The effect of round-the-clock illumination on blood hemoglobin level, body composition and endurance of C57Bl/6 mice

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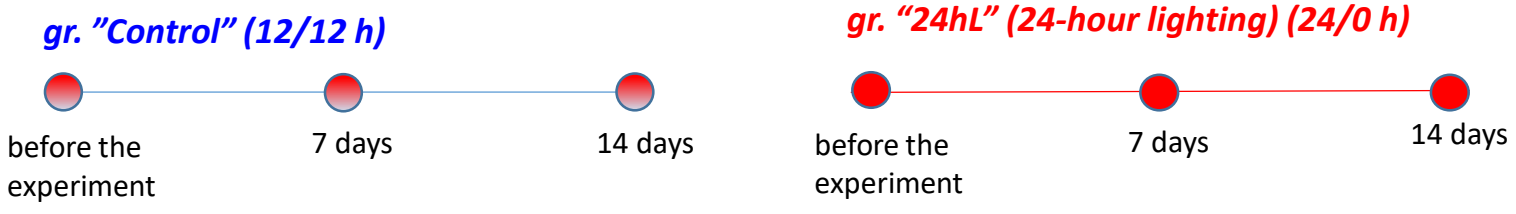
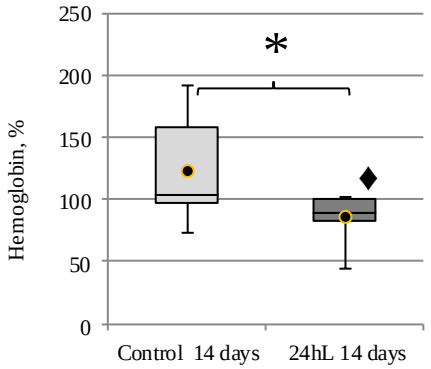
Motivation and Aim. A modern life creates situations for a person (flights through several time zones, shift work, work in the Arctic, artificial increase in the duration of the photoperiod by electric lighting) which violate the circadian rhythms of the body. As a result, the disturbance of the melatonin secretion in pineal gland leads to the development of desynchronosis. In this study, we evaluated the effect of 24-hour lighting (24 hL) on the blood hemoglobin of male C57Bl/6 mice, body composition (fat content, muscle mass and total water mass) and physical endurance.

Material and Methods. Experiments were performed in the SPF-vivarium animals center of ICG SB RAS with C57Bl/6 mice males aged 10-12 weeks. Intact animals (gr."Control", n=10) were kept at the standard lighting mode - **light 14 h/dark 10 h**. The mice from the experimental group (gr. "24hL", n=20) were kept for 14 days under 24-hour lighting (24hL) - **light 24 h/dark 0 h.** We determined blood hemoglobin (before the experiment and after 14 days of the 24hL) and made non-invasive estimation of the body components (body weights and fat, lean and total water masses) with the low-field magnetic resonance imaging EchoMRI-700. The estimation of the body components and the endurance of animals were performed before the experiment, on the 7th and on the 14th day after start of the 24hL.

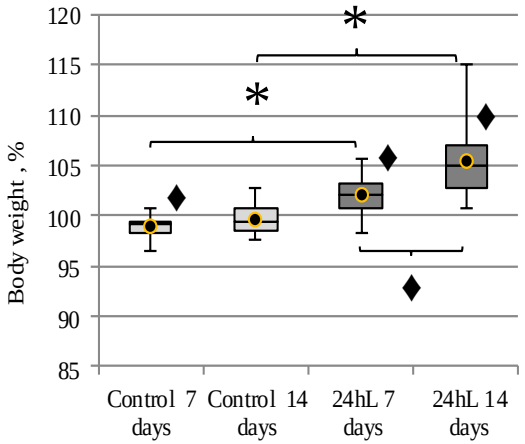
The endurance of animals was studied in the modified suspension test [Aartsma-Rus A., van Putten M., 2014]. We considered: 1) the time of hanging of an animal on grid lower surface for the first approach (T-1), 2) the total time of the hanged stay for three approaches (Ttot). To compare the changes in the studied parameters, the means obtained on the day "before the experiment" in each of the groups - "Control" and "24hL" - were taken as 100%.

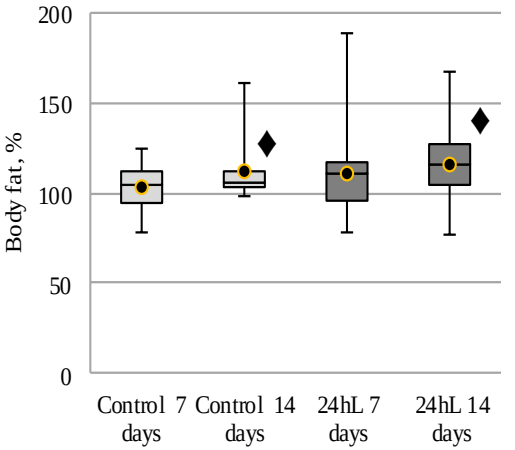


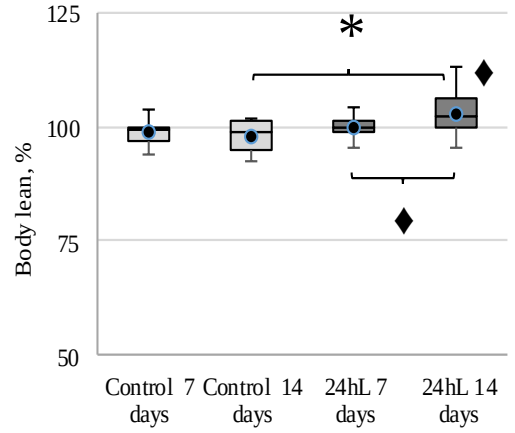


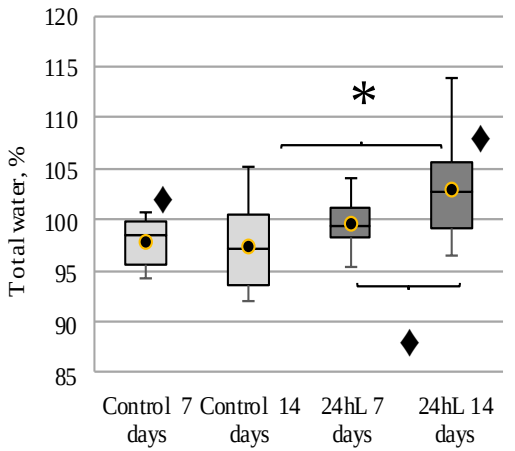
Results and discussion. ***The hemoglobin content*** decreased in mice kept during 14 days under 24hL as compared with intact animals at the time "before the experiment" (♦ the Wilcoxon test; p<0,05) and compared with animals from the "Control" group on the 14th day of the experiment (* the Mann-Whitney U-test; p<0,05). It is known that the light inhibits melatonin production and the decrease in illumination evokes the synthesis and secretion of melatonin. Given that hemoglobin may be a protein carrier for melatonin and helps its transport to target organs [Zinchuk V.V. et al., 2013], revealed by us decrease in blood hemoglobin content in mice kept under 24hL, not only creates conditions for the development of tissue hypoxia, but also inhibits the realization of hormonal and antioxidant effects of melatonin in organs and target tissues.

The body weight in mice under 24hL increased on the 7th and 14th day of the experiment in comparison with control animals (*the Mann-Whitney U-test; p<0,001). ***The lean mass*** and ***the total water*** in the body of mice increased at the end of experiment (*the Mann-Whitney U-test; p<0,001; ♦ the Wilcoxon test; p<0,001). Moreover, within the 24hL group, an increase in the values of these parameters by the end of the experiment was noted compared with the times "before the experiment" and on the 7th day of the 24hL. Analysis of ***the fat content*** did not reveal any significant changes compared with the "Control" group. An increase in the value of this parameter on the 14th day of the experiment was found in both groups, "Control" and "24hL", compared with the moment "before the start of the experiment". There are literature data that presence of mice in conditions of even very dim illumination (5 lux) during the dark phase of the day contributed to body weight increasing [Fonken L.K. et al., 2013]. The impact of unnatural light cycles is associated often with obesity and metabolic syndrome [Fonken L.K. and Nelson R.J., 2014]. Facts of the disturbance of tissue oxygenation processes and prooxidant-antioxidant balance during light regime violation are known in the literature [Baydaş G. et al., 2001; Kooijman S. Et al., 2015].

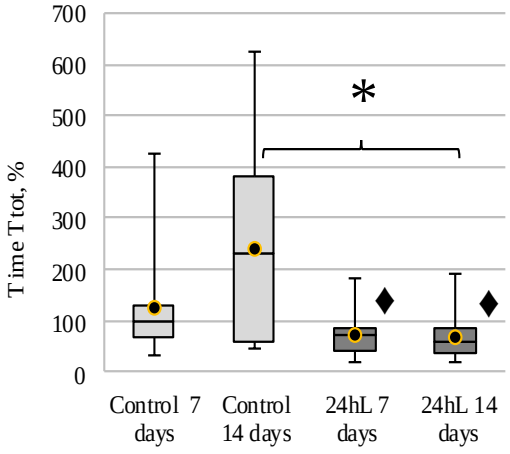


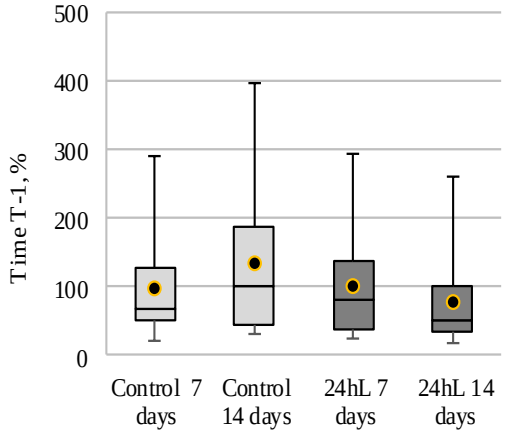






These processes create prerequisites for the development of intercellular space swelling, dilatation of microcirculatory bed, expansion of the sinus system in lymph nodes, thymus, spleen and other organs and, as a consequence, contribute to an increase in the total water content in the body [Borodin Yu.I. et al., 2012; Michurina S.V. et al., 2019].





The study of the 24hL effect on ***the mouse endurance*** showed a decrease in ***the total time (Ttot) of the hanged state of animals on the grid lower surface*** as compared with intact animals at the time "before the experiment" (♦ the Wilcoxon test, $p < 0.01$), and compared with animals from the group "Control" on the 14th day of the experiment (* The Mann-Whitney test, $p < 0.05$). Moreover, a decrease in the Ttot value occurred already on the 7th day of 24hL (♦ the Wilcoxon test, $p < 0.05$). This is possible evidence of a decrease in muscle strength and endurance of animals kept under 24hL since it is known that the fear of falling from a height forces mice remain hanging on the grid until muscle exhaustion [Rafael J.A.et al.,2000]. It is known that skeletal muscles comprise approximately 45% of total body mass. Changes in sarcomere structure, development of mitochondrial dysfunction and disturbance of muscle contractile function are described in skeletal muscle in case of disturbance of circadian rhythms. In addition, metabolic disorders in muscle tissue, including changes in glucose tolerance and insulin sensitivity have been noted [Harfmann B.D. et al., 2015]. Apparently, the cascade of rearrangements in the muscle tissue of animals under round-the-clock lighting (24/0 h) along 14 days leads to the development of mitochondrial dysfunction, tissue hypoxia and violation of the contractile function of the muscles result in a decrease in strength and endurance of C57BL/6 mice.

Conclusion. Thus, keeping C57Bl/6 mice male in conditions of 24h lighting for 14 days leads to an increase in body weight at the end of the experiment, first of all, due to an increase in the content of lean mass and body total water. The latter indicates a violation of the fluid homeostasis of the body. By this time, a decrease in hemoglobin in the blood is detected, that indicates the development of anemia. The decrease in a total hanged state time of animals on the grid lower surface revealed by us on the 14th day of the experiment indicates a decrease in muscle strength and endurance of C57Bl/6 mice under 24-hour lighting compared with animals of the control group.

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