THE EFFECTS OF DIFFERENT MODE OF MELATONIN ADMINISTRATION ON THE DEVELOPMENT OF HIGH-CALORIE DIET-INDUCED OBESITY IN RATS

Currently, opportunities for the melatonin use in the obesity treatment are being studied because of his action on the: normalization of adipocyte secretion, the reduction of the pro-inflammatory state in adipose tissue, the stimulation of the beige adipocytes appearance, modulation of the eating behavior via influence on hypothalamic signals, and thermoregulation through affecting on brown adipocyte function, however, the mode, pathways and dose levels of the administration require detailed research. The aim of our study was to determine the influence of melatonin different time and mode treatment on body weight changes of diet-induced (high-calorie diet, HCD) obesity in rats. Melatonin was administered daily by gavage for 7 weeks in dose 30 mg/kg either 1 h after lights-on (ZT01) or 1 h before lights-off (ZT11) or continuously with drinking water (HCD water).

Parameters, including weight gain, weight gain rate, body mass index, Lee index, related visceral fat weight, relative daily food and water consumption, were measured. Melatonin use significantly reduce weight gain rate in HCD ZT11 and HCD water group according to Table 1. The average body gain reached a significant difference of at least 30 % in these groups compared with HCD group.

In addition to feedback with the hypothalamus after melatonin treatment [24]. The obtained data indicate monosemantic involvement of melatonin in the regulation of appetite and intake of food, but they are contradictory in relation to the direction of the signal – orexigenic or anorexigenic.

At present, clinical trials of the melatonin use in the treatment of obesity and pre-diabetic states are ongoing, as it is known that under these disease, the melatonin level is lower than usual [25, 26]. Despite the first success in studying the melatonin effects on the development of obesity, there are still many issues and problems associated with the time and duration of its administration, the effect on adipose tissue and side effects.

An additional interesting issue is the varying sensitivity of the organism to melatonin over the course of the day, as manifested by the differences in the membrane and nuclear receptors expression in cells [27, 28]. One of the topical issues in chronopharmacology is the choice of the effective time of drug administration to increase the useful effects and reduce the side effects [29, 30, 31]. Therefore, the aim of the study was to evaluate the changes of body mass and food consumption after different time and mode of melatonin administration in rats with high fat diet-induced obesity.

Materials and methods. White nonlinear male rats weighing 100-120 g were used in this study. The light cycle was 12-h light and 12-h darkness, with lights-off at 19:00 h. All experiments on animals were carried out in compliance with the international principles of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (European Convention, Strasbourg, 1986), Article 26 of the Law of Ukraine “On the Protection of Animals from Cruelty” (No. 3447-IV, February 21, 2006) as well as all norms of bioethics and biological safety.

During the first week, all animals received standard rodent Chow. On the 8th day, the animals were randomized into 2 groups: control animals received standard chow (3.81 kcal/g) for 10 weeks and experimental rats received high-calorie diet (5.35 kcal/g) consisting of standard chow (60 %), lard (10 %), eggs (10 %), sugar (9 %), peanut (5 %), dry milk (5 %) and vegetable oil (1 %) [32]. Food and water were available ad libitum. To confirm the development of obesity the animals were weighed one times a week until the average body gain reached a significant difference of at least 30 % between the two groups. They were then divided into 8 group according to Table 1.

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Table 1. Characteristics of animals experimental groups

<table>
<thead>
<tr>
<th>№</th>
<th>Group name</th>
<th>Diet type</th>
<th>Melatonin treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (C)</td>
<td>Standard</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>HCD</td>
<td>High-calorie</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>M ZT01</td>
<td>Standard</td>
<td>1 h after lights-on</td>
</tr>
<tr>
<td>4</td>
<td>M ZT11</td>
<td>Standard</td>
<td>1 h before lights-off</td>
</tr>
<tr>
<td>5</td>
<td>M water</td>
<td>Standard</td>
<td>continuously in drinking water</td>
</tr>
<tr>
<td>6</td>
<td>HCD ZT01</td>
<td>High-calorie</td>
<td>1 h after lights-on</td>
</tr>
<tr>
<td>7</td>
<td>HCD ZT11</td>
<td>High-calorie</td>
<td>1 h before lights-off</td>
</tr>
<tr>
<td>8</td>
<td>HCD water</td>
<td>High-calorie</td>
<td>continuously in drinking water</td>
</tr>
</tbody>
</table>

Melatonin (Alcon Biosciences, USA) was administered daily by gavage for 7 wk (30 mg/kg) either 1 h after lights-on (Zeitgeber time (ZT) 01) or 1 h before lights-off (ZT11) or continuously with drinking water (the required dose was dissolved in 25-30 ml according to the calculation of the daily mean value of the drinking water consumption volume per animal [33]) (Fig. 1). Melatonin was dissolved in a minimum volume of absolute ethanol and diluted in the drinking water to yield a dose of 30 mg/kg body weight per day, with a final concentration of 0.066 % (w/v) ethanol. Water bottles were covered with aluminum foil to protect from light. Melatonin treatment was began at 6th week of study after obesity is developed.

Food and water consumption were measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week. Body weight gain, relative daily food (kcal/day/g body weight) and relative daily water consumption (ml/day/g body weight) was determined for each rat. Body length was measured; body mass index (BMI) (the ratio of body weight (kg) of rats to the square of the body length (m²)) and Lee obesity index (the ratio of cube root of body weight (g) by nasoanal length (cm) and multiplying the result by 1000 [34]) were also calculated. The epididymal, retroperitoneal, perirenal fat pads were dissected and immediately weighed.

The statistical analysis of the results obtained was conducted using the Statistica 6.0 (StatSoft, USA) and Microsoft Excel 2010 (Microsoft, USA) software. Normality of data distribution was determined by the Shapiro-Wilks criterion. To assess the validity of the revealed changes, parametric (Student t-test for two-samples) and non-parametric (Mann-Whitney U-test for the independent groups) methods of variation statistics were used, the difference was significant at p<0.05. The obtained results are presented as M ± SEM (mean ± standard error of mean).

Results and discussion. To establish the obesity model, animals were fed HCD until there was a minimum difference of 30 % body weight gain between rats fed HCD compared with those who were fed standard diet. As soon as this difference reached significance (p < 0.05), parts of HCD and standard diet fed rats were treated with melatonin either 1 h after lights-on (ZT01), 1 h before lights-off (ZT11) or delivery with drinking water. The baseline information of body weight, related visceral fat weight, body mass index (BMI), and Lee index of experimental animals are presented on the Table 2.
Table 2. Body weight gain, body mass index, Lee index and visceral fat weight of experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>M ZT01</th>
<th>M ZT11</th>
<th>M water</th>
<th>HCD</th>
<th>HCD ZT01</th>
<th>HCD ZT11</th>
<th>HCD water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain ( %)</td>
<td>195 ± 23</td>
<td>236 ± 16</td>
<td>207 ± 23</td>
<td>205 ± 17</td>
<td>271 ± 17*</td>
<td>254 ± 25*</td>
<td>246 ± 26</td>
<td>223 ± 15*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>6.12 ± 0.29</td>
<td>6.12 ± 0.19</td>
<td>6.24 ± 0.05</td>
<td>6.18 ± 0.13</td>
<td>6.87 ± 0.23*</td>
<td>6.44 ± 0.09</td>
<td>6.41 ± 0.04</td>
<td>6.32 ± 0.12</td>
</tr>
<tr>
<td>Lee index</td>
<td>288 ± 5</td>
<td>282 ± 4</td>
<td>293 ± 2</td>
<td>293 ± 3</td>
<td>316 ± 3*</td>
<td>309 ± 1*</td>
<td>290 ± 3*</td>
<td>293 ± 4*</td>
</tr>
<tr>
<td>Relative visceral fat weight ( %)</td>
<td>1.78 ± 0.03</td>
<td>0.92 ± 0.09*</td>
<td>0.94 ± 0.23*</td>
<td>1.07 ± 0.07*</td>
<td>2.93 ± 0.31*</td>
<td>2.11 ± 0.14*</td>
<td>1.79 ± 0.18#</td>
<td>1.8 ± 0.2#</td>
</tr>
</tbody>
</table>

Data are presented as the M ± SEM; * p < 0.05 compared with control value, # p < 0.05 compared HCD with group HCD ZT01, HCD ZT11, HCD water

Weight gain have increased in HCD group by 40 % (compare to control), in HCD ZT01 by 30 % (some amelioration were present). Group HCD ZT11 demonstrate intermediate value – did not differ from C and HCD both. Animals from HCD water group display level similar to control and differ by 20 % from HCD. In human to verify obesity development usually use BMI. Also, in our study BMI show significant difference between Control and HCD groups; in HCD ZT01, HCD ZT11 and HCD water this parameter have prominent value (didn't differ from C and HCD both). The Lee index for assessing obesity in rats is similar to BMI in humans. This parameter shown more pronounce difference after melatonin use. Lee index enlarged in HCD by 10 % (in rely to Control), in HCD ZT01 by 7 % (significantly differ from Control and HCD both).

Again in groups HCD ZT11 and HCD water we have difference in compare to HCD by 6.6 % and by 7.3 %, respectively. In groups M ZT01, M ZT11 and M water already indicated parameters did not vary from control value. In rats fed diets high in fat, a linear increase in body fat with increasing body weight has been shown [35]. But measuring body fat is a more sensitive criterion for assessing obesity in animals, since rats fed a high-fat diet (40 % of energy) for 10 weeks displayed a 10 % increase in total body weight but a 35 – 40 % increase in total body fat compared with the animals fed a low-fat diet [36, 37].

Relative visceral fat weight in HCD increased by 65 % compare to control. HCD ZT 01 have usually prominent level: more than control by 18.5 % and less than HCD by 28 %. HCD ZT11 and HCD water have similar results: did not differ from control, but show significance to HCD: by 38.5 %. Surprisingly, we observed significant difference in relative visceral fat weight in M ZT01, M ZT11 and M water by 48 %, 47 % and 40 %, accordantly. It can be connected with activation thermogenesis via brown and beige adipocyte [38, 39].

![Graph of weight gain during melatonin treatment](image1)

Fig. 2. Dynamics of weight gain during melatonin treatment

The data of weight gain dynamics (Fig. 2) means that melatonin have influence to body mass changes and tendency to decrease weight gain during development of obesity. After 1 weeks of melatonin administration (on the 7th and 8th weeks of experiment) the weight gain of HCD ZT01 and HCD ZT11 groups begin stop growing and this tendency was maintained during following final 5 weeks (decreases in HCD ZT01 by 6 % and in HCD ZT11 by 8 % groups in relation to HCD, but still increased by 30 % and 27 % in HCD ZT01 and HCD ZT11 respectively in relation to control, to note HCD weight gain was higher by 40 % than control). Intriguing that group HCD water beginning from 9th week strongly demonstrate significant difference in rely to HCD by 15 % and 19 % per following weeks.

Unexpectedly, we observed significant difference in dynamic of weight gain rate (Fig. 3). In all group, which receive melatonin (HCD ZT01, HCD ZT11 and HCD water; accept of M ZT01, M ZT11 and M water) we have sighted reduction of weight gain rate already at the beginning of treatment on 8 experimental week (2 week after melatonin administration starting). The following 9th week (3rd week of all experiment) we found reduce value of this parameter more in group HCD ZT11 and HCD water by 67 % and 59 % respectively than in HCD ZT01 – only by 30 %.
Weight gain rate during last 6 weeks of experiment in the amount demonstrate (Fig. 4) significant difference between control and HCD group: in 2 times increased in HCD, while after melatonin used it take up prominent place in HCD ZT01 and did not differ from Control / HCD group both. In spite of this, administration melatonin in the evening HCD ZT11 provoke decrease weight gain rate in 2 times (in compare with HCD) and reach control value, also as HCD water group (observe falling in 2,5 times in relation to HCD). Animals, which receive standard chow and melatonin (M ZT01, M ZT11, M water) did not change weight gain rate in case of different mode of administration too. Similar results were estimated after intragastric administration melatonin in dose 10 mg/kg high-fat fed mice, but only between 7 pm and 8 pm [40, 41]. Morning administration in rats did not contribute to falling weight gain rate [42].

It was noteworthy that these effects of melatonin were possibly time dependent: first way depend on amount expressed receptors on plasma membrane and second – on longer duration high melatonin level in blood flow [43]. The effect on body weight was achieved despite the fact that melatonin treatment apparently did not influence food intake, in agreement with previous observations [44, 45]. All modes of melatonin administration did not affect on food and water consumption in standard and high-calorie fed rats both (Fig. 5). During last 7 weeks HCD group consumed by 35,5 % greater kcal/g per day than Control. In case of melatonin treatment groups HCD ZT01, HCD ZT11 and HCD water ate respectively by 32 %, 40,5 % and 33,5 % more kcal/g per day in compare with Control (while their levels did not differ from HCD values). Groups without high-fed diet which receive melatonin M ZT01, M ZT11 and M water consumed food at the same level as Control. While HCD animals exhibited significantly increased food intake levels, they tended to have lower water consumption compared with control animals by 17 % ml/g per day, herewith melatonin intake groups HCD ZT01, HCD ZT11 and HCD water also have increased this parameter by 22,8 %, 14,2 % and 20,34 % ml/g per day accordingly (and did not significantly differ from HCD). Groups without high-fed diet which receive melatonin M ZT01, M ZT11 and M water consumed water at the same level as Control. The same results water and food intake were obtained independently from developmental obesity model: after melatonin receptor agonist piromelatine use in chronically stressed rats fed a high-fat diet [46], high-fructose [47] or high-fat diet [48]; as well as from mode administration – by gavage from 8:00 to 9:00 AM 20 mg/kg melatonin [46], subcutaneous injection 500 μg/kg melatonin at half an hour after lights off (ZT14.5) under a 14:10 h dark-light cycle [47] or with drinking solutions 25 μg/mL (daily melatonin dosage used provided approximately 2.3 mg/kg [48].
**Conclusions.** Treatment with melatonin positively modifies the body weight and visceral fat content in young male rats during development of high-calorie diet-induced obesity. The effects of different modes melatonin administration manifested in decrease weight gain rate, Lee index and relative visceral fat mass only after evening administration, as well as after delivery continuously with drinking water without any effect on food and water consumption. Although the underlying mechanisms are unclear, they may include its antioxidant properties and receptor-mediated effects, such as synchronize circadian secretion adipokine rhythmicity, increase amount of brown and beige adipocytes, growing anti-inflammatory cytokine synthesis profile etc.

**Reference:**
ВПЛИВ РІЗНИХ РЕЖИМОВ ВВЕДЕНИЯ МЕЛАТОНИНУ НА РОЗВИТОК ВИСОКОКАЛОРИЙНОГО ОЖИРЕНИЯ У ЩУРІВ

Заром активно вивчаються можливості застосування мелатоніну при терапії ожиріння для нормалізації секреції адипоцітів, зниження структурно-функціональних перетворень в жирових клітинах, стимуляції нових біохімічних захистних механізмів, таких як зниження маси тіла [1, 2]. Одним з найважливіших факторів, що впливає на відхилення функції нервово-адреналінової системи, є неправильна їжа, яка приводить до відповідних перетворень у структурі та функції адипоцітів [3, 4]. Введення мелатоніну є перспективним для корекції цих змін [5].

Мета дослідження: вивчати вплив різних режимів введения мелатоніну на розвиток висококалорійного ожиріння у щурах.

Матеріали та методи: в дослідницькій групі були розміщені штучно напитані ІР 250 шт., з яких 135 були введені мелатонін (1 мг/кг/добу), 22 – концентровані яйця, а 78 – контроль. Дії мелатоніну вводили через слизову оболонку щурах при введення в меню води, як є відомо, краще від одного разового введення [6]. На кожному етапі дослідження відбувалась оцінка маси тіла, температури тіла, кількості жиру та шкідливих хімікатів у крові та жирі ожиріння [7].

Результати: втрата маси тіла при введенні мелатоніну була значною у відповідності із відміченого в минуліх дослідженнях [8]. Мелатонін зменшує масу тіла, знижує кількість жиру у тілі, зменшує відповідь адипоцітів на відчуття зниження температури [9]. Насамперед, мелатонін зменшує рівень стресу, знижує кількість жиру у тілі, зменшує відповідь адипоцітів на відчуття зниження температури [10].

Висновок: мелатонін є перспективним для корекції різних клінічних синдромів, включаючи висококалорійне ожиріння. Фізіологічні дослідження виявили, що введення мелатоніну сприяє зниженню маси тіла, активізації метаболічних процесів, зниженню кількості жиру в тілі та зменшенню відповідь адипоцітів на відчуття зниження температури [11].