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Effect of Long-Term Light Deprivation on α-Tocopherol Content in Rats during Ontogeny

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We studied the effect of long-term light deprivation which began at different stages of ontogeny on the content of α-tocopherol in rats during the first 3 months of postnatal development. In the offspring postnatally exposed to constant darkness, the level of α-tocopherol in the liver, kidneys, heart, skeletal muscles, and lungs was significantly decreased at the early stages of postnatal ontogeny (2 weeks and 1 month). In rats kept under constant darkness after birth, the content of α-tocopherol in the lungs was also reduced at the age of 1 month. The modulating effect of light deprivation on the level of α-tocopherol can be associated both with the impact of disturbed circadian rhythms and with increased content of melatonin in the body.

Key Words: *α-tocopherol; light deprivation; early postnatal ontogeny; Wistar rats*

The circadian system of the body synchronizes the rhythms of physiological processes with environmental factors, the main of which is the light/dark cycle, and has of great importance in the regulation of metabolism [8]. An important role in this process is played by the pineal gland hormone melatonin, the synthesis of which is activated in the dark [1,7]. Significant changes in lighting conditions lead to disruption of circadian rhythms and the development of desynchronosis, which alter various physiological functions of the body. A large number of studies have established the negative effect of excessive artificial illumination typical of modern life. Exposure to insufficient lighting or constant darkness is less common and also has adverse health effects [2,4,5,14]. These situations are faced by completely blind people [12], as well as employers working under poor lighting conditions, and residents of the Far North during the polar night. Laboratory rats are often used as experimental models for studying the effect of disturbed light conditions on the body [2,7,13]. Despite the fact that rats are mostly nocturnal animals, the rhythm of melatonin synthesis in these animals coincides with that in humans [11].

The circadian system plays a key role in lipid metabolism, providing temporally coordination of the daily rhythms of absorption, transport, and storage of these nutrients with the rest—activity and feeding cycles [8]. Disruption of circadian rhythms leads to changes in various stages of lipid metabolism [8], which in turn can affect the level of fat-soluble vitamins. For instance, in the absence of photoperiodicity associated with keeping under constant darkness for 4 weeks, growing male rats needed increased amount of vitamin E for normal development of gonads [9]. Vitamin E, being a natural antioxidant, plays a significant role in the processes of reproduction, growth, development, in maintaining immunity, and is especially important in the early stages of ontogeny [6].

Our aim was to study the effects of constant darkness starting from the prenatal period or from the moment of birth and lasting during the first 3 months of postnatal ontogeny, on the content of α-tocopherol in rats.

MATERIALS AND METHODS

The experiment was performed on laboratory Wistar rats kept under standard vivarium conditions with free

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access to water and granulated food. This study was carried out in compliance with the Directive 2010/63/EU of the European Parliament and of the Council (On the Protection of Animals used for Scientific Purposes, September 22, 2010).

For breeding, 4-month-old male and female rats were kept under standard fixed lighting conditions (12 h light 750 lux/12 h darkness; LD) or in constant darkness (0-0.5 lux, DD). Females from the LD group and their offspring immediately after birth were randomly divided into two subgroups and either left in the same lighting conditions (LD control) or transferred to constant darkness (LD/DD). Females and offspring born in the DD group were left in constant darkness (DD/DD). At the age of 2 weeks and 1, 2, and 3 months, the animals each group (n=8) were decapitated and samples of liver, kidneys, heart, lungs, spleen, and skeletal muscles were collected. The content of α-tocopherol was measured by HPLC.

The results were processed using Microsoft Excel 2007 and Statgraphics 5.0 software using generally accepted methods of variation statistics. The significance of the differences in the parameters was assessed using the Mann—Whitney U test. The results are presented as $M \pm m$. The differences were significant at $p<0.05$.

RESULTS

In rats of the control group, the content of α-tocopherol in the liver, kidneys, and lungs reached maximum values at the age of 2 weeks and in the heart at the age of 1 month; then, the vitamin level decreased (Fig. 1). In the LD/DD group, the vitamin content was slightly lower than in the control animals during the 1st month of postnatal development in the kidneys and in the heart and lungs during the first 2 months. Significant differences ($p<0.05$) in α-tocopherol content were found in the lungs of 1-month-old rats compared with LD control group. Moreover, the age-related dynamics of changes in the level of α-tocopherol coincided with that in control rats in the liver, heart, and lungs.

In rats of the DD/DD group, the level of α-tocopherol was significantly lower than in animals of the control group, and in some cases of the LD/DD group. Thus, α-tocopherol content in DD/DD rats at the age of 2 weeks significantly differed from the indicators of the other two groups in the liver and kidneys and at the age of 1 month in the kidneys and heart ($p<0.05$). In the heart, the level of α-tocopherol at this age was more than 4-fold lower than in other groups (Fig. 1). During subsequent age periods, this difference was slightly reduced and was minimal in 3-month-old rats, when a significant decrease in vitamin content was observed in the LD control and LD/DD groups. A lower level of α-tocopherol compared to control animals was found in the lungs and skeletal muscle of 1-month-old rats in the DD/DD group ($p<0.05$). The age of the animals and the light conditions did not affect the vitamin content in the spleen.

According to published reports, the absence of light cues in constant darkness leads to the appearance of the so-called "free-running" circadian rhythms, in which the functioning of various physiological systems can be impaired [12,14]. An important role in these processes is played by changes in the secretory function of the pineal gland, which is associated with the duration of constant darkness exposure. Light deprivation for 10 days contributed to an increase in the concentration of melatonin in rat blood [7]. In animals kept under constant darkness for 56 days, the concentration of melatonin in blood remained at the same level (with a slight increase in the hormone content during the day and decrease at night) as in rats under standard lighting conditions [14]. Prolonged exposure to constant darkness (75 days) caused a decrease in the level of melatonin due to depletion of pinealocytes and a decrease in the pineal gland activity [4]. In our experiment, morphological studies of the pineal gland showed that pinealocyte activity in 3-month-old rats in the DD/DD group was increased compared to those in rats of LD control [13]. Most likely, the effect of light deprivation on the content of α-tocopherol in animals during the first month of life is associated with circadian rhythm disturbances in the absence of alternation of light and darkness and an increase in melatonin level in females during pregnancy and lactation and in their offspring.

It has been established that disruption of circadian rhythms significantly affects energy metabolism, including lipid metabolism [8] that is closely related with the metabolism of vitamin E. The process of vitamin E absorption by enterocytes occurs with the participation of pancreatic lipids hydrolyzing enzymes, as well as bile acids, involved in the formation of micelles. Chylomicrons deliver vitamin E from the intestine to the liver, while α-tocopherol is transported to tissues by all classes of lipoproteins [6]. A number of processes regulating the absorption and transport of lipids demonstrate circadian rhythms and are regulated by clock genes. Diurnal changes in plasma lipid levels are associated with diurnal fluctuations in activity of the intestinal and liver microsomal triglyceride transfer protein [8] involved in the formation and secretion of apolipoprotein B (apo B) necessary for the transport of vitamin E both in chylomicrons and in VLDL and LDL. Disturbances in chylomicron formation or production and secretion of VLDL by the liver can lead to vitamin E deficiency [6]. A part of α-tocopherol is delivered to tissues during catabolism of chylomicrons and VLDL by lipoprotein lipase, the activity of which

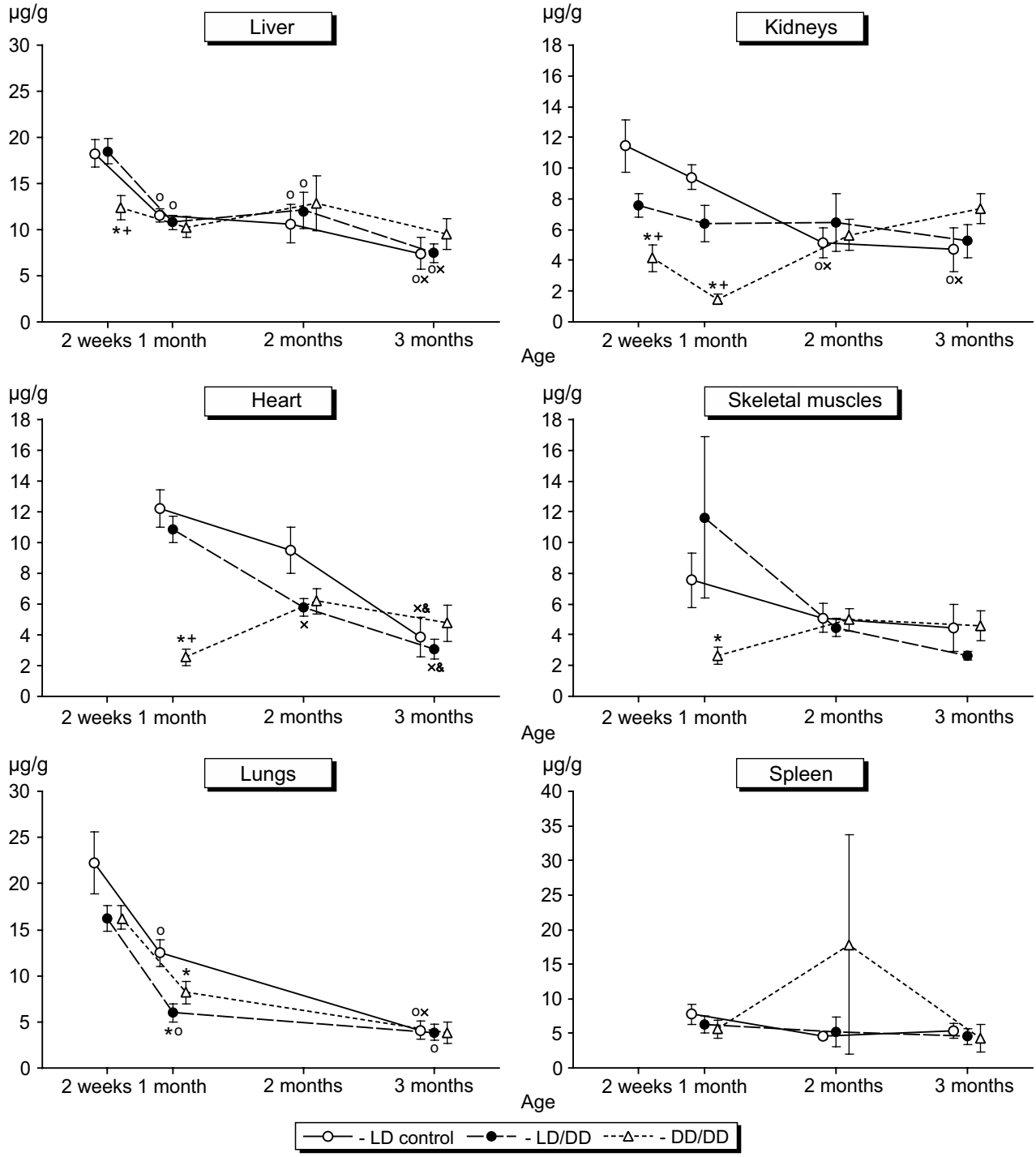

Fig. 1. Concentration of α-tocopherol in organs and tissues of rats kept under different lighting conditions. $p<0.05$ in comparison with *LD group, $^{+}$LD/DD group, rats aged ^{o}2 weeks, ^{x}1 month, $^{\&}$2 months (Mann—Whitney test).

is inhibited by melatonin [10]. This enzyme is directly involved in the regulation of lipid metabolism and affects the tissue-specific accumulation patterns of the lipids [10] associated with the content of α-tocopherol [6]. Thus, lower levels of α-tocopherol in the organs and tissues of rats of the experimental groups can be associated with changes in lipid metabolism caused by disrupted circadian rhythms both at the stage of absorption in the intestine and during transport and metabolism in the liver and other tissues.

In addition to the liver and adipose tissue, active lipid metabolism occurs in the muscle tissue. Cardiomyocytes utilize free fatty acids as the main source of energy [3]. Changes in lipid metabolism be the cause

of low α-tocopherol level in the heart of 1-month-old DD/DD rats found in our study. A significant decrease in the vitamin content in 3-month-old rats in the LD control and LD/DD groups can be a result of structural and metabolic maturation of the heart; in rats, this process is completed by the age of 3 months [3].

Vitamin E plays an important role in the development of the respiratory system [6], which can explain the relatively high level of α-tocopherol in the lungs of 2-week-old rats of all groups studied and its gradual decrease by the age of 3 months (Fig. 1). In rats, alveoli are intensively formed during the period from day 4 to day 21 after birth and continues somewhat slower up to day 60 [15]. Increased oxidative metabolism along with the immaturity of the antioxidant defense system can contribute to active consumption of α-tocopherol, whose antioxidant properties are well known [1,6].

Melatonin and its metabolites also exhibit antioxidant activity that is realized in biological membranes like that of α-tocopherol [1,10]. To maintain the optimal level of oxidative processes necessary for cells functioning, the components of the antioxidant system are in mutually compensatory relationships [1]. In this regard, an increase in the level of melatonin in the body of rats kept in constant darkness could also contribute to a decrease in the content of α-tocopherol in some tissues.

The formation of immature circadian rhythms begins during the fetal period with the participation of the maternal circadian system [4,5]. In newborns, maternal entrainment still plays a role in synchronizing circadian rhythms through melatonin in maternal milk. Therefore, it is likely that more pronounced changes in the level of α-tocopherol in our experiment were observed in rats exposed to constant darkness starting from their intrauterine development and after birth. Maturation of circadian system in rodents occurs during the first 2 postnatal weeks. The photoperiod affecting the body during the first 3 weeks of postnatal development exerts a shaping influence on the functioning of the circadian system in adulthood [5]. Since young rats receive vitamin E with their breast milk during the suckling period, light deprivation of pregnant and/or lactating females could also contribute to a decrease in the content of α-tocopherol in their body and, accordingly, in secreted milk. It should be noted that in the DD group, the number of females that that produced no offspring was more than 2-fold higher than in the control group; the number of stillborns and mortality rate during the first month of life were also higher in this group [2]. It is well known that vitamin E, which is called the reproductive vitamin, is an essential nutrient for normal embryonic development. Since the transfer of α-tocopherol through the placenta is limited, newborns often have its deficiency that contributes to the development of diseases such as hemolytic anemia, chronic lung diseases and reduced survival [6].

The results indicate that in rats, prolonged exposure to constant darkness caused a decrease in the level of α-tocopherol during the early stages of postnatal ontogeny, which is probably associated with disruption of circadian rhythms of metabolic processes and with increased level of melatonin in the body. The content of α-tocopherol was of a greater extent affected by the lack of lighting during the intrauterine development, because in this case, the modulating effect of light deprivation on physiological systems manifested earlier.

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