# CHANGES IN DIURNAL AND NOCTURNAL MELATONIN LEVEL IN WOMEN WITH METABOLIC SYNDROME STRATIFIED BY THE CONCENTRATION OF SERUM TRIGLICERIDES AND HIGHT DENSITY LIPOPROTEIN CHOLESTEROL CONCENTRATION

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**Abstract:** Metabolic syndrome (MetS) is defined as a constellation of interrelated metabolic risk factors that increase the risk of developing type 2 diabetes mellitus and cardiovascular disease. According to the definitions of a number of international organizations and expert groups of MetS, dyslipidaemia is always indicated as one of the main risk factor.
Objective: To compare the night and day concentrations of the hormonal parameters melatonin, leptin, ghrelin in women with MetS and we analyse their relationship according to the concentration of high-density lipoprotein (HDL)-cholesterol and the concentration of triglyceride (TG).
Methods: 41 women with diagnosis MetS according to IDF consensus criteria were enrolled in our study. Melatonin samples were taken at night (02:00 am- 03:00 am; light < 10 lux) and in the morning (08:00 am - 09:00 am). A morning venous blood was drawn after 8 hours of fasting. Serum glucose, total cholesterol, TG, HDL-cholesterol, and low-density lipoprotein (LDL) cholesterol levels were analyzed according to the manufacturer's original programs (Olympys AU 480, Beckman Coulter, USA). Serum melatonin concentrations were measured using ELISA kit (Elabscience Biotechnology Inc, China), serum ghrelin (ELISA kit, Elabscience Biotechnology Inc, China) and leptin (ELISA kit IBL-Hamburg, Germany) on Sirio S micro plate reader (SEAC, Italy). Each participant provided informed consent. All data were analysed by descriptive and nonparametric analysis. Statistical significance was accepted at $P < 0.05$.
Results: Women with TG ≥ 1.7 mmol/l did not have a statistically significant difference in age compared to women with TG < 1.7 mmol/l ($t = 1.28$, $P = 0.209$), in mean waist ($t = 0.11$, $P = 0.916$) and in mean BMI values ($U = 154.50$, $P = 0.652$). Women with TG ≥ 1.7 mmol/l have a higher average concentration of glucose and total cholesterol than women with TG < 1.7 mmol/l. The two subgroups did not differ in melatonin at 03:00am. and 08:00am and in other metabolic and hormonal parameters. Women with HDL-cholesterol < 1.3 mmol/l had a statistically significant larger waist ($t = 3.45$, $P = 0.001$) and higher BMI ($U = 126.50$, $P = 0.029$) and leptin at 08.00am ($U = 134.00$, $P = 0.047$), from women with HDL - cholesterol ≥ 1.3 mmol/l. The two subgroups did not differ in melatonin at 03:00 a. m and 8:00 a.m. and other hormonal and metabolic indicators.
Conclusion: Low HDL-cholesterol and elevated TG are associated with increased waist circumference, BMI and leptin, respectively, rather than with changes in the other investigated hormonal indicators.
**Keywords:** melatonin, leptin, ghrelin, metabolic syndrome, women

## 1. INTRODUCTION

Metabolic syndrome (MetS) become a global epidemic problem (Saklayen M. G. (2018) and is associated with an increased risk of cardiovascular disease (CVD) and type 2 diabetes (T2DM) (Zimmet, P., Magliano, D., Matsuzawa, Y., Alberti, G., & Shaw, J. (2005). According to the definitions of MetS different expert groups, like the European Diabetes Federation (IDF) (Alberti, K. G., Zimmet, P., Shaw, J.,2005) or the expert consensus group of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) (2002), dyslipidemia is always presented as fasting TG level above 1.7 mmol/l, fasting HDL-cholesterol level below <1.03 mmol/l men, < 1.29 mmol/l women. ATPIII also recommends several additional criteria: biomarkers of adipose tissue (adiponectin, leptin), apolipoprotein B, low-density lipoprotein (LDL cholesterol), measurement of insulin resistance and oral glucose tolerance test, endothelial dysfunction, urinary albumin, inflammatory markers (C -reactive protein, tumor necrosis factor α, interleukin 6) and thrombotic markers (plasminogen activator inhibitor type 1, fibrinogen). The

new hormonal markers are investigated in MetS. Melatonin is a methoxyindole synthesized and secreted mainly by the pineal gland at night, the endogenous rhythm of secretion being generated by the suprachiasmatic nuclei and entrained by the light/dark cycle (Claustrat, B., & Leston, J. (2015). Al-Sarraf, I et al. (2018) focused in their study on the importance of the chronobiology, represented by melatonin (MT) and crypto chrome 2 (CRY2), in developing MetS and type 2 diabetes mellitus (T2DM). The role of melatonin on oxidative stress, protein glycation micro albuminuria, and lipid profile in T2DM was presented in the study of Hasan M.H. et al. (2006), but the mechanism remains still unclear.

## 2. MATERIAL AND METHODS

41 women with metabolic syndrome aged 18 to 66 were included in our study. The diagnosis of MeS was made according to IDF criteria in 2006 (3). The biological material for determining serum concentrations of clinical-chemical indicators - glucose, total cholesterol, HDL-cholesterol, TG, and Low-density lipoprotein (LDL) cholesterol and immunoreactive insulin was taken in the morning of fasting between 08:00 – 09:00. The parameters were determined on a clinical chemical analyser Olympus AU480 (Beckman Coulter, USA) according to original programs and with original reagents of the manufacturer. Melatonin and ghrelin were determined with a competitive immunoenzymatic assay of the company Elabscience Biotechnology Inc., and leptin with a sandwich immunoenzymatic assay of the company IBL-Hamburg, Germany. The measurement was performed on a Sirio S micro plate reader, SEAC, Italy. BMI was calculated for all patients (according to the formula by dividing the weight in kilograms by the height in meters squared). Index of insulin resistance (HOMA-IR) was calculated by the formula: fasting serum glucose (mmol/l) x fasting insulin (mIU/l)/ 22.5. The results were analysed with SPSS v.17.0 software package. The Kolmogorov-Smirnov test was used to test the distribution of the quantitative values. Comparisons between groups were performed using the independent samples t-test (for Gaussian distribution of data) and Mann-Whitney U test (for non-Gaussian distribution). Data are presented as mean value and standard error of the mean (mean±SEM), the level of significance was at $P< 0.05$.

## 3. RESULTS

Subdivision of persons according to HDL cholesterol concentration

Women with MetS were divided into two subgroups according to the metabolic indicator HDL cholesterol. The mean age of women with HDL cholesterol < 1.3 mmol/l (n=20) was 30.75 ± 3.03 years, and of women with HDL cholesterol ≥ 1.3 (n = 21) was 36.95 ± 2.52 years. The two groups did not differ in mean age ($t = 1.579$, $P = 0.122$). There was a statistically significant difference between the two groups for BMI ($t = 2.601$, $P = 0.013$) and waist ($t = 3.451$, $P = 0.001$). Data from the comparative analysis of metabolic parameters in the two groups of women with MetS, divided according to HDL cholesterol < 1.3 or ≥ 1.3 mmol/l are presented in Table 1, 2. Data are mean ± standard error of the mean (mean ± SEM). We found no statistically significant difference between the two groups in terms of metabolic parameters.

***Table 1. Metabolic indicators in women with Gaussian distribution type HDL cholesterol < 1.3 or ≥ 1.3 mmol/l***

| Parameters | Women with HDL chol. < 1.3 mmol/l (n = 20) | Women with HDL chol ≥ 1.3 mmol/l (n = 21) | t | P |
|---|---|---|---|---|
| Total cholesterol. (mmol/l) | 5.13 ± 0.230 | 5.37 ± 0.240 | 0.73 | 0.473 |
| LDL - chol. (mmol/l) | 3.53 ± 0.178 | 3.43 ± 0.203 | 0.35 | 0.729 |

* The difference is statistically significant at $P < 0.01$

Source: Own data

***Table 2. Metabolic indicators in women with non-Gaussian distribution type HDL cholesterol < 1.3 or ≥ 1.3 mmol/l***

| Parameters | Women with HDL chol. < 1.3 mmol/l (n = 20) | Women with HDL chol ≥ 1.3 mmol/l (n = 21) | U | P |
|---|---|---|---|---|
| TG (mmol/l) | 1.28 ± 0.229 | 1.44 ± 0.275 | 201.50 | 0.825 |
| IRI (IU/l) | 25.80 ± 12.25 | 11.49 ± 2.600 | 111.00 | 0.182 |
| HOMA-IR | 6.616 ± 3.283 | 2.913 ± 0.613 | 192.00 | 0.638 |
| Glucose (mmol/l) | 5.81 ± 0.081 | 6.23 ± 0.530 | 107.00 | 0.142 |

* The difference is statistically significant at P < 0.01

Source: Own data

The comparative analysis of the hormonal parameters in the two groups of patients showed no statistically significant difference and is presented in Table 2. Data are mean ± standard error of the mean (mean ± SEM).

***Table 3. Hormonal indicators in women with HDL cholesterol. < 1.3 mmol/L or ≥ 1.3 mmol/L***

| Parameters | Women with HDL chol. < 1.3 mmol/l (n = 20) | Women with HDL chol. ≥ 1.3 mmol/l (n = 21) | P value |
|---|---|---|---|
| Melatonin (pg/ml) 3.00 | 143.82 ± 12.93 | 153.35 ± 19.21 | 0.683 |
| Melatonin (pg/ml) 8.00 | 169.68 ± 23.32 | 184.62 ± 125.70 | 0.894 |
| Leptin (ng/ml) 3.00 | 14.21 ± 1.66 | 11.54 ± 2.35 | 0.361 |
| Leptin (ng/ml) 08.00 | 19.61 ± 4.91 | 10.89 ± 2.31 | 0.111 |
| Ghrelin (ng/ml) 03.00 | 2.29 ± 0.86 | 4.95 ± 2.00 | 0.425 |
| Ghrelin (ng/ml) 08.00 | 1.29 ± 0.19 | 1.58 ± 0.29 | 0.411 |

* The difference is statistically significant at P < 0.01

• Subdividing the persons according to the concentration of TG

Source: Own data

The patients included in the study were divided into two subgroups according to TG concentration: one group included 11 women with TG ≥ 1.7 mmol/l, and the other group included 30 women with TG < 1.7 mmol/l. Women with TG ≥ 1.7 mmol/L had a mean age of 38.18 ± 4.73 years, and women with TG < 1.7 mmol/L had a mean age of 32.55 ± 2.04 years (difference not statistically significant, t = 1.28, P = 0.209). We compared the TG concentration in women from the two subgroups with the Mann-Whitney test and found that they differed significantly in mean TG concentration (U = 496.00, P < 0.0001). Women with TG ≥ 1.7 mmol/l have an average waist (mean ± SEM) of 95.09 ± 4.76 cm, and for women with TG < 1.7 mmol/l, it is 94.484 ± 3.15 cm (the difference is not statistically significant, t = 0.11, P = 0.916). Women with TG ≥ 1.7 mmol/l have average BMI values of 28.57 ± 2.12 kg/m2, and for women with TG < 1.7 mmol/l, it is 29.82 ± 1.38 kg/m2 (the difference is not statistically significant, U = 154.50, P = 0.652). Table 3 presents data from the comparative analysis (mean ± SEM) of the metabolic parameters with a Gaussian distribution in the two subgroups of women with MetS according to the concentration of TG and Table 4 presents the data from the analysis of the metabolic parameters with a non-Gaussian distribution with the Mann-Whitney U test.

***Table 4. Metabolic parameters with normal data distribution in women with TG ≥ 1.7 mmol/l and TG < 1.7 mmol/l***

| Parameters | TG ≥ 1.7 mmol/l (n=11) | TG < 1.7 mmol/l (n=30) | t | P |
|---|---|---|---|---|
| Cholesterol (mmol/l) | 5.87±0.38 | 5.04±0.16 | 2.36 | 0.023 |
| HDL- chol. (mmol/l) | 1.36±0.09 | 1.35±0.06 | 0.07 | 0.946 |
| LDL- chol. (mmol/l) | 3.88±0.30 | 3.35±0.14 | 1.82 | 0.077 |

Source: Own data

***Table 5. Metabolic indicators with a non-Gaussian type of data distribution in women with TG ≥ 1.7 mmol/l and TG < 1.7 mmol/l***

| Parameters | TG ≥ 1.7 mmol/l (n=11) | TG < 1.7 mmol/l (n=30) | U | P |
|---|---|---|---|---|
| IRI (μIU/L) | 7.07±0.93 | 5.64±0.12 | 96.00 | 0.241 |
| glucose (mmol/l) | 11.65±3.63 | 22.13±9.01 | 69.00 | 0.003 |
| HOMA-IR | 3.38±1.10 | 5.47±2.37 | 97.00 | 0.256 |

Source: Own data

The comparative analysis of the results shows that patients with TG ≥ 1.7 mmol/l have statistically significantly higher total cholesterol and glucose than women with TG < 1.7 mmol/l (P < 0.05). Table 5 presents the data from the comparative analysis of hormonal parameters, dividing women according to TG concentration.

***Table 5. Hormonal indicators in women with TG ≥ 1.7 mmol/l and TG < 1.7 mmol/l***

| groups / parameters | TG ≥ 1.7 mmol/l (n = 11) | TG < 1.7 mmol/l (n = 30) |
|---|---|---|
| Melatonin (pg/ml) 3.00 | 124.51 ± 20.20 | 157.18 ± 13.59 |
| Melatonin (pg/ml) 8.00 | 190.61 ± 41.96 | 170.22 ± 20.97 |
| Leptin (ng/ml) 3.00 | 10.72 ± 1.76 | 13.65 ± 1.85 |
| Leptin (ng/ml) 08.00 | 11.25 ± 2.29 | 17.16 ± 3.51 |
| Ghrelin (ng/ml) 03.00 | 0.78 ± 0.18 | 1.12 ± 0.11 |
| Ghrelin (ng/ml) 08.00 | 1.58 ± 0.25 | 1.37 ± 0.21 |

Source: Own data

We found that women with TG ≥ 1.7 mmol/l did not differ from women with TG < 1.7 mmol/l in the mean concentration of: • melatonin at 3:00 a.m. (t = 1.27, P = 0.213) and at 8:00 a.m. (U = 170.00, P = 1.00) • leptin at 3:00 a.m. (t = 0.89, P = 0.378) and at 8:00 a.m. (U = 154.50, P = 0.652) • ghrelin at 3:00 a.m. (t = 1.62, P = 0.115) and at 8:00 a.m. (U = 135.50, P = 0.322)

## 4. DISCUSSION

The relationship between melatonin levels and the development of MetS has been of great interest in recent years. The role of pineal gland in the regulation of body energy balance is investigated in several scientific experimental studies, but the mechanism remains to be; elucidated (Santos-Ledo, A. et al 2021). While several experimental model and human studies have shown effect of melatonin on lipid parameters as an adjunctive therapy, only a few human studies have examined endogenous melatonin levels in MetS. Cardinali et al. (2013) demonstrated in their experimental study that melatonin (25 μg/mL drinking solution) counteracted the changes in body weight, systolic blood pressure, plasma LDL-cholesterol, TG, and cholesterol levels in fructose-administered rats, The results in another experimental study gave the possibility of researchers to conclude that melatonin improves dyslipidaemia in a model with MetS (Santos-Ledo, A.2021).The results highlight a possible therapeutic role for melatonin in MetS. Two meta-analysis of Mohammadi-Sartang M et al (2018) and Yuying Li Y.et al. (2024) suggest a significant association between melatonin supplementation and lipid parameters: both shows reduction of total cholesterol levels and TG, while there was no significant effect on LDL-cholesterol (p = 0.615) and HDL -cholesterol (p = 0.197) according to the first research group. The results of Li Y. (2024) showed that melatonin significantly reduced LDL cholesterol, and increased HDL cholesterol levels. Both studies of Goyal et al (2014) Koziróg et al. (2011) evaluate the effects of melatonin supplementation on components of MetS in patients. Even the dosages are different 8mg vs 5 mg both shows favoured melatonin effect on lipid parameters: TG (p = 0.17); HDL cholesterol (p = 0.59); LDL cholesterol (P<0.05). Abood S.J. et al. (2020) presented results at patients with MetS using metformin-melatonin showed improvement in most components of MetS such as fasting serum glucose, lipid profile, and body mass index, decrease in insulin resistance and hyperinsulinemia, increase in serum levels of uric acid, leptin, prolactin, and estradiol, while the serum progesterone level decreases. Partsernyak A.S. et al. (2022) investigated two groups of patients in vitro pineal immunohistochemistry: 25 deceased men with MetS and ischemic cardiomyopathy measured the number of melatonin receptors type 1 (RMA) and type 2 (RMB), 25 deceased by accident; 94 people with MetS three types of treatment measured lipid profile and 6MT, 24 controls. The results showed a lower number of receptors in all with MetS end a decrease in the level of cholesterol and atherogenic lipoproteins (LDL and TG) was observed in all groups. Melatonin is known as circadian synchronizer and the

decreased level in our group of women with Mets can also be liked withe the metabolic disturbances in their lipid and glucose metabolism. Söylemez S, et al. (2019) divided 50 nurses into two groups the night shift workers and the control group and they measured the levels of melatonin, leptin, insulin, fasting blood sugar, cholesterol, TG, HDL cholesterol, and LDL cholesterol. Melatonin was significantly lower in the night shift group compared to the day shift group (p=0.003). In the study the women with MetS leptin level were slightly but not significantly lower in the night shift group (p=0.097). In contrast, the level of ghrelin and other biochemical parameters including triglycerides, fasting blood glucose, insulin, HOMA-IR, and cholesterol were not statistically increased increased in the night shift group. The results of our study show higher leptin level at 8:00 am in the group with HDL< 1.3 mmol/l. The lack of difference of the melatonin concentration in 03:00 am and 8:00 am between the two groups when they are divided according the HDL level and TG concentration can be explained with the fact that all the participants of our study are with MetS. However, further studies need to be performed to decipher melatonin's hypolipidemic role in animal models and humans.

## 5. CONCLUSION

Low HDL-cholesterol in women with MetS is associated with increased waist circumference and high morning leptin. High TGs are associated with high total cholesterol and glucose level. We did not find statistically significant difference of the melatonin level between the two groups with different TG or HDL- cholesterol. Further investigation must interpret the correlation between the melatonin, leptin and ghrelin concentration in women with MetS and healthy control group.

## REFERENCES

Abood, Sattar J., Waleed K. Abdulsahib, Saad A. Hussain, and Sajida H. Ismail. (2020). Melatonin Potentiates the Therapeutic Effects of Metformin in Women with Metabolic Syndrome. Scientia Pharmaceutica 88,2: 28. https://doi.org/10.3390/scipharm88020028

Alberti, K. G., Zimmet, P., Shaw, J., & IDF Epidemiology Task Force Consensus Group (2005). The metabolic syndrome--a new worldwide definition. Lancet (London, England), 366(9491), 1059–1062. https://doi.org/10.1016/S0140-6736(05)67402-8

Al-Sarraf, I. A. K., Kasabri, V., Akour, A., & Naffa, R. (2018). Melatonin and cryptochrome 2 in metabolic syndrome patients with or without diabetes: a cross-sectional study. Hormone molecular biology and clinical investigation, 35(2), /j/hmbci.2018.35.issue-2/hmbci-2018-0016/hmbci-2018-0016.xml. https://doi.org/10.1515/hmbci-2018-0016

Cardinali DP, Bernasconi PAS, Reynoso R, Toso CFR, Scacchi P. (2013). Melatonin May Curtail the Metabolic Syndrome: Studies on Initial and Fully Established Fructose-Induced Metabolic Syndrome in Rats. InternationalJournal of Molecular Sciences. 14(2):2502-2514. https://doi.org/10.3390/ijms14022502

Claustrat, B., & Leston, J. (2015). Melatonin: Physiological effects in humans. Neuro-Chirurgie, 61(2-3), 77–84. https://doi.org/10.1016/j.neuchi.2015.03.002

Goyal, A., Terry, P. D., Superak, H. M., Nell-Dybdahl, C. L., Chowdhury, R., Phillips, L. S., & Kutner, M. H. (2014). Melatonin supplementation to treat the metabolic syndrome: a randomized controlled trial. Diabetology & metabolic syndrome, 6, 124. https://doi.org/10.1186/1758-5996-6-124

Hasan M.H. Al–Mhbashy; Hussain S. A.; Nawfal A.M. (2006) The Effects of Melatonin on The Oxidative Stress, Protein Glycation, Microalbuminuria and Lipid Profile in Type II Diabetes MellitusIraqi J.Pharm.Sci., .15 (1), DOI: https://doi.org/10.31351/vol15iss1pp27-32

Koziróg, M., Poliwczak, A. R., Duchnowicz, P., Koter-Michalak, M., Sikora, J., & Broncel, M. (2011). Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. Journal of pineal research, 50(3), 261–266. https://doi.org/10.1111/j.1600-079X.2010.00835.x

Li, Y., Sun, X., Wang, M., Jiang, Y., Ge, Q. Q., Li, T., Hou, Z., Shi, P., Yao, K., & Yin, J. (2024). Meta-analysis and machine learning reveal the antiobesity effects of melatonin on obese rodents. Obesity reviews: an official journal of the International Association for the Study of Obesity, e13701. Advance online publication. https://doi.org/10.1111/obr.13701

Mohammadi-Sartang, M., Ghorbani, M., & Mazloom, Z. (2018). Effects of melatonin supplementation on blood lipid concentrations: A systematic review and meta-analysis of randomized controlled trials. Clinical nutrition (Edinburgh,Scotland),37,1943–1954. https://doi.org/10.1016/j.clnu.2017.11.003

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (2002). Third Report of the National

Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation, 106(25), 3143–3421.

Partsernyak A.S., Polyakova V.O., Trufanov A.G., Medvedev D.S., Trotsyuk D.V., Markin K., Kurasov E.S., Kuznetsova E.V., Krasichkov A.S. (2022). Melatonin: Manager of psychosomatic and metabolic disorders in polymorbid cardiovascular pathology. Front Neurosci 16:989497. doi: 10.3389/fnins.2022.989497

Saklayen M. G. (2019). The Global Epidemic of the Metabolic Syndrome. Current hypertension reports, 20(2), 12. https://doi.org/10.1007/s11906-018-0812-z

Santos-Ledo, A.; Luxán-Delgado, B.d.; Caballero, B.; Potes, Y.; Rodríguez-González, S.; Boga, J.A.; Coto-Montes, A.; García-Macia, M. (2021).Melatonin Ameliorates Autophagy Impairment in a Metabolic Syndrome Model. Antioxidants, 10, 796. https://doi.org/10.3390/antiox10050796

Söylemez S, Sivri ABÇ, Şimşek E, Polat B, Çakır B. (2019). Melatonin, leptin, and ghrelin levels in nurses working night shifts. J Surg Med. 3(1):22-26. https://doi.org/10.28982/josam.443902

Zimmet, P., Magliano, D., Matsuzawa, Y., Alberti, G., & Shaw, J. (2005). The metabolic syndrome: a global public health problem and a new definition. Journal of atherosclerosis and thrombosis, 12(6), 295–300. https://doi.org/10.5551/jat.12.295