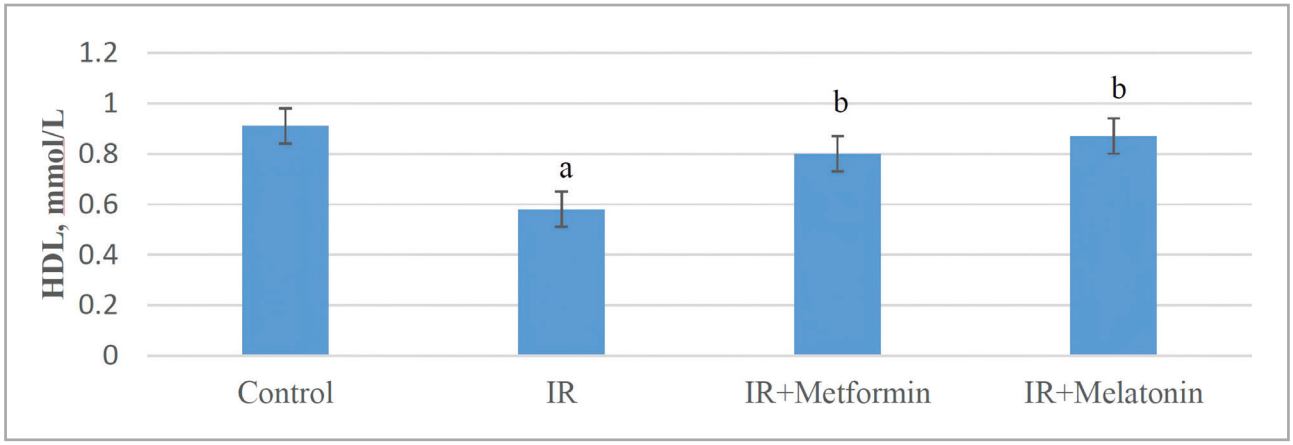

Figure 3: HDL, mmol/L (n=6, x±Sx). Changes are reliable (p≤0.05); a – concerning control; b – concerning IR.

In the group of rats with IR, PK activity (Figure 4) decreased by 15%, while in the group of animals treated with metformin, it increased by 14%, respectively, compared with the control. Melatonin normalized the activity of this enzyme.

The concentration of pyruvate (Figure 5) increased by 173% under conditions of IR compared with the control. The administration of metformin slightly decreased this indicator by 14%; melatonin decreased and normalized it compared with the control.

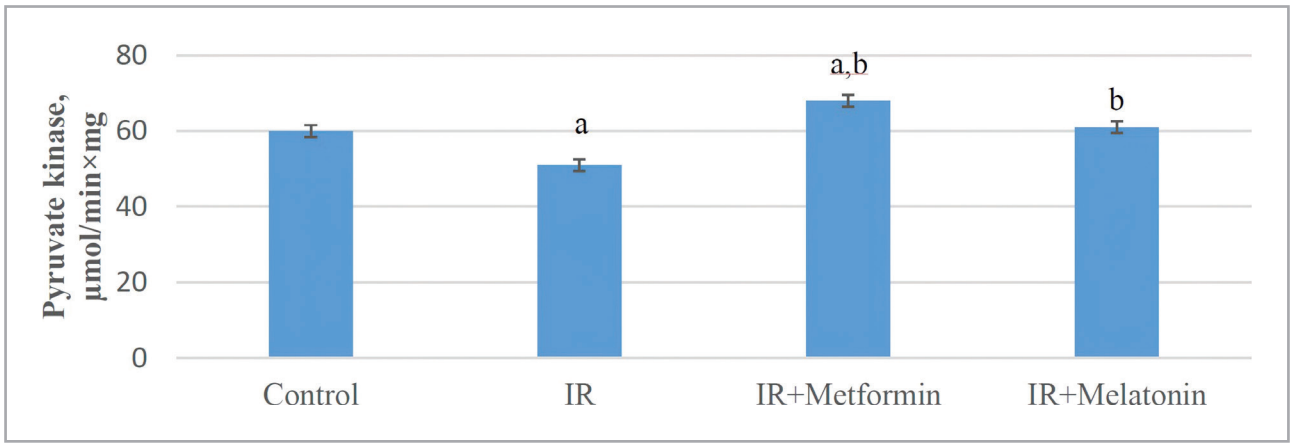

Figure 4: Pyruvate kinase, µmol/min×mg in blood (n=6, x±Sx). a, b – changes are reliable (p≤0.05); a – concerning control rats; b – concerning rats with IR.

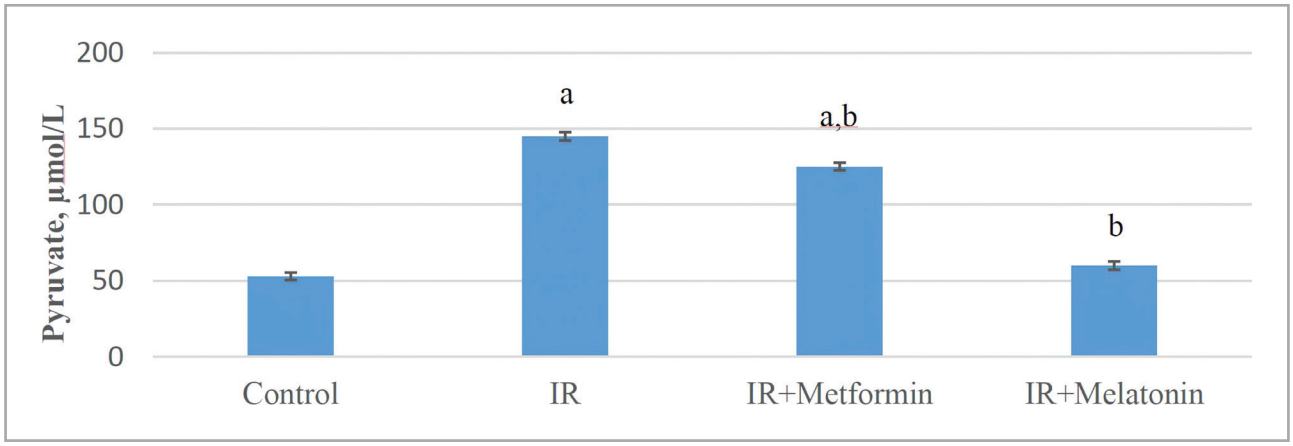

Figure 5: Pyruvate, µmol/L in blood (n=6, x±Sx). a, b – changes are reliable (p≤0.05); a – concerning control rats; b – concerning rats with IR.

Discussion

We observed an increase in glucose levels in response to daily administration of dexamethasone. According to our data, this condition was accompanied by increased insulin concentration and HOMA-IR in the blood. Under real-life conditions in humans, also in the case of stress, glucocorticoid levels cause mitochondrial dysfunction by increasing the activity of glucocorticoid receptors. This is reflected in the formation of hyperglycemia, which is accompanied by hyperinsulinemia IR. Melatonin decreases insulin levels [9]. The results showed that melatonin administration reduces plasma insulin levels *in vivo* and, in addition, there is an insulin-melatonin antagonism [10].

Disruption of the circadian clock as well as melatonin production are known to reduce both insulin secretion and sensitivity and negatively affect glucose homeostasis [1].

The administration of metformin and melatonin in the respective groups normalized glucose levels. This effect of such agents is associated with their ability to suppress gluconeogenesis [11, 12] and improve the supply of glucose to insulin-dependent tissues [13, 14].

Hyperglycemia has been recognized as a trigger for monocytes and macrophages' release of inflammatory cytokines. In addition, the peroxidation of free fatty acids in adipose tissue provoked by hyperglycemia also leads to inflammation. Besides, oxidation of low-density lipoproteins occurs, contributing to the development of atherosclerotic plaques [2].

We observed an increase in total lipids and total cholesterol and a decrease in HDL in response to dexamethasone injections. The key is that dexamethasone stimulates lipogenesis in the liver, inhibits lecithin cholesterol acetyltransferase activity, and raises free cholesterol levels. It can also increase circulating fatty acids and enhance lipolysis in adipose tissue [4]

The administration of melatonin and metformin improved the studied lipid metabolism parameters in the respective groups. Melatonin exhibited this effect, possibly due to its ability to reduce lipid peroxidation and stimulate apoprotein E, which is dependent on lecithin-cholesterol acyl transferase [15–17]. Metformin can be an appropriate treatment option for many diseases in which inflammatory processes and oxidative stress play a role in their pathogenesis. It activates superoxide dismutase [18].

Glucocorticoids stimulate appetite, and the fat deposits that are formed secrete leptin. The latter suppresses appetite, but it has no effect at high concentrations. Melatonin stimulates an improvement in leptin perception [19]. So, lowering total lipids and stopping weight gain was beneficial in the long run.

According to our results, PK's activity decreased under IR conditions. However, the introduction of correction agents contributed to an increase in the activity of this enzyme. This may be due to the hypoglycemic effect of melatonin and metformin, respectively, and the improvement of insulin sensitivity due to reduced total lipids [20]. It should be noted that we observed a more pronounced increase in activity with metformin administration compared to melatonin administration. It is known that metformin inhibits gluconeogenesis and leads to the accumulation of lactate [21]. Melatonin activates the processes of aerobic oxidation in mitochondria, which is an advantage and an opportunity to prevent lactic acidosis [22]. According to the data we obtained, the level of pyruvate in the blood, which increased under IR conditions, was normalized with melatonin administration.

Conclusion

Melatonin administration in insulin-resistant rats normalized fasting glucose, insulin, total lipids, total cholesterol, high-density lipoproteins, pyruvate kinase activity and blood pyruvate levels. This result is positive regarding preventing the risk of endothelial dysfunction and other related disorders. In addition, melatonin administration in insulin-resistant rats had advantages over metformin, which is possibly associated with the activation of aerobic ATP synthesis pathways and the absence of the risk of acidosis.

Conflict of interest

The authors declare no conflict of interest.

References

1. Hong Thuan Tran, Takeru Kondo Takeru Kondo, Amal Ashry. Effect of circadian clock disruption on type 2 diabetes. Front. Physiol., 2024; Sec. Chronobiology: 15 – 2024. doi.org/10.3389/fphys.2024.1435848
2. Laura Matuschik, Vladimir Riabov, Christina Schmuttermaier et al. Hyperglycemia Induces Inflammatory Response of Human Macrophages to CD163-Mediated Scavenging of Hemoglobin-Haptoglobin Complexes. Int J Mol Sci. 2022;23(3):1385. doi: 10.3390/ijms23031385

 https://doi.org/10.46389/rjd-2025-1890

3. Mariana Moreira Andraschko, Mara Taís de Carvalho. Melatonin attenuates glucocorticoid effect induced by medroxyprogesterone acetate in rats. Gen Comp Endocrinol. 2022;316:113959. doi: 10.1016/j.ygcen.2021.113959
4. Mona F. Mahmoud, Noura Ali, Islam Mostafa, Rehab A. Hasan, Mansour Sobeh. Coriander Oil Reverses Dexamethasone-Induced Insulin Resistance in Rats. Antioxidants 2022, 11(3), 441. doi.org/10.3390/antiox11030441
5. Clifford J. Bailey Metformin: Therapeutic profile in the treatment of type 2 diabetes. Diabetes, Obesity and Metabolism. 2024; 26 (S3): 3-19. doi.org/10.1111/dom.15663
6. Dalia M Kopustinskiene, Jurga Bernatoniene Molecular Mechanisms of Melatonin-Mediated Cell Protection and Signaling in Health and Disease. Pharmaceutics. 2021;13(2):129. doi: 10.3390/pharmaceutics13020129
7. Fatima G, Sharma V P, Verma N S. Circadian variations in melatonin and cortisol in patients with cervical spinal cord injury. Spinal Cord. 2016; 54, 364–367
8. Kushnir, O., Yaremii, I. Effect of melatonin on fibriniolysis in the brain of rats with dexamethasone diabetes. Scientific Journal of Polonia University. 2023; 56(1), 414-418. doi.org/10.23856/5656
9. Min Jeong Kim, Gee Euhn Choi, Chang Woo Chae, Jae Ryong Lim, Young Hyun Jung, Jee Hyeon Yoon, Ji Yong Park & Ho Jae Han. Melatonin-mediated FKBP4 downregulation protects against stress-induced neuronal mitochondria dysfunctions by blocking nuclear translocation of GR. Cell Death & Disease. 2023; 14: 146.
10. Elmar Peschke, Helena Schucht, Eckhard Mühlbauer Long-term enteral administration of melatonin reduces plasma insulin and increases expression of pineal insulin receptors in both Wistar and type 2-diabetic Goto-Kakizaki rats. Journal of pineal research, 2010; 49(4): 373-381. doi: 10.1111/j.1600-079X.2010.00804.x
11. Seungwon An, Balachandar Nedumaran, Hong Koh, Dong Jin Joo, Hyungjo Lee, Chul-Seung Park, Robert A. Harris, Keong Sub Shin, Ali R. Djalilian, Yong Deuk Kim. Enhancement of the SESN2-SHP cascade by melatonin ameliorates hepatic gluconeogenesis by inhibiting the CRBN-BTG2-CREBH signaling pathway. Experimental & Molecular Medicine. 2023; 55:1556–1569.
12. Jin Feng, Xiaohui Wang, Xiaochun Ye Mitochondria as an important target of metformin: The mechanism of action, toxic and side effects, and new therapeutic applications. 2022; 177:106114 doi.org/10.1016/j.phrs.2022.106114
13. Anupama Nath, Songita Ghosh, Debasish Bandyopadhyay. Role of melatonin in mitigation of insulin resistance and ensuing diabetic cardiomyopathy. Life Sci. 2024; 355:122993. doi: 10.1016/j.lfs.2024.122993
14. Severino C., Brizzi P., Solinas A., Secchi G., Maioli M., Tonolo G. Low-dose dexamethasone in the rat: a model to study insulin resistance. American Physiological Society. Endocrinology and Metabolism. 2002;283(2), E367-E373. doi.org/10.1152/ajpendo.00185.2001
15. Guillermo Santos-Sánchez, Ana Isabel Álvarez-López, Eduardo Ponce-España. Melatonin Modulates Lipid Metabolism and Reduces Cardiovascular Risk in Apolipoprotein E-Deficient Mice Fed a Western Diet. Nutraceuticals 2024, 4(2), 260-272; doi.org/10.3390/nutraceuticals4020016,
16. Mateo Amaya-Montoya, Jairo A. Pinzón-Cortés, Lina S. Silva-Bermúdez ApoE and apoC-III-defined HDL subtypes: a descriptive study of their lecithin cholesterol acyl transferase and cholesteryl ester transfer protein content and activity. Lipids in Health and Disease. 2020;19(106): doi.org/10.1186/s12944-020-01291-x
17. Jun Xiang, Wen Zhu, Feng Yang Melatonin-induced ApoE expression in mouse astrocytes protects endothelial cells from OGD-R induced injuries. Transl Psychiatry. 2020;10:181. doi: 10.1038/s41398-020-00864-9
18. Ali Hasanpour Dehkordi, Abolfazl Abbaszadeh, Samareh Mir. Amin Hasanvand Metformin and its anti-inflammatory and anti-oxidative effects; new concepts. J Renal Inj Prev. 2019;8(1): 54-61. doi: 10.15171/jrip.2019.11
19. Vennila Suriagandhi, Vasanthi Nachiappan. Protective Effects of Melatonin against Obesity-Induced by Leptin Resistance. Behav Brain Res. 2022:417:113598. doi: 10.1016/j.bbr.2021.113598.
20. Abudukadier Abulizi; Rebecca l. Cardone; Stephan Siebel; Charles Kung; Richard Kibbey. Small molecule activator of pyruvate kinase regulates in vivo glucose homeostasis. Diabetes. 2020;69(1):120-LB. doi.org/10.2337/db20-120-LB
21. Tulipano G. Integrated or Independent Actions of Metformin in Target Tissues Underlying Its Current Use and New Possible Applications in the Endocrine and Metabolic Disorder Area. Int J Mol Sci. 2021;22(23):13068. doi: 10.3390/ijms222313068.PMID: 34884872
22. Siddhi, J. · Sherkhane, B. · Kalavala, A.K. Melatonin prevents diabetes-induced nephropathy by modulating the AMPK/SIRT1 axis: focus on autophagy and mitochondrial dysfunction. Cell Biol. Int. 2022; 46:2142-2157.