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ΦΑΡΜΑΚΕΥΤΙΚΗ, 37, ΙΙ, 2025 | 178-184 ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

PHARMAKEFTIKI, 37, II, 2025 | 178-184 REVIEW ARTICLE

Effect of Melatonin on Body Weight and Hormones in Male Rats

Muhammed M. Askar*, Anwar Y. Zaaen

Department of Pharmacology, College of Pharmacy, University of Anbar, Ramadi, Iraq.

DOI: 10.60988/p.v37i2.74

KEY WORDS: Melatonin, body weight, insulin, growth hormone, ACTH, metabolism.

ARTICLE INFO:
Received: October 12, 2024
Revised: February 25, 2025
Accepted: March 3, 2025
Available on line: June 30, 2025

*** CORRESPONDING AUTHOR:**
Ph.muhammed.malik@uoanbar.edu.iq

ABSTRACT

Background and objectives: Melatonin is a neurohormone secreted mainly by the pineal gland, where it is synthesized and secreted at night. Many research studies have shown that melatonin supplementation enhanced growth hormone secretion in rats, from this point the current research aimed to explore the effect of melatonin on other hormones and some endogenous substances related to metabolism. **Methods:** Current study uses 30 rats distributed into 3 groups (10 rats each); T1 group treated with melatonin (0.4 mg/kg) as a therapeutic dose, T2 group treated with melatonin (0.8 mg/kg) as double dose, and T0 group administered distilled water as a control group. **Results:** The administration of melatonin in double dose (T2 group) revealed a significant ($P<0.01$) reduction in body weight (189.20 g) at the end of the experiment when compared to T1 or control groups. Insulin showed a significant ($P<0.01$) increase in the T2 group (112.99 μIU/mL) when compared with T1 or control groups. The results showed there is an oblivious role of melatonin in inhibiting the secretion of the ACTH hormone, especially in the T2 group by revealing a significant reduction ($P<0.01$) of ACTH level in the T2 group (6.97 pg/ml). Further, results revealed a significant ($P<0.01$) increase in GH clearly in the T2 group (39.36 pmol/L). **Conclusion:** Melatonin has a negative effect on hormones that are responsible for normal growth and metabolism and may explain why melatonin is involved in regulating obesity in various species as well this effect may extend to humans and imitate loss of body weight when used chronically in children.

Introduction

In recent years, attention increased to the links between melatonin, sleep disorders, and metabolic syndrome. Insomnia and dyssomnia are important risk factors for obesity, insulin resistance, metabolism, and type 2 diabetes mellitus. Therefore, the treatment and regulation of circadian rhythms and sleep by exogenic therapy by using melatonin drugs can take an important role in the treatment and prevention of metabolic syndrome and obesity controlling[1]. The most common risk factors that lead to obesity is sleep deficit, the deprivation of sleep can affect the level of hunger hormone (ghrelin), by increasing the hormone level and hunger feeling[2]. Melatonin can affect various physiological functions, e.g. obesity by affecting body mass, energy consumption and energy expenditure by growth hormone (GH)[3]. These effects may vary depending on the species, it either reduces as in rats or increases body fat mass as in hamster[4]. GH have a chief role in the stimulation of longitudinal growth especially in postnatal by promoting bone growth[5]. Another study reveals that GH can reduce body fat[6]. Furthermore, it can regulate the metabolism of fats, minerals, electrolytes, carbohydrates, and nitrogen[7]. GH stimulates lipolysis in adipocytes, which leads to decreases in body fat; it also helps in the uptake of amino acids by muscle and the retention of nitrogen, which conserves muscular strength and mass[8]. GH increased when melatonin was administrated to mice, on the other hand, melatonin can increase the levels of leptin hormone which has an important function in the process of weight loss. Melatonin can act directly on the adrenal gland, repress the glucocorticoid response to ACTH (adrenocorticotropic hormone) in some animals such as sheep, and rats and this may also extend to humans[10], by a theory that explains the production of glucocorticoids in response to ACTH stimulation is not only influenced by external factors (such as stress) but is also subject to an internal factors e.g. circadian rhythm, which controlled by melatonin within the adrenal glands[11]. There is a potential relationship between melatonin and the human adrenal cortisol response to ACTH, this may contribute to reducing the adverse effects of cortisol such as stress and body weight[12]. In a study on insulin-melatonin relations in rats after inducing diabetes has concluded a converse association: when the level of melatonin decreases can lead to up-regulation of insulin secretion and vice versa and illustrates this inverse correlation can open new ways in diabetes treatment in humans[13]. The present study sought to identify the role of melatonin at therapeutic doses on the metabolic hormonal changes on rat model.

Materials and Methods

Experimental design: Thirty rats were divided into 3 equal groups with weights ranging from 250-280 grams and were placed in the Animal House of Anbar University - College of Pharmacy, under standard conditions with a temperature of 25°C and light cycle 12 hours/day[14,15]. The first group T1: 10 rats treated with melatonin at a therapeutic dose of 0.4 mg/kg, the second group T2: was treated with melatonin at 0.8 mg/kg as a 2-folded dose, and the third group T0: was administered distilled water as control group. The experiment was for 30 days, beginning from 7/3/2023 to 7/4/2023. Melatonin was dissolved in water at concentration of 1mg/ml to be administered at two doses of 0.4 mg/kg and 0.8 mg/kg rats body weight[16,17].

Ending the experiment and blood sampling: Two blood samples were withdrawn; the first blood sample was withdrawn initially before melatonin administration from their tail. At the end of the experiment the rats were fasting for 24 hrs. By using xylazine and ketamine the rats were anaesthetized intramuscularly at doses (0.04 g/kg and 0.09 g/kg body weight), respectively. The second blood sample were withdrawn by using the heart puncture technique collected in regular tubes and used to separate the serum to conduct special tests in the current experiment.

Parameters studied: rats body weight were recorded and compared. The serum growth hormone, ACTH, and insulin were measured as per manufacturer instructions using rat ELISA kits supplied by Toso (Japan). The principle of assay used to quantified target hormones (growth hormone, ACTH, and insulin) were based on sandwich ELISA technique, which do involve several steps starting with covering the microplate surface with capture antibodies overnight, followed by sequential

Table 1. Effect of melatonin administration to rats in different doses on body weight

Melatonin (mg/kg)	Body weight (g)	
	Zero time	End of experiment
T0	270.00±5.45a	304.40±2.48a
T1	261.80±2.82a	241.60±2.62b
T2	266.60±3.32a	189.20±2.15c
LSD 0.05	13.35	7.559
CV.	3.64	2.214

Data expressed as mean±SD, p<0.05 is considered significant using One-way analysis of variance (ANOVA) with Tukey's multiple comparison tests, T0: control group, (T1): 0.4 mg/kg melatonin and (T2): 0.8 mg/kg melatonin, (LSD): lowest significance difference, (CV): coefficient of variation.

Table 2. Effect of melatonin administration to rats in different doses on Growth hormone, Insulin and ACTH for 30 days compared with the control group

Melatonin (mg kg^{-1})	Growth hormone (pmol L)	Insulin (µIU mL)	ACTH (pg ml)
M_0	25.99± 0.98a*	86.45± 0.73a	13.53± 0.55a
$M_{0.4}$	31.09± 0.87b	92.06± 0.97b	10.79± 0.53b
$M_{0.8}$	39.36± 0.74c	112.99± 1.08c	6.97± 0.59c
$LSD_{0.05}$	2.71	2.92	1.73
CV.	6.04	2.16	11.92

Data expressed as mean±SD, p<0.05 is considered as significant using One-way analysis of variance (ANOVA) with Tukey's multiple comparison tests,T0: control group, (T1): 0.4 mg/kg melatonin and (T2): 0.8 mg/kg melatonin, (LSD): lowest significance difference, (CV): coefficient of variation.

Table 3. Pearson coefficient r of intercorrelation of studied properties. (BWZ): body weight at zero time of experiment, (BWE): body weight at end of experiment.

properties	BWZ	BWE	GH	Insulin	ACTH
BWZ		0.696	0.816	0.934	0.790
BWE	0.460		0.120	0.238	0.094
GH	-0.285	-0.982		0.118	0.026
INSULIN	-0.104	-0.931	0.983		0.145
ACTH	0.325	0.989	-0.999	-0.974	

washing steps in the morning to be followed by sample and standard loading to the well for 2 hours. A second washing step started, followed by enzyme conjugation step of addition of streptavidin-horseradish peroxidase to react with the target protein, the excess enzyme removed by plate washing. The bounded enzyme reacted with added substrate 3,3',5,5'-tetramethylbenzidine giving light yellow color to be spectrophotometrically quantified at 450nm[12,13].

Statistical Analysis: Data presented as means ± standard deviation (SD). For serum growth hormone, ACTH, and insulin, One-way analysis of variance (ANOVA) was used, and Tukey's multiple comparison tests were used whenever the ANOVA test turned significant. SPSS (version 23, USA) statistics software was used for statistical analysis. A p-value less than 0.05 was considered significant.

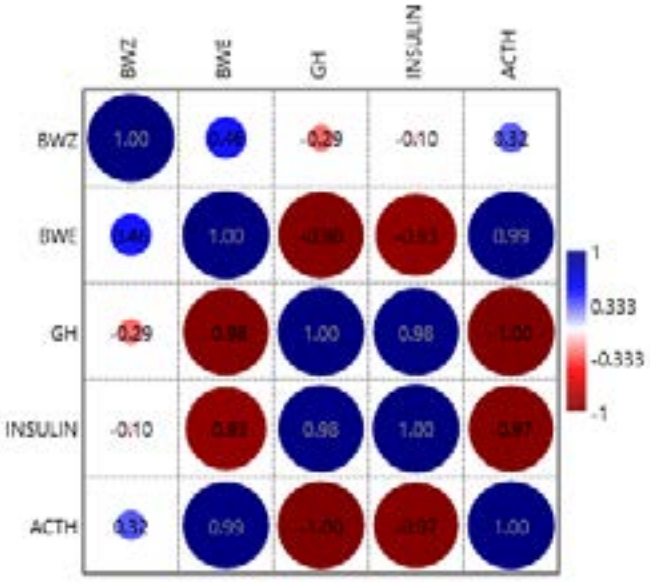

***Figure 1**. Color plot correlation of studied properties (BWZ): body weight at zero time of experiment, (BWE): body weight at end of experiment.*

Results

Body weight: The results in (Table 1) below showed the effect of melatonin on the body weight of the rats, there was no significant difference (P>0.01) in the body weight of all groups before starting the experiment (zero time). The T0 group body weight showed a significant increase (P<0.01) at the end of the experiment when compared with the same group at zero-time T1 and T2 groups in all periods of the experiment. During the 30 days of the experiment, the body weight results of the T2 group showed a significant reduction (P<0.01) in body weight compared to the same group at zero time, the T1 and T0 groups.

Growth hormone (GH), insulin, and ACTH: The results in (Table 2) below showed the effect of melatonin on growth hormone (GH) (pmol/L), insulin (μIU/mL) and ACTH (pg/ml). The results showed the administration of melatonin at a dose of 0.8 mg/kg in the T2 group had the highest significant (P<0.01) increasing level of growth hormone (39.36) pmol/L as well as insulin level (112.99) μIU/mL when compared with T0 and T1 groups. While the results of ACTH level were significant (P<0.01) largest decrease in the T2 group (6.97) pg/ml when compared with T0 and T1 groups.

Parameter correlation: The body weight at zero-time positively correlated with body weight at end of trial (0.46) and ACTH (0.325), while its negatively correlated with GH (-0.29) and insulin (-0.10). whereas, GH negatively significant correlated with body weight at end of trial (-0.98) and ACTH (-1.00) (Table 3, Figure 1).

Discussion

The results of this study confirmed that melatonin supplementation can help to reduce body weight and improve metabolic function in overweight individuals, improve insulin resistance, and lipid profiles in obese rats, even when used at low therapeutic standard doses when administered to rats on regular basis in a relatively short period of time.

The present study has confirmed that the body weight of rats in total were reduced at therapeutic and over therapeutic doses, this has been confirmed by earlier studies, who have found that melatonin sup-

plementation can improved body weight and reduced abdominal fat in overweight in human possibly by metabolite acceleration pathways[18,19]. Animal studies have indicated that melatonin may affect metabolism and energy balance and weight loss through its effect on the activity of some enzymes involved in fat metabolism[20].

Another animal studies discussed the effect of melatonin on body weight by different mechanisms e.g. metabolic effects by receptors of melatonin that present in various tissues, including adipose tissue in addition to its effect on the activity of some enzymes involved in fat metabolism e.g. lipoprotein lipase (LPL) and hepatic TG lipase) and by the ability of melatonin in activation of brown adipose tissue (BAT) causing burn more calories to generate heat[21]. Several studies have shown that the pineal gland can affect GH-IGF-1 function, and it appears that the change in endogenous melatonin concentration plays an important role in this mechanism[22].

Several years ago, melatonin was found to lead to higher levels of growth hormone (GH) in humans undergoing reconstruction[23]. Sleep cycle disturbances affect the release of cortisol. It has been shown that daily intake of melatonin reduces the response to ACTH secretion in cases of acute and chronic stress in rats[24]. Melatonin has an important role in insulin resistance in peripheral tissues, including adipose tissue[25]. Patients with obesity taking melatonin for 12 weeks show a pronounced decrease in the Insulin resistance index[26]. In the case of existing Insulin Resistance, melatonin treatment improves glucose metabolism in the insulin resistance model by restoring the effect of insulin on the cardiovascular system[27-29]. Melatonin participates in improving Insulin resistance may via MT1 receptors or by preventing mitochondrial dysfunction[30].

Conclusion

It's important to take seriously the role of melatonin in reduction of body weight by affecting metabolism through stimulating growth hormone, ACTH and insulin secretion especially when taken for long time as therapy for children to induction of sleep. In other hand, these characteristics of melatonin may helpful as therapeutic doses can enhance the medication that used for obesity and weight control as adjuvant therapy.. More explanation needed for effects of melatonin on other endocrine system and hormonal balances. □

References

1. Singh S., Sarma S., Kashyap A., Chakrabarti A. Alteration of melatonin receptor expression associated with melatonin-mediated amelioration of oxidative stress in the spleen of hyperthyroid mice. *Physiol. Pharmacol.* 28(3), 324-337, 2024.
2. Raja N., Ganesan A., Krithika C.L., Aniyan Y. Effect of saffron extract on sleep quality and melatonin concentrations in adults with temporomandibular disorders-an observational study. *Texila Int. J .Pub. Heal.* 12(4), 1-10, 2024.
3. Dorosh D., Liadova T., Popov M., Volobuieva O., Pavlikova K., Tsivenko O., Volobuiev D. The effect of melatonin on the serum level of interleukin 31 in herpesvirus skin diseases on the background of HIV. *Georgian Med. News.* 347, 181-184, 2024.
4. Rashidi S.K., Ansari Dezfouli M., Khodagholi F., Dadashpour M., Shabani A.A. Melatonin Attenuates Methamphetamine Neurotoxicity through inhibition of NLRP3 and pyroptosis pathway. *Physiol. Pharmacol*. 28 (4), 430-439, 2024.
5. Dixit M., Poudel S. B., Yakar S. Effects of GH/IGF axis on bone and cartilage. *Mol. Cell Endocrinol.* 519, 1-8, 2021.
6. Reinehr T., Lindberg, A., Koltowska-Häggström M., Ranke M. Is growth hormone treatment in children associated with weight gain?–longitudinal analysis of KIGS data. *Clin. Endocrin*. 81(5), 721-726, 2014.
7. Guevara-Aguirre J., Guevara A., Palacios I., Pérez M., Prócel P., Terán E. GH and GHR signaling in human disease. *Growth Horm. IGF R*. 38, 34-38, 2018.
8. Mustafa B., Shareef A., Mahmood M. Comparison of bone maturation response to treatment with short and long-term growth hormone therapy in short-stature pediatric patients. *Georgian Med. News*. 331(10), 27-30, 2022.
9. Jbarah R.M., Ali L.Q. The Effect of Melatonin on Leptin and Growth Hormone in Mice*. J. Univ. Anbar. Pure Sci*. 18(1), 116 – 123, 2024.
10. Dorosh D., Liadova T., Popov M., Volobuieva O., Pavlikova K., Tsivenko O., Volobuiev D. The effect of melatonin on the serum level of interleukin 31 in herpesvirus skin diseases on the background of HIV. *Georgian Med. News*. 347(2), 181-184, 2024.
11. Torres-Farfan C., Abarzua-Catalan L., Valenzuela F.J., Mendez N., Richter H.G., Valenzuela G.J., Serón-Ferré M. Cryptochrome 2 expression level is critical for adrenocorticotropin stimulation of cortisol production in the capuchin monkey adrenal. *Endocrin*. 150(6), 2717-2722, 2009.
12. Valenzuela F.J., Torres-Farfan C., Richter H.G., Mendez N., Campino C., Torrealba F., Serón-Ferré M. Clock gene expression in adult primate suprachiasmatic nuclei and adrenal: is the adrenal a peripheral clock responsive to melatonin? *Endocrin*.149(4), 1454-1461, 2008.
13. Swadi A., Hilal N., Abdul-Aziz M. The role of melatonin and vitamin D in Iraqi premenopausal women osteoarthritis patients. *Georgian Med. News*. 338(5), 53-56, 2023.
14. Al-Thanoon Z. A., Merkhan M. CoQ10 improved liver function and redox status in pollution-exposed workers. *Pharmakeftiki*. 36(2), 67-76, 2024.
15. Qasim H.O., Jabber I.M., Ahmed S.M., Merkhan M.M. Aripiprazole cognitive effects on attention deficit hyperactivity disorder (ADHD) in experimental mouse model. *Rev. Clin. Pharmacol. Pharmacokinet. Int*. 38 (2), 197-204, 2024.
16. Shida C.S., Castrucci A.M.D.L., Lamy-Freund M.T. High melatonin solubility in aqueous medium. *J. Pineal Res*. 16(4), 198-201, 1994.
17. Kandimalla K., Kanikkannan N., Andega S., Singh M. Effect of fatty acids on the permeation of melatonin across rat and pig skin in-vitro and on the transepidermal water loss in rats in-vivo*. J. Pharm. Pharmacol*. 51(7), 783-790, 1999.
18. Laudon M., Frydman-Marom A. Therapeutic effects of melatonin receptor agonists on sleep and comorbid disorders. *Int. J. Mol. Sci.* 15(9), 15924-15950, 2014.
19. Amstrup A. K., Sikjaer T., Pedersen S. B., Heickendorff L., Mosekilde L., Rejnmark L. Reduced fat mass and increased lean mass in response to 1 year of melatonin treatment in postmenopausal women: A randomized placebo-controlled trial. *Clin. Endocrin*. 84(3), 342-347, 2016.
20. Farias T.S.M., Cruz M.M., Sa R.C.C., Severi I., Perugini J., Senzacqua M., Alonso-Vale M.I.C. Melatonin supplementation decreases hypertrophic obesity

and inflammation induced by high-fat diet in mice. *Front. Endocrin*ol. 10, 750, 2019.
21. Shareef M.S., Charfeddine B., Roomi A.B. The Association between Spexin Levels, Lipid Profiles, Glucose Metabolism, and Immune Function in Newly Diagnosed Diabetes in Postmenopausal Iraqi Women. *Rev. Diab. Stud*. 19(3), 166-170, 2023.
22. Jindal P., Kant K., Kaur N., Gupta S., Ali A., Naeem M. Melatonin: Discovery, biosynthesis, phytohormones crosstalk, and roles in agricultural crops under abiotic stress conditions. *Environ. Exp. Bot*. 226,105942, 2024.
23. Ahmad S.B., Ali A., Bilal, M., Rashid S.M., Wani, A.B., Bhat R.R., Rehman M.U. Melatonin and health: insights of melatonin action, biological functions, and associated disorders. *Cell Mol. Neurobiol.* 43(6), 2437-2458, 2023.
24. Juszczak M., Roszczyk M., Kowalczyk E., Stempniak B. The influence of melatonin receptors antagonists, luzindole and 4-phenyl-2-propionamidotetralin (4-P-PDOT), on melatonin-dependent vasopressin and adrenocorticotropic hormone (ACTH) release from the rat hypothalamo-hypophysial system. vitro and in vivo studies. *J. Physiol. Pharmacol*. 65, 777-784, 2014.
25. de Oliveira A.C., Andreotti S., Farias, T.S., Torres-Leal F.L., de Proença A.R., Campaña A.B., Lima F.B. Metabolic disorders and adipose tissue insulin responsiveness in neonatally STZ-induced diabetic rats are improved by long-term melatonin treatment. *Endocrin*ology153(5), 2178-2188, 2012.
26. Sun H., Wang X., Chen J., Gusdon A.M., Song K., Li, L., Qu S. Melatonin treatment improves insulin resistance and pigmentation in obese patients with Acanthosis nigricans. *Int. J. Endocrinol*. 2018(1), 2304746, 2018.
27. Sartori C., Dessen P., Mathieu C., Monney A., Bloch J., Nicod P., Duplain, H. Melatonin improves glucose homeostasis and endothelial vascular function in high-fat diet-fed insulin-resistant mice. *Endocrinology* 150(12), 5311-5317, 2009.
28. Esmaeil F.K., Alnori M.K., Ajeel M.A. Uncovering the Mechanistic Role of vitamin D in Modulating Glycemic Pertained Parameters in Insulin-resistant Patients. *Rev. Diab. Stud*. 20(1), 32-38, 2024.
29. Mohammad S., Fadhil N., Mahmood M. Effects of metformin and cinnamon on 1, 5 anhydroglucitol, adiponectin and ghrelin on newly diagnosed type 2 diabetes mellitus patients. *Jordan Med. J*. 55(4), 225-233, 2021.
30. de Luis D.A., Izaola O., Primo D., Aller R. A circadian rhythm-related MTNR1B genetic variant (rs10830963) modulate body weight change and insulin resistance after 9 months of a high protein/low carbohydrate vs a standard hypocaloric diet. *J. Diabetes Complications*. 34(4), 1-13, 2020.