ORIGINAL ARTICLE

CONTENTS

Carbohydrate metabolism in the rats' liver under conditions of light and dark deprivation and correction by melatonin

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ABSTRACT

Aim: This study aimed to investigate changes in carbohydrate metabolism in the liver of rats under light and dark deprivation and evaluate the effects of melatonin treatment.

Materials and Methods: Photoperiodic changes were simulated over two weeks: the natural equinox (March 16-29); the artificial equinox (12:12 light-dark cycle, 500 lux); constant light (24 hours, 500 lux) for dark deprivation; and constant dark (24 hours, 0-0.5 lux) for light deprivation. Forty-eight rats were divided into control and melatonin-treated groups (5 mg/kg daily for 14 days). Enzyme activities and glycogen content in the liver were measured using standard methods. Statistical analysis was performed using the Student's t-test.

Results: Glucose-6-phosphate dehydrogenase activity decreased by 18% under constant light but increased by 35% under constant dark compared to the equinox. Pyruvate kinase activity decreased by 17%, while glucose-6-phosphatase and lactate dehydrogenase activities increased by 9% and 20%, respectively, under constant light. Constant dark and melatonin treatment reduced glucose levels by 26% across all conditions, activated aerobic pathways, and increased glycogen content by 13% compared to the equinox.

Conclusions: Melatonin treatment improved carbohydrate metabolism in the liver of rats under light and dark deprivation, suggesting its role in metabolic adaptation to altered photoperiods.

KEY WORDS: energy metabolism, liver, rats, melatonin, circadian rhythm

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INTRODUCTION

The liver plays a central role in metabolism, particularly in maintaining glucose homeostasis [1]. Hepatocytes contain the necessary enzymes for glycolysis, gluconeogenesis, glycogen metabolism, and the pentose phosphate pathway of glucose-6-phosphate oxidation, among others.

Glucose-6-phosphate dehydrogenase is an enzyme involved in the pentose phosphate pathway, where it supplies NADPH for the reduction of glutathione and supports the antioxidant defense system. Pyruvate kinase is a key enzyme in the glycolytic pathway responsible for glucose breakdown. Lactate dehydrogenase serves as an indicator of anaerobic glycolysis. Glucose-6-phosphatase is the terminal enzyme in both gluconeogenesis and glycogenolysis. Glycogen, primarily stored in the liver, acts as a glucose reserve.

Jet lag is known to contribute to the development of various pathologies, including metabolic syndrome, gastric and duodenal ulcers, fibromyoma, and myocardial infarction [2]. As far as we know, melatonin is a well-known regulator of circadian rhythms [3]. This hormone is produced by pinealocytes in the pineal gland, as well as in cells of the gastrointestinal tract, among other tissues [4].

Changes to the circadian rhythm often occur when individuals travel across time zones, work night shifts, or even due to unhealthy habits like prolonged computer use or late-night television watching. These activities disrupt the natural production of melatonin. It is well-established that melatonin synthesis [5] can be suppressed by constant exposure to artificial lighting, leading to impaired melatonin formation. This disruption results in dyssynchrony, which stems from hormonal regulation disorders, particularly an increase in steroid hormone production. This can contribute to mental health issues, such as anxiety, chronic fatigue, and eating disorders. Prolonged exposure to this dyssynchrony can eventually lead to the development of diseases [6].

Recent studies also suggest that the pituitary stalk (pars tuberalis) and hypothalamic tanycytes play key roles in seasonal timing. The pars tuberalis expresses a high density of melatonin receptors, making it highly responsive to

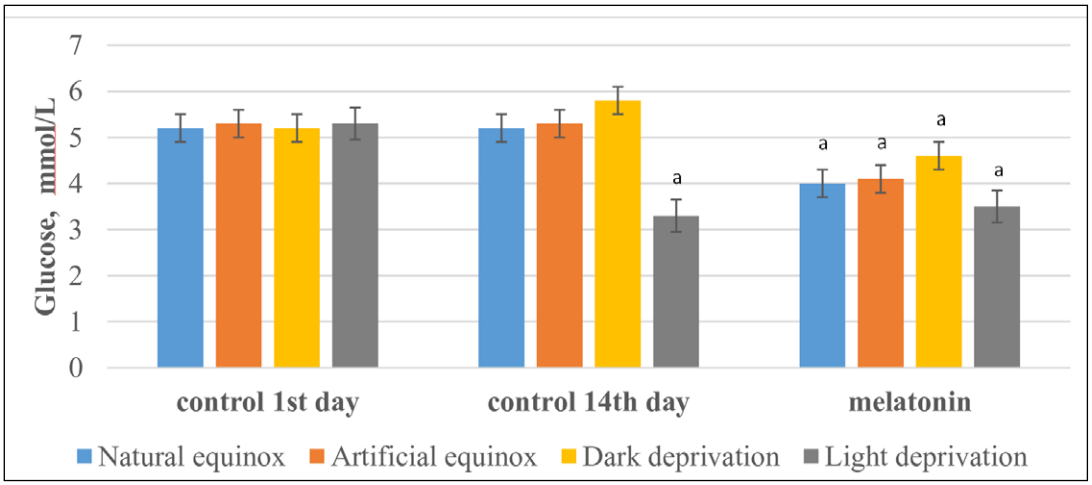

Fig. 1. Glucose level in the blood of rats, mmol/L (n=6, x±Sx)
Note: a - changes are reliable concerning control 1st day (p≤0,05)

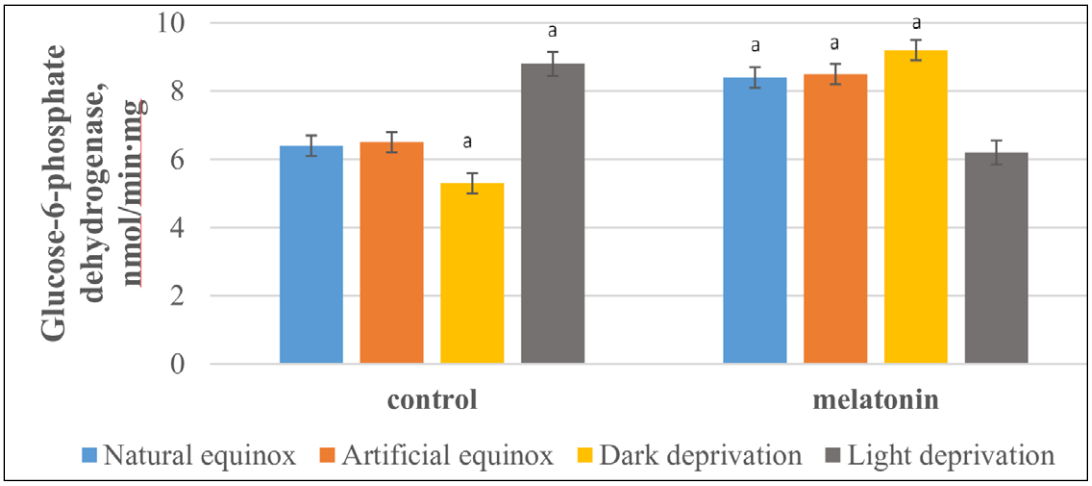

Fig. 2. Glucose-6-phosphate dehydrogenase, nmol/min·mg (n=6, x±Sx)
Note: a - changes are reliable concerning natural equinox control (p≤0,05)

changes in the nocturnal secretion of melatonin from the pineal gland as the photoperiod varies throughout the year [7]. One of the most profound seasonal changes observed in rodents is the expression of deiodinase type 2 (Dio2) genes. In many seasonal species studied, T3 levels decrease under short photoperiods, a process mediated by downregulation of Dio2 and upregulation of Dio3 in tanycytes [8].

A thorough understanding of the mechanisms behind circadian rhythm disturbances and the potential corrective role of melatonin could help prevent inevitable pathologies and restore a healthy lifestyle.

AIM

The aim of our study was to determine changes in carbohydrate metabolism in the liver of rats under conditions of light and dark deprivation and correction with melatonin.

MATERIALS AND METHODS

The experiments were conducted on 48 male outbred white rats, each weighing between 0.18 and 0.20 kg. The rats were treated in accordance with ethical standards, including the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), the guidelines of the International Committee of Medical Journal Editors (ICMJE), and the "Bioethical Expertise of Preclinical and Other Scientific Research Conducted on Animals" (Kyiv, 2006).

Photoperiodic changes were simulated over a two-week period: 1) the natural equinox, from March 16 to 29, with an average photoperiod of 12:12 hours; 2) the artificial equinox, with light from 08:00 to 20:00 and an illumination of 500 lux at the cell level (Cummings M.A., Berga S.L., et al., 1989), also maintaining a 12:12 hour light-dark cycle; 3) constant light throughout the day (500 lux) – dark deprivation; 4) 24-hour darkness (0-0.5 lux) – light deprivation [4, 6]. Some animals in each group were administered melatonin (Sigma, USA) intraperitoneally at a dose of 5 mg/kg daily for 14 days [9]. Two weeks was the optimal duration for detecting metabolic changes in the liver of rats resulting from altered light regimes and melatonin administration, as this period encompasses adaptive, regulatory, and metabolic responses. The remaining animals served as the control group.

Glycemia levels were determined using the One Touch device (Johnson & Johnson, USA). On the 14th day of the experiment, the animals were euthanized by decapitation under light ether anesthesia. The supernatant obtained after centrifugation of a 5% liver homogenate at 900g was analyzed for the activity of the enzymes

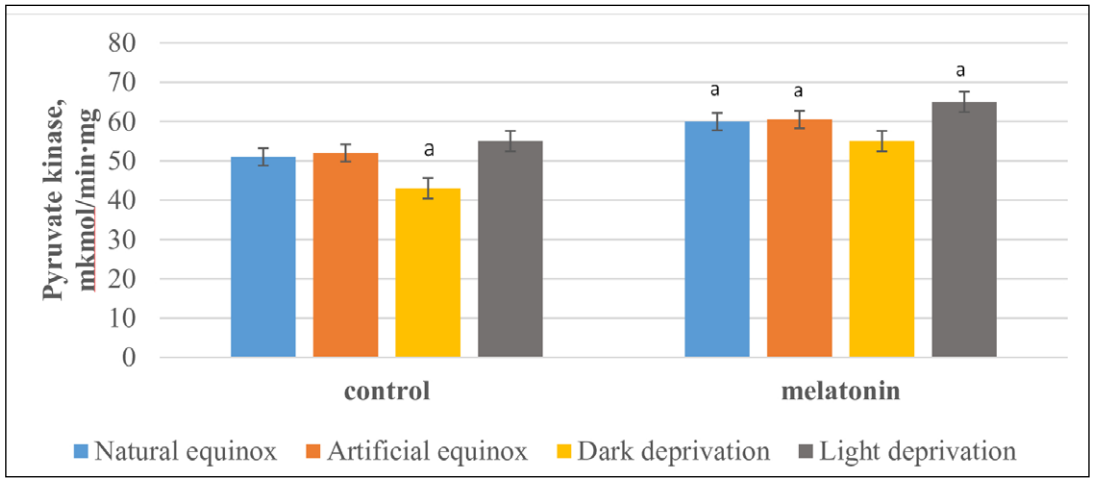

Fig. 3. Pyruvate kinase, mkmol/min·mg (n=6, x±Sx)
Note: a - changes are reliable concerning natural equinox control (p≤0,05)

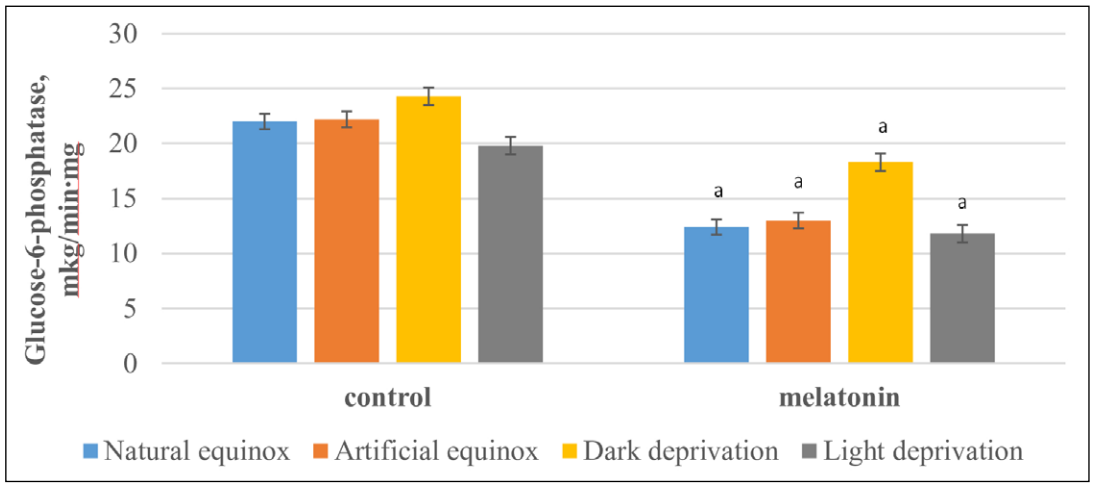

Fig. 4. Glucose-6-phosphatase, mkg/min·mg (n=6, x±Sx)
Note: a - changes are reliable concerning natural equinox control (p≤0,05)

glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PDH), pyruvate kinase (EC 2.7.1.40, PK), lactate dehydrogenase (EC 1.1.1.27, LDH), and glucose-6-phosphatase (EC 3.1.3.9, G6Pase) using standard methods [10].

To determine glycogen content, tissues were digested with a 30% KOH solution, followed by the addition of ethanol [11]. The glycogen was then hydrolyzed with sulfuric acid, and glucose levels were measured as an indicator of glycogen content.

Statistical analysis was performed using Statistica 10 (StatSoft Inc.). The arithmetic mean (x) and standard deviation (Sx) were calculated for all indicators, expressed as x ± Sx. To assess the reliability of the differences between the experimental and control groups, the Student's t-test was applied. The probability of the difference between the samples (p) and the confidence intervals for the mean were also determined. A preliminary check for normality in the data distribution was conducted using the Shapiro-Wilk test, which showed no significant deviation from normal ($p > 0.05$). Based on this, the use of the Student's t-test was considered valid. The non-parametric Mann-Whitney U test was also performed to confirm the reliability of the results, yielding similar p-values. The critical significance level for hypothesis testing was set at 0.05.

RESULTS

Under different lighting conditions over a two-week period, we observed a 37% decrease in glycemia levels under constant darkness (Fig. 1) compared to control animals under equinox conditions. In contrast, exposure to constant light showed a tendency toward an increase in glycemia levels when compared to the equinox group.

Melatonin administration at a dose of 5 mg/kg led to a decrease in glycemia levels: an average reduction of 22% under equinox conditions, 12% under constant light, and 34% under constant darkness. These changes were significantly different from the control group on the first day of the experiment.

Additionally, metabolic pathway indicators regulating blood glucose levels also varied under different lighting conditions. G6PDH activity (Fig. 2) decreased by 18% under constant light but increased by 35% under constant darkness compared to the control under equinox conditions.

PK activity (Fig. 3) decreased by 17% under dark deprivation but remained unchanged under light deprivation compared to the equinox control. G6Pase (Fig. 4) and LDH (Fig. 5) activities increased by 9% and 20%, respectively, under dark deprivation, while they showed a tendency to decrease under light deprivation compared to the equinox control.

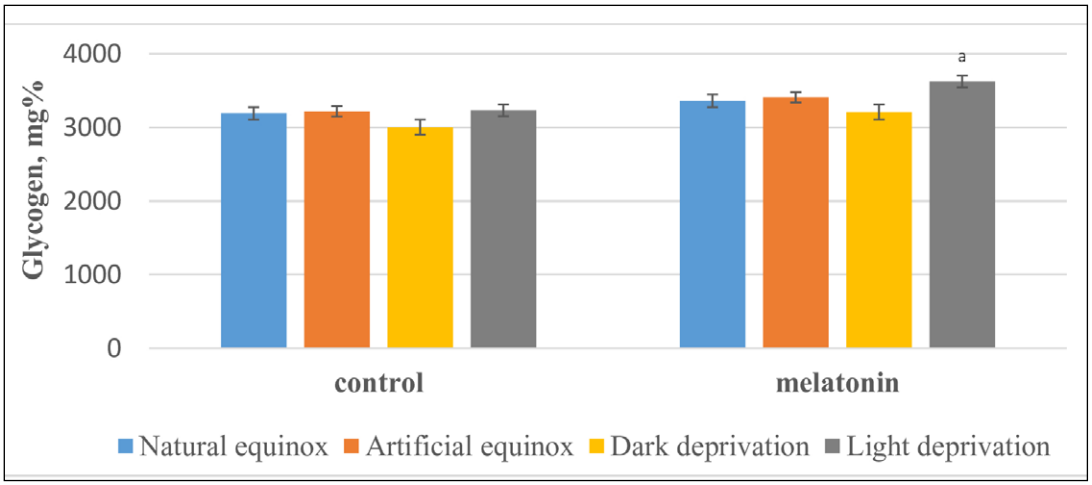

Fig. 5. Lactate dehydrogenase, nmol/min·mg (n=6, x±Sx)
Note: a - changes are reliable concerning natural equinox control (p≤0,05)

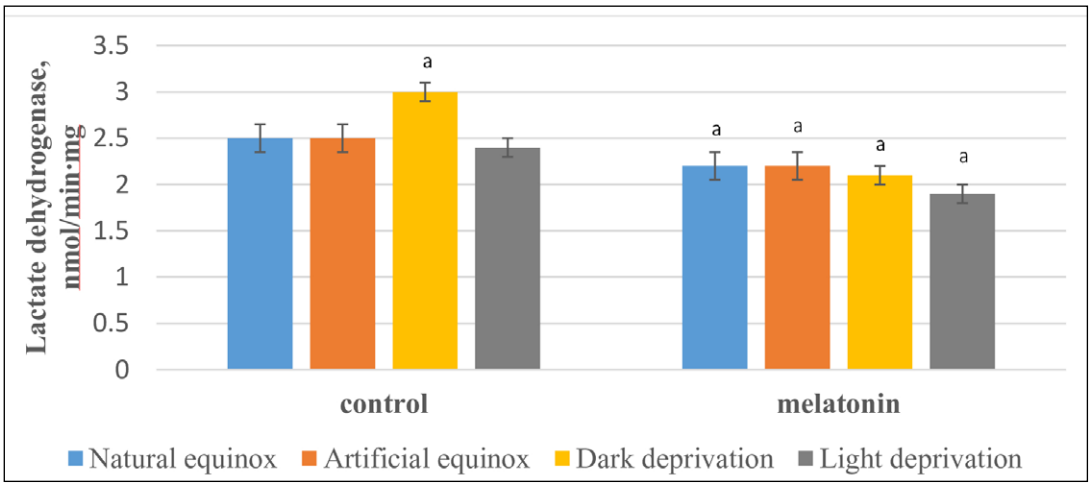

Fig. 6. Glycogen, mg% (n=6, x±Sx)
Note: a - changes are reliable concerning natural equinox control (p≤0,05)

Melatonin administration to animals resulted in: 1) a 32% increase in G6PDH activity (Fig. 2), an 18% increase in PK activity (Fig. 3), a 43% decrease in G6Pase activity (Fig. 4), and a 12% decrease in LDH activity (Fig. 5) under equinox conditions, compared to the control group;

2) a 42% increase in G6PDH activity, along with a 15% decrease in LDH activity and a 17% decrease in G6Pase activity, under constant light conditions, compared to the control group;

3) a decrease in G6PDH activity (which did not differ from the control group under equinox conditions), a 13% increase in glycogen content (Fig. 6), a 25% increase in PK activity and a 25% decrease in LDH activity, and a 48% decrease in G6Pase activity, under constant darkness, compared to the control group.

DISSCUSION

Recent studies show that altering the metabolic enzymes G6PDH, PK, and LDH can partially reverse metabolic reprogramming and promote adult cardiomyocyte proliferation [12, 13]. Furthermore, melatonin has been shown to downregulate hepatic genes involved in gluconeogenesis, such as fructose-1,6-bisphosphatase 1, forkhead box O1 alpha, thioredoxin-interacting protein, phosphoenolpyruvate carboxykinase, and the G6Pase catalytic subunit, supporting the idea that melatonin improves glucose metabolism [14].

G6PDH is crucial for maintaining glutathione in its reduced state, and enhancing its activity is of significant interest. In our study, melatonin administration increased G6PDH (Fig. 2) activity under dark deprivation conditions, while activity returned to normal levels under light deprivation. These results suggest that melatonin activates compensatory mechanisms during dark deprivation while restoring pro- and antioxidant balance during light deprivation.

Under constant light, melatonin production is likely reduced, leading to decreased G6PDH activity (Fig. 2). Pinealectomy or pineal gland hypofunction due to constant light impairs melatonin synthesis, reducing insulin sensitivity and GLUT4 expression [15]. Therefore, it is logical that G6PDH activity decreases under constant light, while melatonin administration reverses this effect.

Insulin plays a key role in regulating glucose metabolism, activating PK and G6PDH, and inhibiting G6Pase. However, disruptions in circadian rhythms can impair this insulin-glucose relationship. Coomans et al. (2013) found that 24-hour light exposure decreases the amplitude of the central pacemaker in the SCN, leading to increased food intake, reduced energy expenditure, and weight gain. This reduction in SCN

amplitude can disrupt circadian rhythms in energy metabolism and insulin sensitivity.

In line with our results, exposure to constant darkness and melatonin administration under equinox and constant light conditions reduced blood glucose (Fig. 1) and increased glycogen (Fig. 6) deposition in the liver. This may be due to melatonin's ability to stimulate glucose uptake in the brain and liver during the night, when insulin levels are naturally low [16]. These findings suggest melatonin may act as a hypoglycemic hormone, similar to insulin, further enhanced by a decrease in G6Pase activity (Fig. 4) with melatonin treatment.

Additionally, melatonin administration resulted in increased PK activity (Fig. 3) and decreased LDH activity (Fig. 5) during both equinox and altered photoperiods. This could be explained by melatonin entering mitochondria at night, where it inhibits pyruvate dehydrogenase kinase, thereby promoting the upregulation of the pyruvate dehydrogenase complex [17]. This process allows pyruvate formed in glycolysis to enter the aerobic pathway, forming Acetyl-CoA for the tricarboxylic acid cycle and favoring energy production. Importantly, melatonin's enhancement of aerobic glucose degradation does not lead to uncontrolled reactive oxygen species (ROS) production.

This study established that exposure to constant darkness and melatonin administration during equinox and constant light conditions increases G6PDH activity (Fig. 2). Melatonin likely stimulates G6PDH activity by modulating gene expression [18], maintaining a balanced pro- and antioxidant system and supporting the glutathione protective system. Consequently, melatonin enhances G6PDH activity, supplying NADPH2 for the subsequent formation of reduced glutathione.

Finally, melatonin is recognized as an adaptogen and cell-protective agent [19]. Under physiological conditions, it enhances ATP formation through the aerobic pathway, which is particularly beneficial under physical stress [20]. In constant darkness, melatonin, possibly through tanycytes, reduces the synthesis of triiodothyronine [21, 22], which leads to decreased G6Pase and LDH activity (Fig. 4, 5). In contrast, under constant light, the opposite effect is observed.

CONCLUSIONS

Thus, we observed a deterioration in the aerobic glucose oxidation pathway and an increase in gluconeogenesis under constant light conditions. In contrast, there was an increase in glucose consumption via the aerobic pathway, alongside a reduction in its production under constant dark conditions. Additionally, melatonin administration improved glucose metabolism under all lighting conditions. This improvement in carbohydrate metabolism in the liver of rats, under both light and dark deprivation, was associated with the activation of the pentose phosphate pathway for glucose-6-phosphate oxidation, which is likely linked to enhanced antioxidant defense mechanisms across all types of illumination.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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