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Melatonin reduces structural disorders of the thyroid gland in rats with induced visceral obesity

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Abstract. Visceral obesity (VO) can cause many diseases in the body, including leading to thyroid pathology. Therefore, the search and development of new effective methods for preventing of thyroid dysfunction in obesity is a relevant research area. One of these methods can be the use of the pineal gland hormone – melatonin. **The aim** was to study the effect of melatonin on the histomorphometric changes in the thyroid gland of rats with VO and to evaluate the possibility of its use as a prophylactic agent for gland dysfunction in this pathology. **Material and methods.** For the experiment, 30 male Wistar rats aged 3 months were selected. VO in animals was modeled by maintaining them on a high-calorie diet (HCD). 3 groups of rats were formed: group I – control, group II – animals that were on the HCD for 12 weeks, and group III – rats that were on the HCD for 8 weeks, and then for another 4 weeks, in addition to this diet, orally received melatonin (Unipharm Inc., USA) at a dose of 5 mg/kg. Histological preparations were made from the thyroid tissue. Histomorphometry was performed using the «ImageJ» computer program. The concentration of total lipids, triglycerides and cholesterol was determined in blood serum. **Results.** Rats received HCD showed clear signs of VO: the weight of visceral fat and the concentration of lipid metabolism indicators probably increased. Structural changes were found in the thyroid gland, which indicated its hypofunction. Follicles were emptied of colloid and its accumulation was inhibited. Hyperplasia of the thyroid gland was observed, which led to an increase in its size. Rats that received melatonin in addition to HCD had significantly less structural thyroid dysfunction. In addition, melatonin contributed to reducing the degree of obesity development, namely, it reduced the weight of visceral fat and returned lipid metabolism indicators to control values. **Conclusion.** Administration of melatonin to rats has a protective effect on the thyroid gland against the harmful effects of obesity. This may be of practical interest when using melatonin as a prophylactic agent for disorders of the structure and function of the thyroid gland caused by VO.

Keywords: thyroid gland, melatonin, visceral obesity.

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The global increase in obesity among the population has become a serious problem in health care. Obesity can cause many diseases in the body, including leading to thyroid pathology [1]. Studies have shown that there may be a connection between obesity and thyroid activity [2]. It has been studied that hypothyroidism can cause the development of obesity. And obesity, in turn, can lead to a decrease in gland activity and further weight gain [3].

Obese people often have thyroid dysfunction. With obesity, the hypothalamic-pituitary-thyroid axis is activated, which leads to an increase in the level of thyroid-stimulating hormone. At the same time, the synthesis of thyroid hormones is at a low level [4]. In addition, obesity is often associated with insulin resistance, which can affect thyroid function, preventing the penetration of thyroid hormones into cells and the manifestation of their metabolic effects. In conclusion, obesity is the main risk factor for the development of thyroid dysfunction [5]. Therefore, the search and development of new effective methods for preventing thyroid function disorders in obesity is an actual direction of research. One of these methods can be the use of the pineal gland hormone – melatonin.

It is known from the literature data that exogenous melatonin supplements reduce weight gain and visceral fat deposition in many animal models associated with HCD feeding [6]. It has also been studied that melatonin plays an important role in glycemic homeostasis, mitochondrial activity, increases the activity of brown adipose tissue, and has antioxidant and anti-inflammatory properties [7]. While other works did not reveal a significant effect of melatonin on the development of obesity [8]. Therefore, additional studies are needed for further clinical confirmation of the melatonin effect in this pathology. The dose, time and duration of melatonin administration should be considered as a treatment option among the factors that determine its effectiveness.

Among scientists, there is no single point of view regarding the influence of melatonin on thyroid activity. Some researchers note the suppressive effect of melatonin on the thyroid gland [9], others – a stimulating effect [10], and some others deny any involvement of melatonin in the gland functioning [11]. Also, there is still no consensus on the possible role of melatonin as an adjunctive drug for the treatment of structural disorders of the thyroid gland in VO, although it shows great potential in this aspect. For a better understanding the mechanisms of melatonin's effect on the state of the thyroid gland in obesity, further researches are needed in this direction.

The aim of this work was to study the effect of melatonin on the histomorphometric changes in the thyroid gland of rats with VO and to evaluate the possibility of its use as a prophylactic agent for gland dysfunction in this pathology.

Material and methods

Experiment was conducted on 30 male Wistar rats aged 3 months. VO in animals was modeled by maintaining them on a HCD. 3 groups of rats were formed: group I – control, group II – animals that were on HCD, and group III – rats that were on HCD for 8 weeks, and then for another 4 weeks orally received melatonin (Unipharm Inc., USA) at a dose of 5 mg/kg in addition to HCD.

Each rat of the control group received 20 g of standard feed daily (66 kcal), which contained: 6% fat, 23% protein, and 55% carbohydrates (recipe K120-1 «Rezon-1», Ukraine). Animals of this group had free access to water. Experimental rats of the II and III groups received HCD with a total daily calorie content of 116 kcal per animal. The composition of such a diet, in addition to standard mixed feed, included pork lard, white breadcrumbs and sunflower seeds (fats – 45%, proteins – 9% and carbohydrates – 31%). In addition, experimental rats received a 10% fructose solution after a day instead of water, which increased the caloric content to 140 kcal and accelerated the development of VO [12].

Rats were removed from the experiment by decapitation under isoflurane anesthesia. All manipulations with rats were carried out in accordance with the principles of the Helsinki Declaration of 1975 and its revision of 1983, as well as in accordance with the «Rules for the performance of work using experimental animals» approved by the Ministry of Health of Ukraine. All protocols were also approved by the Committee on Biomedical Ethics of Animal Care and Use of the Bogomoletz Institute of Physiology of the NAS of Ukraine (protocol No. 5 dated 31.11.19).

For histomorphometric studies of the thyroid gland, samples of its tissue were taken from the central part, from which histological preparations were made according to the standard method [13]. The samples were fixed in Bouin's liquid, dehydrated in alcohols of increasing concentration and embed-

ded in paraffin. Paraffin sections 6 μm thick were made on a sled microtome (MS-2, Reichert, Austria). Staining of the obtained sections was carried out according to Van Gieson. Using a digital camera, micropreparations were photographed on an «Eclipse E100» microscope (Nikon, Japan). Morphometry on digital images of micropreparations was performed using the «ImageJ» program.

The cross-sectional area of follicles, colloid and follicular epithelium, external and internal diameters of follicles, the height of the follicular epithelium on histological sections of the thyroid gland were measured. The average number of thyrocytes in the follicles was calculated. The follicular-colloid index (the ratio of the area of the follicular epithelium to the area of the colloid), the stereological resorption index (4/h, where h is the average chord length of the line segments falling on the colloid) and the colloid accumulation index (the ratio of the average internal diameter to twice the height of the epithelium) were determined. Using the method of superimposing point morphometric grids, the relative area of the connective and parenchymal tissue of the gland was determined and the stromal-parenchymal index was calculated (the ratio of the relative area of the connective tissue to the relative area of the parenchyma of the gland). The width of interlobar, interlobular and interfollicular connective tissue layers was measured [14, 15].

The concentration of total lipids, triglycerides, and cholesterol was determined in the blood serum of rats by a colorimetric-enzymatic method using standard sets of reagents («Filisit-Diagnostika», Ukraine) on a biochemical analyzer («Sinnowa», China). The obtained data were processed by the methods of variational statistics using the software Statistica 6.0 for Windows («StatSoft», USA) and Excel 2010 («Microsoft», USA). The normality of the distribution of digital arrays was checked using the Shapiro-Wilk W-test. One-way analysis of variance was used to compare the samples. Differences at $p<0.05$ were considered significant.

Results and discussion

The presence of VO in rats was determined by the weight of visceral fat and by indicators of lipid metabolism in the blood serum. In the rats that received HCD for 12 weeks (group II), clear signs of VO were observed. This was evidenced by a significant greater weight of visceral fat by 133% (**Fig. 1**). Serum lipid parameters were also significantly increased, namely total lipids – by 50%, triglycerides – by 76%, and cholesterol – by 28% compared to controls. Rats that received melatonin in addition to HCD (group III) had less pronounced signs of VO. Thus, their visceral fat weight was 45% higher ($p<0.05$), and the indicators of lipid metabolism, in general, did not differ from the control values. That is, the administration of exogenous melatonin suppresses the development of VO in rats. Other authors also noted that administration of melatonin to the HCD can reduce the concentration of triglycerides and cholesterol in the blood of animals [16].

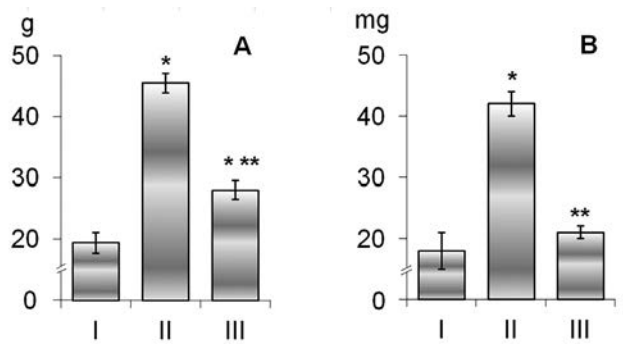

Fig. 1. Weight of visceral fat (A) and thyroid gland (B).

*Note: I – control rats, II – rats receiving HCD and III – rats receiving HCD and melatonin; *p<0.05 compared to the group I, ** p<0.05 compared to the group II; one way ANOVA followed by Bonferroni post hoc test, n=10 rats/group.*

At the end of the experiment, the rats of the group II showed an increase in the weight of the thyroid gland by 133% ($p<0.05$) compared to the control, which indicates its hyperplasia. One of the main reasons for the development of thyroid hyperplasia is insufficient synthesis of hormones and a decrease in their concentration in the blood. This leads to active stimulation of the gland, cell proliferation with subsequent increase in its weight and size [17]. Other authors also noted the increase in thyroid volume during obesity, associating it with an increase in the number of gland nodes [18]. In another study, after a 6-month stay of rats on the HCD, an increase in the size of the thyroid gland, an increase in the concentration of triglycerides in it, and a decrease in the concentration of thyroxine in the blood serum were found. In addition, the thyroid gland of these rats decreased the concentration of proteins associated with hormone synthesis [19]. Thyroid weight in animals of the group III did not differ from the control (Fig. 1).

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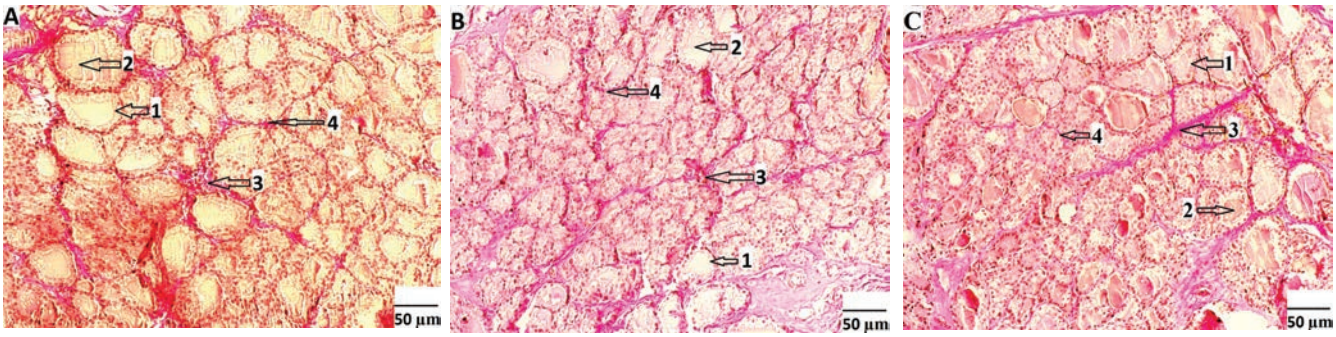

Fig. 2. Photomicrograph of a thyroid section of a control animal (A), after exposure to a HCD (B) and a rat that received a HCD and melatonin (C). Van Gieson's stain ×400.

Note. 1 – follicle; 2 – colloid; 3 – interlobular connective tissue; 4 – interfollicular connective tissue.

It was found that the thyroid gland of rats, which were on the HCD, preserved the lobular structure. The follicles were mostly small in size. Intensive processes of colloid resorption and reduction of its area were observed. This indicates inhibition of colloid accumulation, depletion of the gland and its hypofunction. Thyrocytes were mostly prismatic, rarely cubic in shape. An increase in the number of interfollicular islands (aggregation of thyrocytes without colloid) was detected. This may be related to compensatory mechanisms of the gland (in connection with its insufficient hormonal secretion), which are manifested by hyperplasia of thyrocytes **(Fig. 2)**.

Histomorphometric analysis of the thyroid gland in group II of rats revealed a significantly smaller area of follicles, colloid, and follicular epithelium by 36, 57, and 24%, respectively, than in controls. The external and internal diameters of follicles in these rats were smaller by 23 and 39% ($p<0.05$), respectively. The number of thyrocytes lining the follicle wall was also 13% less than in the control ($p<0.05$) **(Table)**.

Table. Histomorphometric parameters of the thyroid gland (M±m, n=10)

Parameters	Control	HCD	HCD+Melatonin
The relative area of parenchyma, %	77.9±1.8	76.2±1.3	76.3±1.6
Area, µm²:			
follicle	2590±76	1650±41*	2389±55^
colloid	940±46	400±20*	701±26*^
follicular epithelium	1650±70	1250±49*	1688±36^
Follicle diameter, µm:			
external	52.2±1.0	40.1±1.1*	50.5±1.5^
interior	30.7±1.3	18.7±1.5*	26.3±1.0^
The height of thyrocytes, µm	10.8±0.3	10.7±0.8	12.1±0.4
The number of thyrocytes in the follicle, pcs	21.5±0.7	18.8±0.5*	21.6±0.3
The relative area of stroma, %	22.1±1.1	23.8±1.0	23.7±1.8
Stromal-parenchymal index	0.28±0.02	0.31±0.03	0.31±0.01
The width of the interlayers of the connective tissue, µm:			
interlobar	22.1±1.5	23.3±1.0	20.8±1.2
interlobular	8.1±0.3	13.0±1.2*	8.3±0.3^
interfollicular	1.45±0.09	1.52±0.07	1.38±0.06

*Note. * – P<0.05 significant difference compared to the control, ^ – P<0.05 significant difference compared to the rats received HCD.*

Other authors found that the follicles in the thyroid gland of rats that were on the HCD were mostly large in size with excessive accumulation of colloid. The height of thyrocytes decreased, the cell cytoplasm was vacuolated and had a broken membrane [20]. The discrepancy in the obtained data may be related to different conditions of the experiments.

Rats that received melatonin in addition to HCD had smaller thyroid structural changes compared to animals in the group II. In the thyroid gland of these rats, the area of follicles, colloid, and follicular epithelium was found to be 45, 75, and 35% larger, respectively, than in animals of the group II. The external and internal diameter of the follicles in rats of the group III were larger than those of the group II by 26 and 41% ($p<0.05$), respectively (Table, Fig. 2).

Follicular-colloid index, stereological resorption index and colloid accumulation index were indicators that characterize the dynamics of colloid accumulation and removal. It was found that the follicular-colloid index and the stereological resorption index were significantly higher by 78 and 65%, respectively, in the rats that were placed on the HCD, compared to the control. The index of colloid accumulation, on the contrary, was lower by 39% ($p<0.05$). Changes in these parameters indicate the emptying of follicles from colloid and inhibition of its accumulation. In the thyroid gland of rats that received melatonin in addition to HCD, these indicators underwent less intense changes. Thus, the follicular-colloid index and the stereological resorption index were 23 and 29% lower ($p<0.05$), respectively, and the colloid accumulation index, on the contrary, was 23% ($p<0.05$) higher than in animals of the group II **(Fig. 3)**.

In the rats that were on the HCD, a tendency towards an increase in the amount of connective tissue in the gland was revealed. And the width of interlobular connective tissue layers was significant by 60% larger compared to the control. This is also a sign of gland hypofunction. In the thyroid gland of rats treated with melatonin, the width of the interlobular connective tissue did not differ from that of the control and was smaller than that of the group II by 36% ($p<0.05$). Other morphometric parameters of the connective tissue were close to the control values (Table).

It is known from the literature data that excessive obesity can lead to an increase in the interfollicular fat depot or steatosis in the thyroid follicular cells. Thus, steatosis and ultrastructural changes, including stretching of the endoplasmic reticulum and mitochondrial disorders in follicular cells of the thyroid gland, were constantly observed in mice with VO [21].

There are many works devoted to the influence of melatonin on the state of the thyroid gland. However, the results obtained by different authors are ambiguous and often opposite. In the majority of studies based on numerous experiments with direct administration of melatonin into the body, its suppressive effect on the functional activity of the thyroid gland has been shown [9]. Some authors give melatonin the role of a stimulating factor in the thyroid gland. Thus, Gevorgyan A.R. et al. found that injections of melatonin (for 10 days) at a dose of 0.05 mg/kg and 0.5 mg/kg (to a greater extent) led to the stimulation of thyroid hormone secretion in old rats [10]. The antitumor properties of melatonin in the thyroid gland were also revealed [22]. Other re-

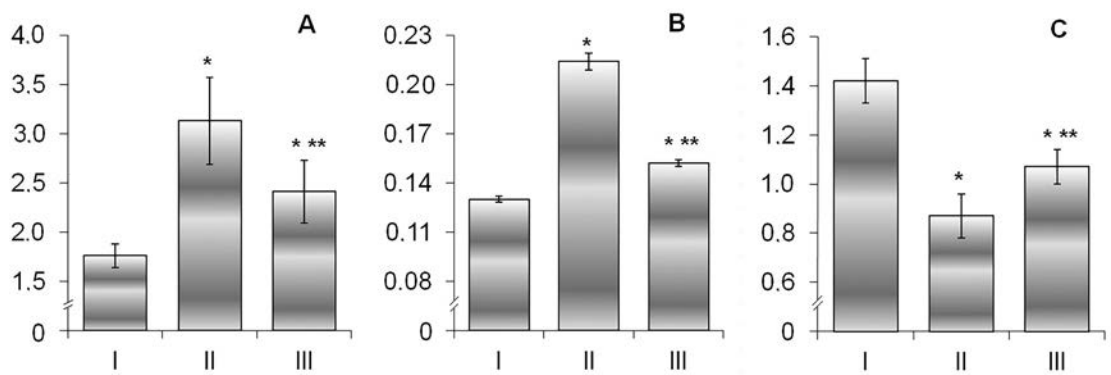

Fig. 3. Follicular-colloid index (A), stereological resorption index (B) and colloid accumulation index (C).

*Note: I – control rats, II – rats receiving HCD and III – rats receiving HCD and melatonin; *p<0.05 compared to the group I, ** p<0.05 compared to the group II; one way ANOVA followed by Bonferroni post hoc test, n=10 rats/group.*

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searchers did not note the effect of melatonin in the regulation of thyroid function [11].

Previously, we conducted experiments to investigate the effect of melatonin on the state of the thyroid gland in rats fed a standard diet. According to most of the obtained histomorphometric indicators, it was established that the 28th daily administration of melatonin (5 mg/kg) reduced the functional activity and physiological regeneration of the thyroid gland [15].

Therefore, the ambiguity of the literature data regarding the influence of obesity or melatonin on the structure and function of the thyroid gland may be related to: the use of different models of the thyroid gland, the degree of obesity, different dosages and duration of melatonin administration, age and sex of animals, etc. In our opinion the effect of melatonin on the structural state of the thyroid gland with VO is generally poorly studied. Therefore, the study of the role and mechanisms of melatonin's effect on the thyroid gland in obesity and the possibility of using it for preventive purposes in this pathology remains a relevant area of research.

Conclusions

In rats that received HCD for 12 weeks, clear signs of VO and histomorphometric changes in the thyroid gland, which indicated its hypofunction were observed.

Rats that received melatonin (5 mg/kg) in addition to HCD had significantly less thyroid structural disorders compared to animals that were only on HCD. Thus, administration of melatonin to rats has a protective effect on the thyroid gland against the harmful effects of obesity. In addition, melatonin contributes to reduce the degree of obesity, namely, it reduces the weight of visceral fat and returns indicators of lipid metabolism to control values.

The obtained data may be of practical interest when using melatonin as a prophylactic agent for disorders of the thyroid gland function caused by VO.

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Abbreviations

HCD – high-calorie diet
VO – visceral obesity

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Мелатонін знижує структурні розлади щитоподібної залози в щурів з індукованим вісцеральним ожирінням

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Резюме. Вісцеральне ожиріння (ВО) може викликати безліч захворювань в організмі, зокрема призводити до патології щитоподібної залози (ЩЗ). Тому, пошук та розробка нових ефективних методів профілактики порушень функції ЩЗ при ожирінні є актуальним напрямком досліджень. Одним із таких методів може бути використання гормону шишкоподібної залози – мелатоніну. **Мета** – дослідити вплив мелатоніну на гістоморфометричні зміни ЩЗ щурів із ВО та оцінити можливість його використання як профілактичного засобу при порушеннях функції залози при цій патології. **Матеріал і методи.** Для експерименту було відібрано 30 щурів самців лінії Wistar у віці 3 міс. ВО у тварин моделювали шляхом їх утримання на висококалорійному раціоні (ВКР). Було сформовано 3 групи щурів: група I – контроль, група II – тварини, які знаходилися на ВКР протягом 12 тижнів, і група III – щури, які 8 тижнів знаходилися на ВКР, а потім ще 4 тижні, на додаток до цього раціону, перорально отримували мелатонін (Unipharm Inc., США) у дозі 5 мг/кг. З тканини ЩЗ виготовляли гістологічні препарати. Гістоморфометрію здійснювали за допомогою комп'ютерної програми «ImageJ». У сироватці крові визначали концентрацію загальних ліпідів, тригліцеридів та холестерину. **Результати.** У щурів, які були на ВКР, спостерігали явні ознаки ВО: вірогідно зросла маса вісцерального жиру та концентрація показників ліпідного обміну. У ЩЗ виявили структурні зміни, які свідчили про її гіпофункцію. Відбувалось спустошення фолікулів від колоїду та пригнічення його накопичення. Спостерігали гіперплазію ЩЗ, що призводила до збільшення її розмірів. Щури, які на додаток до ВКР отримували мелатонін, мали значно менші структурні розлади ЩЗ. Крім того, мелатонін сприяв зменшенню ступеня розвитку ожиріння, а саме знижував масу вісцерального жиру та повертав показники ліпідного обміну до контрольних значень. **Висновок.** Введення щурам мелатоніну має захисний ефект на ЩЗ від згубного впливу ожиріння. Це може становити практичний інтерес при використанні мелатоніну як профілактичного засобу при порушеннях структури та функції ЩЗ, викликаних ВО.

Ключові слова: щитоподібна залоза, мелатонін, вісцеральне ожиріння.

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