The effects of melatonin administration in determined times of day on the kidney in rats with high-calorie diet-induced obesity

**Keywords:** melatonin, obesity, chronotherapy, kidney, high-fat diet, glomeruli.
resistance and other factors [3]. At present, obesity has not identified a clear specific clinical and morphological variant of kidney damage, but, according to the study, the most frequent manifestations of nephropathy in obesity in adults are microalbuminuria, proteinuria, hyperfiltration and, less often – a decrease in the filtration function of the kidneys [4]. Obesity is a proven factor for the progression of many variants of chronic kidney disease and terminal renal failure. The formation of kidney damage in obesity is realized in several ways [5]:

- auto- and paracrine effects of adipose tissue hormones and cytokines;
- the effect of insulin resistance, hyperinsulinemia and dyslipidemia;
- disorders of systemic and renal hemodynamics;
- the role of relative oligonephronia with the formation of intra-glomerular hypertension.

Melatonin is an indole hormone produced by the pineal gland and carries out a number of functions in the body: metabolic (lipolytic and hypoglycemic); rhythm control; immunomodulatory antioxidant and mitochondrial-protector. The effect of melatonin on weight loss can be explained by numerous physiological processes of indolamine, which are provided by various mechanisms [6–10]:

- activation of ATP synthesis in mitochondria;
- regulation of expression of the insulin receptor gene, providing a normal glucose metabolism;
- participating in the activation of gonadotropins secretion in the pituitary gland, which leads to suitable producing of testosterone – an important fat burning hormone;
- complicity in the metabolism of serotonin and vitamin D, essential for the implementation of eating behavior within the framework of the functioning of the "brain reward system", as well as for maintaining a normal body shape, primarily due to the optimal amount and quality of muscle mass;
- Antioxidant effect;
- Interaction with leptin.

Thus, melatonin is a universal endogenous adaptogen that regulates homeostasis according to changes in the environment and the influence of pathogenic factors on the body.

In humans blood pressure, body temperature, blood concentrations of melatonin, insulin, corticosteroids and adrenaline oscillate nearly 24-hour (circadian) cyclic rhythms. Chronotherapy aims to reduce adverse drug reactions and optimize drug efficacy by timing drug administrations in accordance with the body's circadian rhythms [11–12]. Peak of melatonin synthesis at mid night, and also receptors mainly are presented after light-off mostly. Thus, melatonin administration after 1 hour light-off supposedly will be more effective than others routes of administrations [13].

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, Strasburg, 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 %) [14]. 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 and the respective animals were classified as having the normal body mass (Control) and those with development of obesity (HCD). Rats of HCD and Control groups were divided into two subgroups: one subgroup received no MT, animals of the second and subgroups obtained MT administrations – single peroral by gavage introductions, 1 h before light-off (group HCD ZT11 and M ZT11). Thus, the experimental subgroups are indicated below as normal body mass (control), HCD, M ZT11, HCD ZT11.

Melatonin (Alcon Biosciences, USA) was administered daily by gavage for 7 wk (30 mg/kg) 1 h before light-off (ZT11). 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.

On the last day of the experiment, the animals were decapitated, and then the kidney was isolated. Also the visceral (epididymal, retroperitoneal, mesenteric, perirenal) fat pads were dissected and immediately weighed.

Histological examination was performed to characterize the morphology and functional status of kidney. Fragments of kidney in the size of 1 x 1 cm were fixed in 4 % of paraformaldehyde in 0.1 M phosphate buffer for 72 hours, after which they were dehydrated and embedded into paraffin according to a standard procedure. From the paraffin blocks, 5 μm sections were performed and stained with Bemer's hematoxylin and eosin. Further examination of sections was performed using a light microscope BX41 (Olympus, Japan). Microphotographs were taken using the DP20 (Olympus, Japan) digital camera and the QuickPHOTO MICRO software (Promicra, Czech Republic).

The cross-sectional area of the kidney glomeruli and the numerical density of the glomeruli were used as criteria for assessing the morphology and functional kidney cortex status of glomerular zone. All parameters were measured using the ImageJ software (National Institutes of Heath, USA).

Statistical data analysis was performed using the Statistica 6.0 (Stat- Soft, USA) and Microsoft Excel 2010 software (Microsoft, USA). The distribution of values was estimated using Shapiro-Wilk test. Since the deviation of these values distribution from the normality was minor, to evaluate the differences between the values we used Student's t-test. The differences with probability of the null hypothesis p < 0.05 were considered significant. The obtained results are presented as the mean ± standard error of mean.

Results and discussion. In the sections of the kidney cortical part (tubular zone) HCD group rats were fixed changes in tubular epitheliocytes, namely, cytoplasm accommodate a few amount lipid droplets (Fig. 1, B, arrow), which did not stain with water-soluble dyes. Accumulation of lipid droplets is due to the fact that during obesity as a result of intensive lipolysis, a large number of circulating free fatty acids have been in blood flow. Low
levels of adiponectin, tissue resistance to leptin, and cytokines prevent the capture of free fatty acids by mitochondria, inhibit their oxidation and contribute to the accumulation of free fatty acids in the cell [19].

In HCD group also (Fig. 1 B, asterisk) marked protein aggregates in the lumen of the renal tubule, which may be the first signs of proteinuria. One mechanism for the appearance of such changes is an elevated angiotensin II level during obesity development, which can directly increase the permeability of the basal membrane of the glomeruli, thereby contributing to increased proteinuria [16].

Fig. 1. Microphotographs of rats' kidney sections of the cortical part (tubules):
A – control group, B – HCD group; hematoxylin-eosin staining; oc. x10, ob. x100
Notes: * – lipid droplets, asterisk – protein aggregates in the lumen of the renal tubule

In the kidney cortical part (glomerular zone) under conditions of obesity, expanded space of the capsule (Fig. 2, B, asterisk) and enlarged mesangial region from extra-glomerular cells (Fig. 2, B, arrow) are observed. One of the mechanisms for the development of such changes is the increase in the level of proinflammatory cytokines, in particular TNF-α, as one of the key pro-inflammatory factors that stimulates the activation of proliferation and sclerosis in mesangial cells of the renal tissue [20], since obesity leads to infiltration of adipose tissue by macrophages. TNF-α is expressed and secreted by adipose tissue. Its concentration correlates with the degree of obesity and its associated insulin resistance, reducing the activity of the insulin receptor. In addition, an increase in the levels of TNF-αpromotes the generation of reactive oxygen species (ROS) in glomerular and proximal tubular cells. The enhancement of ROS activity leads to kidney damage in several ways, which include increased renal endothelial dysfunction, microalbuminuria, mesangial expansion, and fibrosis [21]. Another idea to increase the mesangial area is the endothelia (ET) signal system [22].

Internal renal arteries are characterized by the highest sensitivity to ET-1 compared with other organs. Like AT II, ET-1 causes spasm of the glomeruli arterioles, moreover constrict efferent arterioles more pronounced than the degree of narrowing afferent. Together with the ability to modulate the tone of the vascular wall, ET-1 has the properties of a growth factor that promotes proliferation of mesangial cells, smooth muscle cells of vessels, fibroblasts and endothelial cells, and enhances the production of fibronectin and collagen IV by mesangial cells, as well as the synthesis of soluble and insoluble fibrin by smooth vascular cells of the vessels [23].

The obtained data indicate the negative effect of obesity on the morpho-functional structure of the kidneys (Fig. 3). Thus, the cross-sectional area of the renal glomeruli for the HCD group is by 20 % less than for the control group. In general, there is a decrease in the number of renal glomeruli by 36% compared with control values. An explanation for such a decrease in the number of renal glomeruli is that obesity leads to the formation of a relative deficit of nephrons to total body weight, therefore, even with the normal number of nephrons at birth, the condition of comparative oligonephria [24]. During obesity the total area of the filtration surface in a nephrons with normal number can not withstand against metabolites excessive loading at an adequate level for a long time. The deficiency of nephrons in relation to body weight at the initial stages is offset by hypertrophy of glomeruli and hyperfiltration, which develop under the influence of hormones and growth factors, that are produced by adipose tissue, and leads to an increase in total renal filtration relative to the body surface [25].

Melatonin administration during obesity development has led to an increase the renal glomeruli area and the number of glomeruli – 10 % and 35 % both, respectively in compare with HCD group. Interestingly, the glomeruli number reached control values (no significant difference), and the glomeruli area in the HCD ZT11 group take an intermediate position – that is, they significantly differ from the control by 10 %. This mechanism initially is compensatory, but with prolonged increase in the volume of adipose tissue, a steady violation of hemodynamics in each individual nephron is formed. As a result, intraglomerular hypertension develops, which is considered one of the main factors in the progression of kidney damage [26]. Prolonged action of increased hydrodynamic pressure as a result leads to renal glomeruli fibrosis, reduction of the active nephrons mass, attenuation of functional renal reserve, the development of true oligonephria [27].

Melatonin administration to rats with standard diet (M ZT11) did not affect the renal glomeruli area and the number of glomeruli in relation to control group.
The results of this study about the high-calorie diet-induced obesity effects on the morpho-functional state of rats' kidney, in many manifestations, coincide with the studies of other authors. In a significant number of studies, catch out intracellular lipid inclusion in renal epithelium cells [28–30], reduction the total area and number of glomeruli, expansion of the Bowman space, accumulation of fibrin [31–32]. Thus, an increased mesangial region was noted in this work, also was detected in the kidneys of rats with obesity-induced type 2 diabetes [33].

Other studies also noted the beneficial effects of melatonin therapy on the kidney morpho-functional state of in animals with obesity and other metabolic diseases. In various animal models associated with experimental pyelonephritis, renal insufficiency, hypertension, diabetes and various variants of nephrotoxicity, melatonin reduces oxidative stress, suppresses chronic inflammation and limit apoptosis [34–36].

Melatonin had also effect on the general body weight. During obesity development relative visceral fat weight is the most sensitive criterion for assessing obesity in animals [37]. Relative visceral fat weight (Fig. 4) in HCD increased by 65 % compare to control. HCD ZT11 did not differ from control, but show significance to HCD: by 38.5 %. Melatonin administration in group M ZT11 did not influence on relative visceral fat weight.
In the same study melanatonin also demonstrate attenuation of pathology changes during obesity: in rabbits with HCD induced obesity melanatonin administration (subcutaneously in a dose of 1 mg/kg daily 2–3 h before lights-off for 4 weeks) provide disappearance of interstitial foam cells, with multiple tiny intracytoplasmic and fat droplets that displace in the renal tubules [38]. In alloxan-treated diabetic rats after melanatonin (200 µg/animal/day by gavage for 8 weeks in the morning between 9 AM and 11 AM) intervention ameliorated degenerative changes in Bowman’s capsule with marked expansion in Bowman’s space and necrosed tubular wall [39]. Amelioration of streptozocin-induced diabetic nephropathy in rats by melanotonin (dose of 10 mg/kg/day for 30 day by intraperitoneal injection) that manifestation in tubules was markedly reduced of TGFβ-1, but also explored mild epithelial desquamation in tubules and hydropic degeneration in some tubular cells [40]. For the choose a uni effective protocol of melatonin administration further study is needed in the chronotherapy key [41].

Conclusions. Thus, it has been established that obesity induced high-calorie diet leads to significant changes in the morphological structure of the kidneys. It has been shown that daily administration of melanatonin in a dose of 30 mg/kg for 1 hour before light-off to obese rats leads to improvement of the kidneys morpho-functional state. Namely, the reduction of the pathological changes manifestation in the tubules (the disappearance of intracellular lipid droplets and protein aggregates in the lumen of the tubule) and in the glomeruli (reduction of mesangial site with extraglomerular cells and capsule space) of the kidneys compared with obese animals. In this case, the cross-sectional area of the kidney glomeruli grows, but does not reach the level of control values. While the number of renal glomeruli returns to the level of control groups.

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ВПЛИВ ВВЕДЕННЯ МЕЛАТОНИНУ В ПЕВНИЙ ЧАС ДОБИ НА НИРИ КРЫС ІЗ ОЖИРІННЯМ, ІНДУКОВАНИМ ВИСОКОКАРБОНОВОЮ ДІЄТОЮ

Нирки, як і серцево-судинна система, є одним із основних органів-мішеней, уражених до ожиріння, тому що першими беруть на се- бе функцію компенсації метаболізму при надлишку жирової тканини в організмі. Ураження нирок при ожирінні є багатофакторним про- цесом, що викликає ряд подій, що включають запалення, оксидативний стрес, порушення ліпідного обміну, активізацію системи ренін-ангіотензин-альдостерон, інсулинорезистентність тощо. Оптимальним кандидатом для зниження швидкого впливу ожиріння на нирки має бути сполука, яка одночасно видає протизапалювальні та антиоксидантні властивості, контролює циркадний ритм, а також впливає на секрецію адипонітину. Молекула, що відповідає цим умовам, — це мелатонін. Методи нашого дослідження було досліджено мор- фофункціональний стан (morphofunctional characteristics клубоців та каналічі нирок, оцінено морфометричними параметрами: площу та кількість клубоців) нирок у віці 6, 9, 12 та 14 ведення відповідає групам контролю (КРУСи), контрольних (КРУСк), нирок, що отримані в групах подальших введення мелатоніну, відповідає групам (МЕВ, МЕВк). Дослідження, що проводилося у відрохту грибів Pseudonadsoniella brunnea (Basidiomycota, Agaricomycotina, Agaricomycetes, Polyporales, Meripilaceae) вперше було включено до оптимальних умов викиду меланіну, L-тирозину.

О. Калмыкова, асп., Т. Кушмирук, студ., М. Дзержинський, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ, Україна

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