

Original Article

Restoring the sleep-wake cycle helps overcome insulin resistance in rats

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Abstract

Our goal was to establish the possibility of increasing insulin sensitivity as a result of restoring the sleep-wake cycle in rats after the formation of insulin resistance under conditions of circadian rhythm disruption due to weekly 24-hour light exposure. Rats were kept under different circumstances: natural day-night change for a week, then – in conditions of 24-hour lighting (>30 lux white 460–480 nm) during one more week, and then rats were transferred to a natural day-night change for another seven days. Animals were subjected to blood sampling from the tail vein on the eighth, fifteenth and twenty second days of the experiment. In the blood samples were determined: fasting glucose level, insulin level, HOMA-IR index, total triglycerides, glucose tolerance test. Statistical processing of the obtained results was carried out using the Student's t-test and the Mann-Whitney method. The value of $p \leq 0.05$ was considered reliable. Changing the regime from natural day and night to 24-hour lighting led to disturbances at the level of dysregulation of metabolic processes in experimental rats. Under these conditions, we observed an increase in the levels of glucose, an increase of insulin concentration and the HOMA-IR index as well as total triglycerides. The restoration of normal living conditions allowed the restoration of indicators. In conclusion, a change in the sleep-wake cycle was manifested in some changes. The return to a normal sleep-wake cycle was marked by the restoration of blood glucose and insulin levels, indicating the overcoming of insulin resistance.

Keywords: circadian rhythm, glucose, blood, rats

Introduction

Synchronization of the endocrine glands is the basis of well-being and prevention of disease. It is known that working at night causes disorders. These include fatigue, stress. This leads to changes in eating behavior, obesity, and the development of insulin resistance [1].

Melatonin is a hormone that promotes sleep, has adaptogenic and antioxidant properties. Its production synchronizes the activity of the endocrine glands during sleep [2].

Melatonin regulates the production of glucocorticoids through MT 1 receptors. Thus, when melatonin concentrations are high (with the onset of darkness), cortisol concentrations decrease and *vice versa*. It is known that melatonin is suppressed by artificial lighting [3].

Abnormalities in cortisol secretion due to persistent melatonin suppression lead to activation of gluconeogenesis and lipolysis with the formation of indicators characteristic of the development of metabolic syndrome. This is also facilitated by disorders of



leptin-ghrelin interaction. The result of such changes is the appearance of insulin resistance with the formation of hyperglycemia and hyperinsulinemia, which lead to an increase in inflammatory cytokines and impaired endothelial function. This is the development of insulin resistance and the appearance of metabolic syndrome [4].

The use of β -adrenergic antagonists reduces melatonin synthesis [5]. The use of antidepressants, due to the reduction in monoamine oxidase activity, contributes to an increase in the amount of norepinephrine and, as a result, increases melatonin production. Tranquilizers, acting through agonism of gamma-aminobutyric acid formation, lead to a decrease in the formation of the sleep hormone. By the way, physical exercise can increase melatonin synthesis. When working with insulin, melatonin exhibits a counter-effect: when insulin increases, melatonin levels fall, and vice versa [6]. Therefore, the formation of melatonin is influenced by various internal and external environmental factors.

Changing behavior and developing healthy habits instead of harmful ones can be not only prevention, but also an opportunity to restore the body's normal functioning.

Our goal was to establish the possibility of increasing insulin sensitivity as a result of restoring the sleep-wake cycle in rats after the formation of insulin resistance under conditions of circadian rhythm disruption due to weekly 24-hour light exposure.

Material and methods

Experiments on rats were conducted in accordance with "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), according to directions of International Committee of Medical Journals Editors (ICMJE), as well as "Bioethical expertise of preclinical and other scientific research conducted on animals" (Kyiv, 2006). We used in our work 30 white non-linear Wistar rats, which were kept under different circumstances: at the beginning in conditions of natural day-night change for a week, then after – in conditions of 24-hour lighting (>30 lux white 460–480 nm at the level of the bottom of the cage) during one more week, and then rats were transferred to a natural day-night change for another seven days [7]. Animals were subjected to blood sampling from the tail vein on the eighth, fifteenth and twenty second days of the experiment. In the blood samples were determined

such parameters: glucose level by One Touch Ultra Easy, LifeScan, USA, insulin level by immunoluminescent analyzer Snibe Co., Ltd, China using the Maglumi test kit, China, HOMA-IR index (blood glucose (mg/dL) \times fasting insulin (μ U/mL) \div 405) [8], total triglycerides ("Reagent" Research and Production Enterprise, Ukraine), oral glucose tolerance test. Statistical processing of the obtained results was carried out using the Student's t-test and the Mann-Whitney method. The value of $p \leq 0.05$ was considered reliable.

Results

Under conditions of 24-hour lighting during the week, fasting glucose levels (Figure 1) increased by 57% compared to those under normal rhythm conditions.

Restoration of physiological sleep-wake conditions in the next seven days was marked by normalization of glucose levels on the twenty-second day of measurement.

Under conditions of altered circadian rhythm, glycemic curve values (Figure 1) significantly differed by 51% after 0.5 hours and by 17% after 2 hours compared to those under normal rhythm conditions. Restoration of normal conditions affected the normalization of the glycemic curve.

The insulin content (Figure 2) in the blood of rats after exposure to 24-hour light increased by twofold. After the restoration of a normal rhythm, this indicator also returned to normal.

The HOMA-IR index (Figure 3) increased 2.2 times due to the change in rhythm when exposed to light. Restored circadian rhythm was marked by the decline in HOMA-IR because of insulin and glucose levels changes.

Followed by glucose level, the level of triglycerides markedly rise up on 76% after sitting in conditions of 24-hours light. After turning back to normal rhythm this parameter went down and did not differ from initial one.

Discussion

Changing the regime from natural day and night to 24-hour lighting led to disturbances at the level of dysregulation of metabolic processes in experimental rats. Under these conditions, we observed an increase in the levels of prandial and postprandial glucose based on the results of OGTT. This phenomenon was accompanied

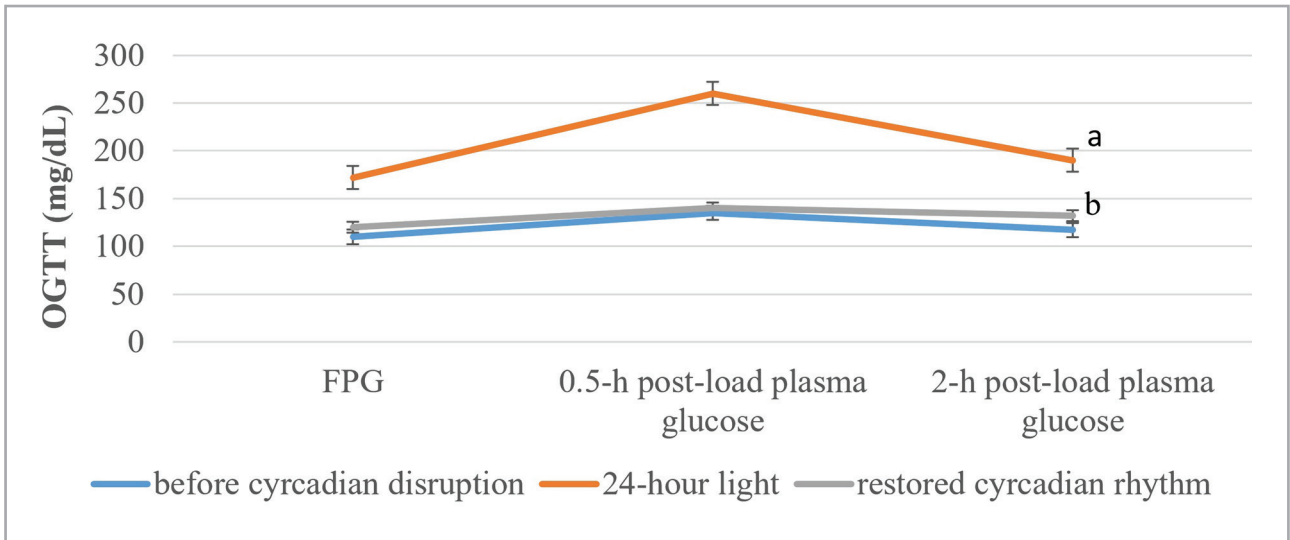


Figure 1: Oral glucose tolerance test (OGTT), mmol/L (n=30, x±Sx): 1. Changes are reliable (p<0.05). 2. a – concerning control; b – concerning IR.

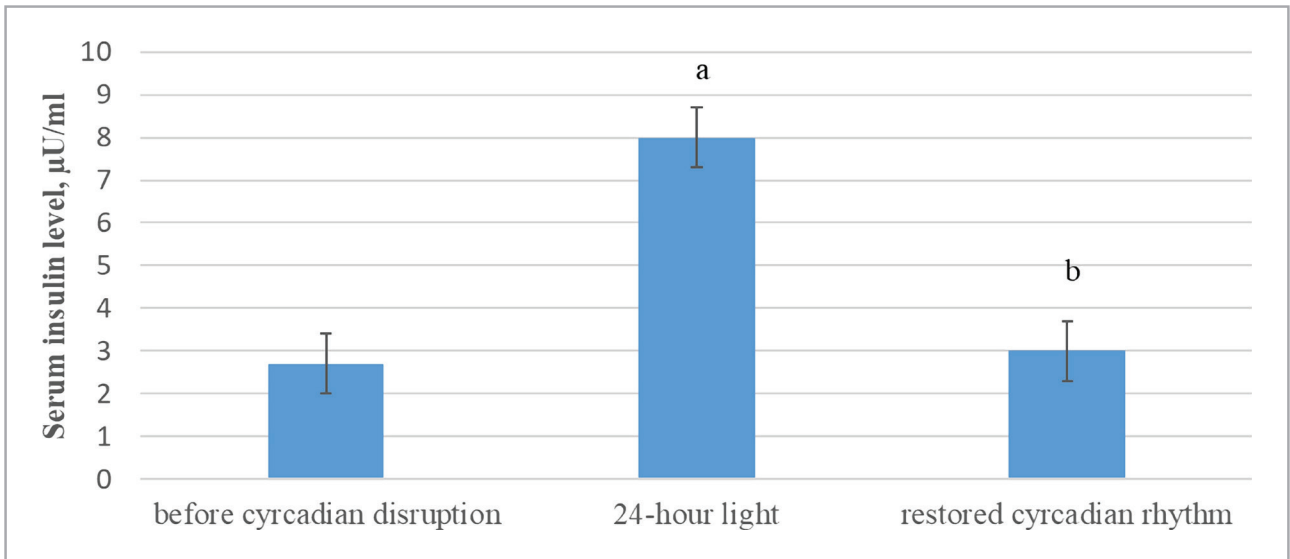


Figure 2: Serum insulin level, μU/ml (n=30, x±Sx): 1. Changes are reliable (p<0.05). 2. a – concerning control; b – concerning IR.

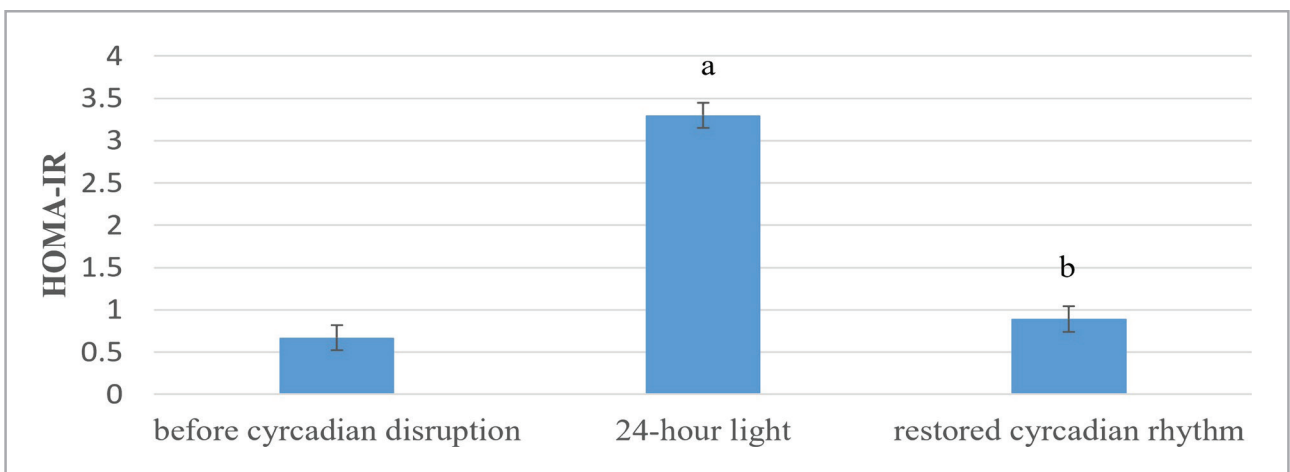


Figure 3: HOMA-IR (n=30, x±Sx): 1. Changes are reliable (p<0.05). 2. a – concerning control; b – concerning IR.

by an increase in the level of insulin and the HOMA-IR index. The level of total triglycerides also increased. The authors described [9] that staying in constant illumination leads to disorders in melatonin synthesis and disorders of regulation of metabolic processes with hormones. The suprachiasmatic nucleus controls the production of melatonin, so when its function is disrupted, for example, with 24-hour lighting, it transmits a distorted signal to organs and tissues, which disrupts the course of physiological processes. Thus, the appearance of diseases during aging is associated with the involution of the pineal gland.

In our case, the change in conditions from natural to 24-hour lighting contributed to the increase in glucose due to impaired control over glucocorticoids and leptin. As is known, this activates gluconeogenesis and suppresses the feeling of satiety, increasing food consumption. This is accompanied by increased lipolysis in adipose tissue and lipogenesis in the liver, which is perhaps why we observed an increase in total triglycerides in the blood. Melatonin secretion disorders lead to impaired insulin formation, which in our experiment was reflected in an increase in insulin concentration in the blood and showed the presence of insulin resistance when determining HOMA-IR [10].

It is known that hyperglycemia causes the risk of developing prooxidant stress [11], which is even more dangerous against the background of a decrease in melatonin, as a strong antioxidant. The widespread distribution of triglycerides in the bloodstream worsens rheology and is subject to peroxidation. All this leads to vascular damage and ischemia. In turn, the restoration of normal living conditions allowed the restoration of indicators that did not differ from those that existed before the change of day and night cycle.

Conclusion

A change in the sleep-wake cycle leads to the formation of insulin resistance, which was manifested in an increase in glucose levels against the background of an increase in insulin levels, which was reflected in the HOMA-IR index and glucose tolerance curves. The rats' return to a normal sleep-wake cycle was marked by the restoration of blood glucose and insulin levels, indicating the overcoming of insulin resistance.

Conflict of interest

The authors declare no conflict of interest.

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