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RESEARCH

Effect of continuous illumination on the ultrastructure of rat hepatocytes during lifetime

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Abstract

Chronic light exposure is a known factor accelerating mammalian aging, primarily through circadian rhythm disruption, oxidative stress, and inflammation. The liver, a central organ in systemic aging, undergoes structural and functional decline with age, including mitochondrial dysfunction, lipid accumulation, and impaired detoxification. This study investigates the impact of constant light exposure on the ultrastructural changes in hepatocytes of Wistar rats across different ontogenetic stages. Male Wistar rats were divided into control (natural light cycle) and experimental (constant light) groups. Liver samples were collected at 6, 12, 18, and 24 months, processed for electron microscopy, and analyzed for hepatocyte morphology, mitochondrial integrity, and Golgi apparatus structure using stereometric methods. Constant light exposure induced premature hepatocyte aging, marked by mitochondrial dysfunction, lipid accumulation, and impaired organelle turnover. These changes suggest accelerated oxidative damage and metabolic dysregulation, likely due to melatonin suppression and circadian disruption. The findings highlight the role of environmental light pollution in promoting liver aging and underscore the need for chronobiological interventions to mitigate age-related hepatic decline.
Keywords: Aging, hepatocytes, constant light exposure, mitochondria, ultrastructure

Introduction

It is now well known that constant illumination is one of the factors that accelerates the rate of aging in mammals. The influence of lighting on the rate of aging is based on several mechanisms with several factors, the leading ones being disruption of circadian rhythms, oxidative stress and other biological processes.

Circadian rhythms (CRs) provide synchronization of biological processes of living organisms with external factors, which has decisive importance for maintaining of homeostasis in changing environmental conditions [1, 2]. CRs are connected with light-darkness cycle and are generated by 24-hour period of rotation of Earth, determining existence of cycles of sleep and wakefulness, feeding and fasting, secretion of hormones, energy resources exchange, body temperature fluctuations [3-6].

In humans, increasing exposure to light pollution, which causes desynchronosis, is due to a number of social reasons. The digitalization of modern life makes long-term interaction with digital technology inevitable; for a significant number of professions, the need for overtime and shift work remains relevant; jet lag due to the high prevalence of transmeridian flights also contributes to the development of desynchronosis [7, 8]. These and other reasons for the disruption of the light regime make it a normal, almost inevitable part of the life of a modern person. It is all the more important to keep in mind that, according to the “circadian destruction” hypothesis accepted by most chronobiologists, exposure to light at night disrupts the endogenous circadian rhythm and also suppresses the nocturnal secretion of melatonin by the pineal gland, which leads to a decrease in its concentration in the blood [9-11]. Light, especially the blue portion of the spectrum (produced by screens, LED lamps), suppresses the production of melatonin, a hormone that regulates sleep and has antioxidant properties. Chronic melatonin deficiency accelerates cellular aging in all tissues of a mammalian organism. Disruption of normal circadian gene function (*e.g. Clock*, *Bmal1*) affects cellular metabolism, DNA repair, and immunity; changes in their expression are associated with

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accelerated aging and the development of age-related diseases [12-14].

Artificial light can increase the formation of reactive oxygen species (ROS), which damage cells structures. Long-term exposure of the retina to light promotes the accumulation of lipofuscin (the "aging pigment") and cell degeneration [15-18]. Excessive light leads to development of chronic inflammation - a key factor in aging, as well as to a decrease in the efficiency of autophagy [19-22]. Thus, chronic exposure to artificial light, especially at night, may accelerate aging through dysregulation of circadian rhythms, development of oxidative stress and inflammatory processes.

In hepatocytes, as well as in cells of other organs, molecular-genetic level of biological clock includes *Bmal* gene, functioning in pair with *Clock* gene, and also *Per* (*Per1*, *Per2*, *Per3*) and *Cry* (*Cry1*, *Cry2*, encoding cryptochrome proteins) gene families, and also a number of other genes [23, 24]. The second feedback loop in mammals, conditioning additional system reliability, is provided by competitive and multidirectional interactions of proteins *REV-ERBα* and *RORA* with *RORE* element [25].

Light, without being critical factor for existing of circadian rhythms, influences their period and amplitude, expression of number of genes and mutual coordination of rhythms [21, 26, 27]. Most liver functions have circadian rhythmicity [28-37]. Expression of genes providing wide spectrum of liver functions may be regulated directly by autonomic circadian system of hepatocytes, by rhythmic signals from external environment (lighting, food intake) or by combination of both mechanisms [38, 39]. At the same time, high degree of dependence of liver functioning from normally synchronized control of its CRs by suprachiasmatic nuclei and pineal gland has been proven [33, 40, 41]. The liver is a central organ that links cellular aging with systemic age-related changes. Maintaining its health is critical to prolonging healthy life.

Changes observed in the liver during the aging process are closely related to a decrease in enzyme activity, accumulation of toxins, changes in metabolism, a decrease in the regenerative capacity of the liver, and also to the development of chronic inflammation [42-49]. Mitochondrial dysfunction is one of the signs of cellular aging, which is one of the main causes of aging of the organism as a whole. Mitochondria play a central role in aging due to their influence on energy metabolism, oxidative stress, apoptosis and cellular communication. With age, their function is impaired, which contributes to the development of age-related diseases (neurodegeneration, sarcopenia, diabetes, cardiovascular pathologies) [50-52].

Age-related mitochondrial disorders are manifested in the development of electron leakage, accumulation of reactive oxygen species (ROS), and decreased mitophagy [53-60]. Thus, constant lighting may lead to disruption of circadian rhythmicity of hepatocytes, acting on them through the system of time givers, and also directly, by the level of pineal melatonin. During aging, there is a disruption in synchronization of internal biological rhythms of organism with external cycles of light and darkness; circadian rhythms often become less stable, which may accelerate an aging process and increase the risk of senile diseases [61-63].

Our previous studies showed that under condition of constant lighting hepatocytes undergo fatty dystrophy; edema of hepatocytes, swelling of mitochondria and wrinkling of cell nuclei were observed at ultrastructural level; and also decrease in expression of *Bmal1* and *Clock* and increase in expression of *Per2* were revealed. Darkness deprivation led to restructuring of circadian rhythms of expression of *Bmal1*, *Per2*, *Clock*, and also the rhythms of number of micromorphometric parameters of hepatocytes [64-66]. Based on the above, we considered it interesting to study the effect of dark deprivation on the ultrastructural features of hepatocytes and their organelles in model animals (Wistar rats) at different periods of lifetime.

Materials and methods

Wistar rats were the object of the study. The animals were 90 days old at the beginning of the experiment. Animals were taken from the “Stolbovaya” affiliate of the FSBIS

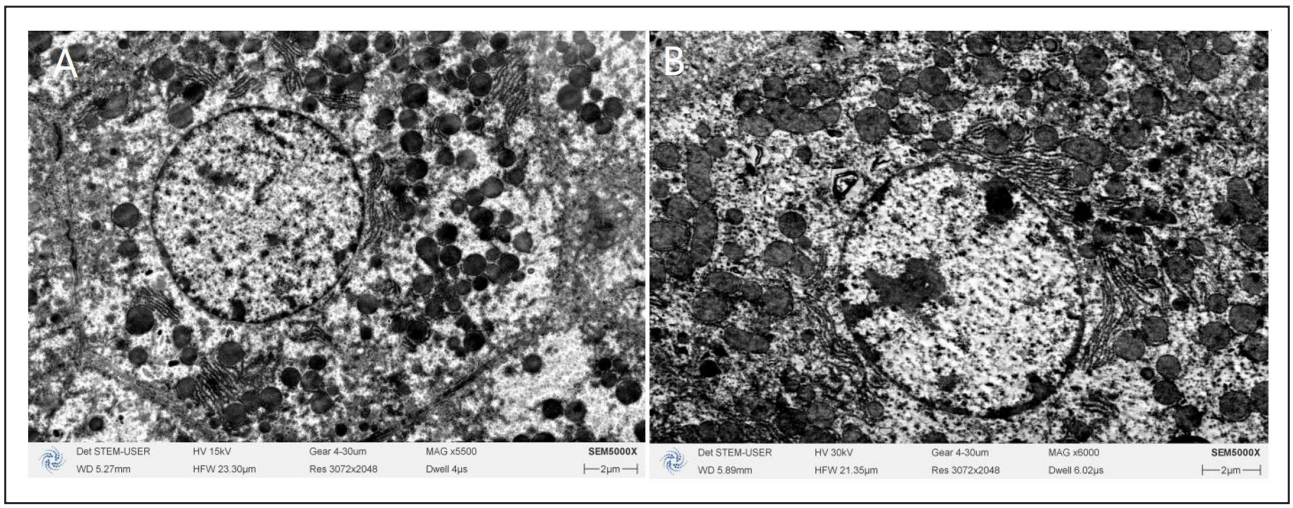

Figure 1. Hepatocytes of animals of control group. (**A**) 6 months. (**B**) 12 months. TEM, A: ×5500, B: ×6000.

Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency. No signs of pathogens or unexplained deaths were observed.

All animals were kept in plastic cages at a temperature of 20-22°C and a relative humidity of 60-70%. Rats had free access to drinking water and briquetted feed. The animals were given tap water that complied with Russian sanitary standards for drinking. (SanPiN 2.1.4.1074-01 — sanitary and epidemiological rules and regulations "Drinking water. Hygienic requirements for the quality of water in centralized drinking water supply systems. Quality control Resolution of the Chief State Sanitary Doctor of the Russian Federation). Drinking bowls were checked regularly to ensure proper maintenance, cleanliness and functionality. The source of feed was briquetted feed PK-120-1 (OOO Laboratorsnab, certificate of conformity No. POCCRU.nO81.B00113, GOST P50258-92).

Animal keeping and experiments were performed in accordance with the The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 18, 1986). Permission for the study was obtained from the Bioethics Committee of Avtsyn Research Institute of Human Morphology, protocol #38(10) from 14.03.2022.

The study was conducted on 320 male rats, randomly divided into 2 equal groups. The animals were kept under the same standard vivarium conditions in the same rooms, the only difference being the lighting regime. The 1st group (control, n = 160) was kept under natural light. Since our studies involve the study of circadian rhythms of various parameters of the rats' organism, for standardization the control group was kept under conditions of a light/dark regime of 12/12 hours. The light source included 6 fluorescent lamps SL 36/26-735, located at a height of 2.59 m above the surface of the cages, providing illumination of 300 lux at the level of the cages with animals. The 2nd group (experimental, n = 160) was kept under constant lighting. Operating around the clock, located at a height of 2.59 m above the surface of the cages, providing illumination of 300 lux at the level of the cages with animals. Round-the-clock illumination with a strength greater than 100 lux disrupts melatonin production. The luminous flux intensity was 3350 lm in both cases.

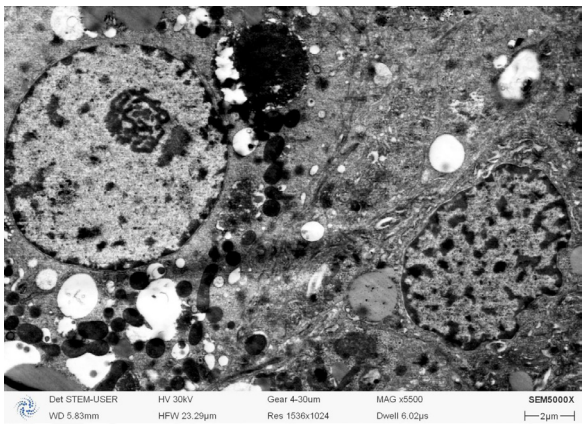

Figure 2. Hepatocytes of animals of control group, 18 months. TEM, ×5500.

The animals were withdrawn from the experiment at the age of 6, 12, 18 and 24 months. The selection of animals for age groups was random. The exclusion criterion was the presence of external signs of pathologies. By the time the animals were withdrawn from the experiment at the age of 24 months, there were 28 animals in the control group and 21 rats in the experimental group. When animals were euthanized, the liver was eviscerated. Euthanasia was carried out three weeks after the start of the experiment in a carbon dioxide chamber Shanghai TOW Intelligent Technology Co.,Ltd equipped with a device for the upper gas supply (100% CO_2) The chamber volume was filled with gas at a rate of 20% per minute to avoid dyspnea and pain in animals. A liver sample was removed immediately after euthanasia (2-3 min) to avoid the occurrence of postmortem artifacts.

Liver samples (2 mm^3) were fixed with 2.5% glutaraldehyde solution on phosphate buffer (pH 7.4), then additionally fixed in 1% osmium oxide solution (OsO_4), dehydrated in ethanol, contrasted with 1% uranyl acetate solution in 70% ethanol during dehydration and embedded into epon-araldite mixture according to the standard technique. Ultrathin sections obtained on a UC Enuity ultramicrotome (Leica Microsystems CMS GmbH, Germany) were additionally contrasted with use of Reynold's lead citrate stain, viewed in a field emission scanning electron microscope HIMERA EM50X (CIQUTEK, China), and photofixed.

The dissector method was used for stereometric studies [67]. Using the QuPath program, we determined: the cross-sectional area of hepatocytes, the cross-sectional area of nuclei, nuclear-cytoplasmic ratio (NCR), the numerical density of mitochondria, mitochondrial cross-sectional area, mitochondrial perimeter, number of cristae in mitochondria, ratio of mitochondrial area to mitochondrial perimeter, mitochondrial roundness coefficient (circularity index), Golgi complex cross-sectional area, number of Golgi complex's vesicles, number of lipid droplets in cytoplasm of 1 hepatocyte. All measurements were performed on the cross-section of mitochondria. For analysis, uniform areas were selected - the intermediate zone of the liver lobules. The analysis was performed in 10 random fields of view (×10,000, area 25 µm² each) to determine the numerical density of mitochondria and the sizes of hepatocytes and their nuclei, and in 10 random fields of view (×20,000, area 6.25 µm² each) for micromorphometry of mitochondria and the Golgi complex [11, 68].

Statistical analysis

Statistical processing of the results was performed using GraphPad Prism v8.41 (USA). The D'Agostino-Pearson test was used to identify the distribution type. For a normal distribution, the Student's t-test was used; for an abnormal distribution, the Mann-Whitney test for paired comparison was used. Differences were considered statistically significant if the level of statistical significance (α) or the probability of error of deviation from the null

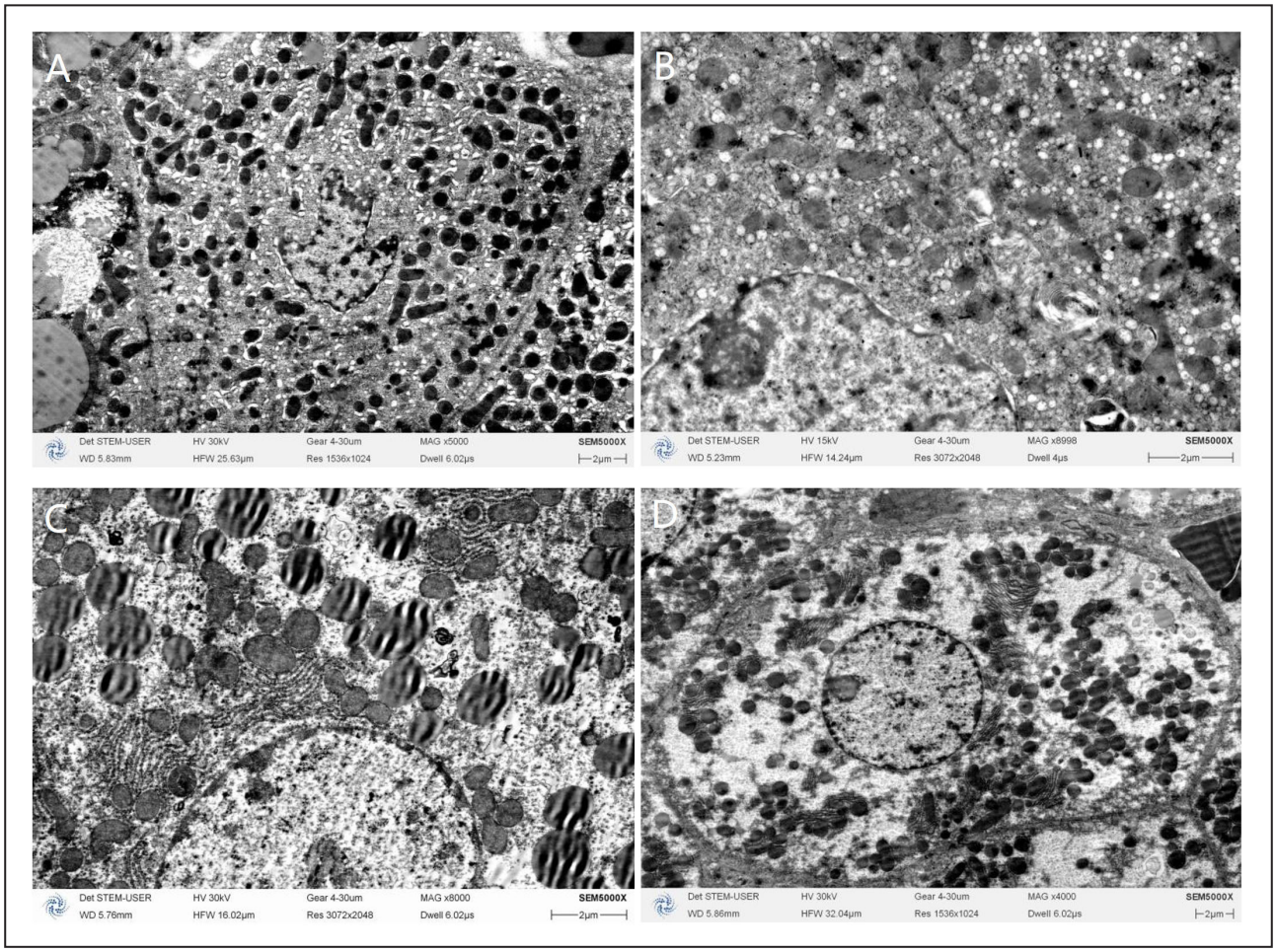

Figure 3. Hepatocytes of animals of control group, 24 months. TEM, D: ×4000, A: ×5000, C: ×8000, B: ×8998.

hypothesis was below 5% ($p < 0.05$). The arithmetic mean and standard deviation were used to plot the graphs. The strength of the differences was indicated as follows: "*" corresponds to $P < 0.05$; "**" corresponds to $P < 0.005$; "***" corresponds to $P < 0.0005$.

Results

Ultrastructural pattern of hepatocytes of 6 and 12 months old animals of control group corresponded to norm: round-shaped nuclei, mitochondria with dense matrix, well-developed endoplasmic reticulum and non-vacuolated Golgi complex, glycogen grains were observed. (Figure 1A & B).

At 18 months, in animals of this group, with a practically unchanged structure of hepatocytes, single necrotic cells are observed, as well as cells with signs of small-drop fatty degeneration. Ultrastructure of hepatocytes of rats of this group shows the transformation of shape of nuclei accompanied by swelling of the cytoplasm and the presence of lipid-containing vacuoles. Mitochondria are characterized by size polymorphism and high electron density (Figure 2).

At 24 months, condensation and radial distribution of chromatin along the karyolemma were noted in the hepatocytes of rats of this group, numerous lipid-containing vacuoles and mitochondrial polymorphism were observed in the cytoplasm of the cells at the ultrastructural level. In addition, a significant number of pycnotic nuclei were also detected in the hepatocytes of these animals, which was accompanied by accumulation of peroxisomes. The granular endoplasmic reticulum was characterized by pronounced vesiculation and dilation of cisterns; an increase in the number of vacuoles, including lipid-containing ones, was noted in the cytoplasm of hepatocytes. Swelling of mitochondria was locally observed, accompanied by lysis of their cristae and matrix and the presence of unevenness of the outer membrane (Figure 3A-C). Signs of reparative regeneration were noted: newly formed hepatocytes, a result of reparative mitosis, had characteristic signs in the form of a rounded cell shape with large light-colored nuclei, dilation of the endoplasmic reticulum, small mitochondria; the presence of lipid globules, a deficiency of glycogen in the cytoplasm, the inability to identify the Golgi complex, and an extension of intercellular contacts were also observed. (Figure 3D).

In animals of the experimental group, changes observed in the control at 18 months were already observed from 6 months (Figure 4A). And from 18 months, the picture of the ultrastructure of hepatocytes in experimental rats corresponded to that in two-year-old animals of the control group, but no characteristic signs of newly formed hepatocytes were observed (Figure 4B-D).

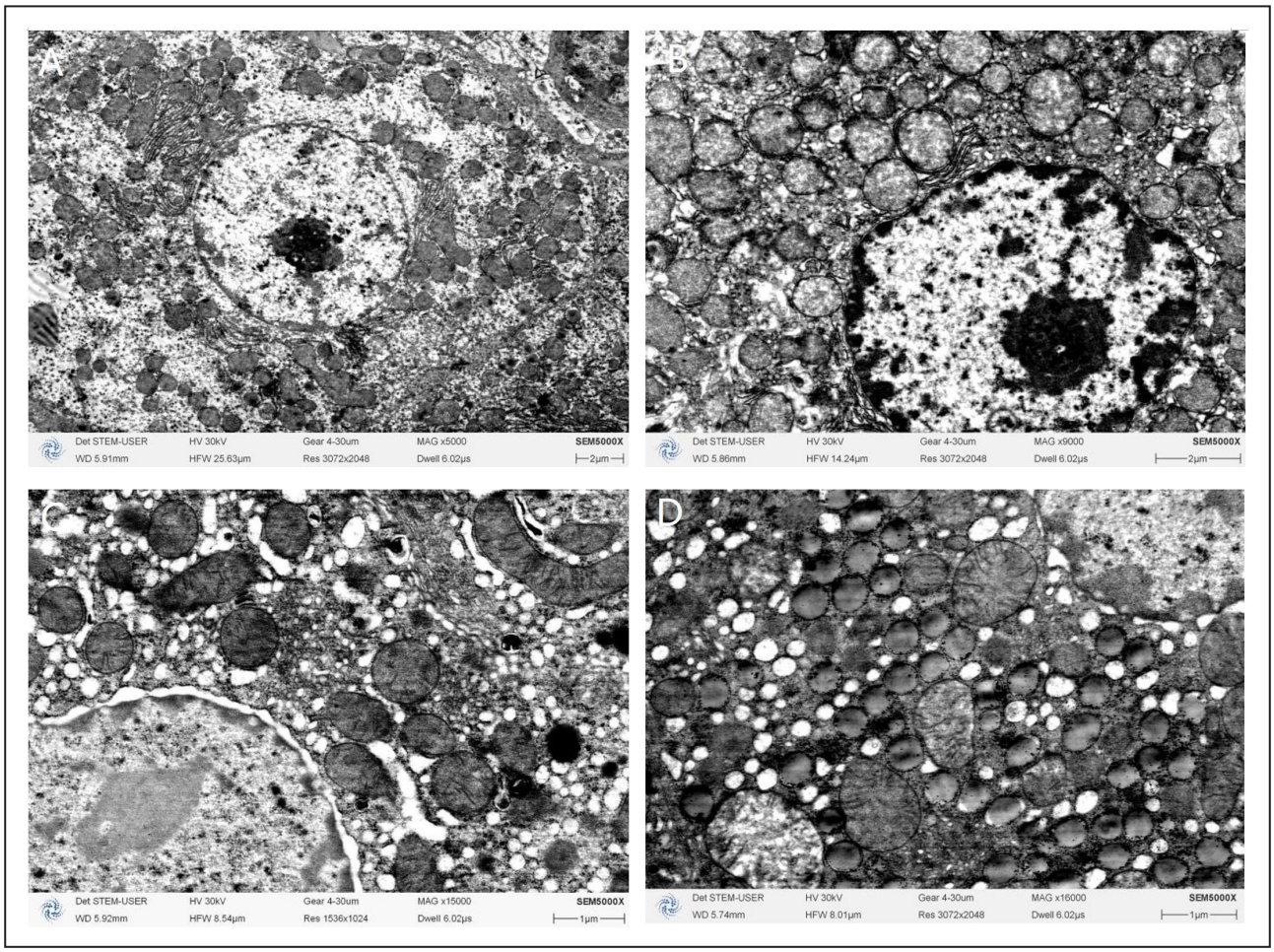

Figure 4. Hepatocytes of animals of experimental group. (**A**) 6 months. (**B-D**) 18 months. TEM, A: ×5000, B: ×9000, C: ×15000, D: ×16000.

The study showed that both in the control and in the experiment, there was an increase in the cross-sectional area of hepatocytes throughout life (Table 1). The increase in size relative to the previous age period is significant at 12 and 18 months, while the changes that occurred by the 24th month are not significant. At the same time, the values of the hepatocyte cross-sectional area in animals of the experimental group are higher than in rats of the control group in all studied periods of ontogenesis (Table 1).

The dynamics of the cross-sectional area of the hepatocyte nucleus was characterized by an increase by 12 months with a subsequent decrease by 18 months, while, as in the case of cell size, the control indicators were lower than those of experimental animals during the same periods of

Table 1. Dynamics of micromorphometric parameters of hepatocytes during lifetime.

Group	6 months	12 months	18 months	24 months
	Cross-sectional area of hepatocyte (µm^2)			
Control	171.0 ± 20.77	202.1 ± 21.42*	265.8 ± 24.95***	287.2 ± 23.75
Experiment	222.90 ± 10.09$^{\bullet\bullet\bullet}$	251.1 ± 11.59$^{*,\bullet\bullet\bullet}$	278.3 ± 29.10$^{*,\bullet\bullet}$	320.8 ± 51.21$^{\bullet\bullet}$
	Cross-sectional area of hepatocyte nuclei, (µm^2)			
Control	42.26 ± 7.94	48.47 ± 9.79***	34.11 ± 8.87***	36.26 ± 6.21
Experiment	52.76 ± 7.98$^{\bullet\bullet\bullet}$	64.17 ± 4.55$^{***,\bullet\bullet\bullet}$	57.01 ± 4.37$^{***,\bullet\bullet\bullet}$	57.94 ± 6.83$^{\bullet\bullet\bullet}$
	NCR			
Control	0.245 ± 0.022	0.239 ± 0.020	0.128 ± 0.011***	0.126 ± 0.014
Experiment	0.236 ± 0.021	0.255 ± 0.034$^{**,\bullet\bullet}$	0.204 ± 0.015$^{**,\bullet\bullet}$	0.181 ± 0.021$^{***,\bullet\bullet\bullet}$

Note: "*", $P < 0.05$; "**", $P < 0.005$; "***", $P < 0.0005$ in comparison with 6-months-old animals at the same age; "$^{\bullet}$", $P < 0.05$; "$^{\bullet\bullet}$", $P < 0.005$; "$^{\bullet\bullet\bullet}$", $P < 0.0005$ in comparison with parameters of control group at the same age.

Table 2. Dynamics of micromorphometric parameters of mitochondria of hepatocytes during lifetime.

Group	6 months	12 months	18 months	24 months
	Average numerical density of mitochondria per 1 μm^2, units			
Control	1.69 ± 0.26	1.31 ± 0.17***	0.99 ± 0.09***	0.80 ± 0.09***
Experiment	1.44 ± 0.23$^{\bullet\bullet\bullet}$	1.22 ± 0.17$^{***,\bullet}$	0.84 ± 0.09$^{***,\bullet\bullet\bullet}$	0.72 ± 0.11$^{***,\bullet\bullet}$
	Cross-sectional area of mitochondria (μm^2)			
Control	0.319 ± 0.036	0.359 ± 0.012***	0.30 ± 0.016***	0.284 ± 0.051
Experiment	0.314 ± 0.088$^{\bullet\bullet\bullet}$	0.327 ± 0.057$^{***,\bullet\bullet\bullet}$	0.241 ± 0.036$^{***,\bullet\bullet\bullet}$	0.189 ± 0.067$^{\bullet\bullet\bullet}$
	Perimeter of mitochondria (μm)			
Control	1.93 ± 0.16	2.02 ± 0.19**	1.87 ± 0.14	1.80 ± 0.11
Experiment	1.98 ± 0.13	2.16 ± 0.19$^{***,\bullet\bullet\bullet}$	1.94 ± 0.08$^{***,\bullet\bullet\bullet}$	1.68 ± 0.14$^{***,\bullet\bullet}$
	Number of cristae in mitochondria (units)			
Control	23.45 ± 3.58	18.81 ± 3.98**	16.13 ± 3.12**	12.66 ± 2.12*
Experiment	21.52 ± 3.88$^{\bullet\bullet}$	14.17 ± 2.89$^{***,\bullet\bullet\bullet}$	12.59 ± 3.16$^{***,\bullet\bullet\bullet}$	11.82 ± 2.98
	Ratio of mitochondrial area to perimeter			
Control	0.23 ± 0.03	0.22 ± 0.02	0.28 ± 0.03**	0.36 ± 0.04**
Experiment	0.24 ± 0.04	0.22 ± 0.03	0.29 ± 0.04**	0.32 ± 0.03*
	Circularity index			
Control	1.03 ± 0.04	1.25 ± 0.05*	1.19 ± 0.11	1.27 ± 0.14
Experiment	1.0 ± 0.06	0.93 ± 0.05	0.93 ± 0.09	0.84 ± 0.13$^{***,\bullet\bullet\bullet}$

Note: "*", $P < 0.05$; "**", $P < 0.005$; "***", $P < 0.0005$ in comparison with 6-months-old animals at the same age; "$^{\bullet}$", $P < 0.05$; "$^{\bullet\bullet}$", $P < 0.005$; "$^{\bullet\bullet\bullet}$", $P < 0.0005$ in comparison with parameters of control group at the same age.

the experiment (Table 1).

When analyzing the value of NCR, it was found that in animals of the control group there is a gradual decrease in this parameter, however, when comparing the values in different periods, it is reliable only at 18 months. Similar changes were noted in the hepatocytes of experimental rats, but in this case the difference between the indicators in all the studied periods was reliable. In addition, in the hepatocytes of rats of the experimental group, NCR is higher than in the control at all studied ages, except for 6 months (Table 1).

Mean numerical density of mitochondria decreases during ontogenesis both in hepatocytes of rats in the control group and in the experimental group; however, in the control it turns out to be higher than in experimental animals during the same periods of ontogenesis (Table 2).

The cross-sectional area of mitochondria in hepatocytes of animals of both groups increases by 12 months, but then by 18 months there is a reliable decrease. At the same time, the parameters of experimental animals are always higher than in the control (Table 2).

The perimeter of mitochondria in hepatocytes of maturing animals increases in the period from 6 to 12 months, but then decreases by 18 months in the control and up to 24 months in the experimental group. The experimental group parameters are greater than the control at all studied ages, except 6 months (Table 2).

The number of cristae in mitochondria during an individual development of an organism is higher in the organoids of rats of the experimental group, with the exception of 24 months. In the mitochondria of liver cells of rats of both groups, there is an age-related decrease in the number of cristae, but in the control this process proceeds more smoothly, in particular, a reliable difference is noted between the indicators at 18 and 24 months, which is not noted in the experiment (Table 2).

The ratio of mitochondrial area to perimeter does not differ significantly between groups, but in both the control and the experiment, it increases by the 18th month of life.

The circularity index of mitochondria of hepatocytes of rats in the control group increases by 12 months and remains virtually unchanged, while in the experiment it remains unchanged for up to 24 months.

The average number of Golgi complex dictyosomes in the field of view decreases during ontogenesis; significant differences are observed between 6 and 12 months, both in the control and in the experiment (Table 3). The number of cisterns in hepatocytes of the control group decreases reliably by 12 and 18 months, and in the experiment – by 12 and 24 months. At all ages studied in the experiment, the number of cisterns is less than in the control. The area of the Golgi complex dictyosome profile in the control is always higher than in the experiment, and in animals of both groups there is a constant decrease in this parameter

Table 3. Dynamics of micromorphometric parameters of Golgi complex of hepatocytes during lifetime.

Group	6 months	12 months	18 months	24 months
	Number of dictyosomes per field of view (units)			
Control	4.02 ± 0.30	3.04 ± 0.61**	3.01 ± 0.39	2.88 ± 0.71
Experiment	3.91 ± 0.38	3.10 ± 0.42**	3.16 ± 0.51	2.91 ± 0.41
	Number of Golgi complex cisterns (units)			
Control	8.07 ± 0.99	6.85 ± 0.89***	6.02 ± 0.40***	6.09 ± 0.69
Experiment	7.08 ± 1.03‴	4.97 ± 1.21***,‴	5.12 ± 0.71‴	2.97 ± 1.17***,‴
	Golgi complex profile area (μm^2)			
Control	3.27 ± 0.48	2.65 ± 0.22***	2.31 ± 0.24***	1.82 ± 0.31***
Experiment	2.75 ± 0.38‴	2.18 ± 0.43***,‴	1.61 ± 0.23***,‴	1.15 ± 0.41***,‴

Note: "*", $P < 0.05$; "**", $P < 0.005$; "***", $P < 0.0005$ in comparison with 6-months-old animals at the same age; "˙", $P < 0.05$; "˙˙", $P < 0.005$; "˙˙˙", $P < 0.0005$ in comparison with parameters of control group at the same age.

during ontogenesis (Table 3).

Discussion

The conducted study revealed significant age-related changes of ultrastructure and morphometric parameters of hepatocytes of rats of both control and experimental groups. The obtained data allow us to assume that observed changes are based on molecular mechanisms associated with oxidative stress, mitochondrial dysfunction, violation of lipid metabolism and processes of cell aging.

In the control group, hepatocytes retained normal ultrastructure up to 12 months: round nuclei, dense mitochondrial matrix, developed endoplasmic reticulum (ER) and unchanged Golgi complex. However, by 18 months, signs of fatty degeneration, swelling of the cytoplasm, mitochondrial polymorphism and vacuolization of the ER appeared. By 24 months, these changes had worsened, with chromatin condensation, an increase in the number of peroxisomes, accumulation of lipid vacuoles, and degenerative changes in mitochondria noted.

These processes may be associated with the accumulation of reactive oxygen species (ROS) and a decrease in the efficiency of antioxidant systems due to a decrease in melatonin levels with age, which is normally concentrated in the mitochondria, preventing oxidative damage to DNA, lipids and proteins. With age, the electron transport chain in mitochondria is disrupted, which leads to increased production of superoxide anion and hydrogen peroxide. This, in turn, causes oxidative modification of proteins and lipids, which explains the degeneration of mitochondrial cristae and fragmentation of their membranes [69].

In the experimental group, similar changes were observed as early as 6 months, and by 18 months the picture corresponded to that of 24-month-old control animals. This indicates premature aging of hepatocytes, possibly due to increased oxidative stress or disruption of DNA repair mechanisms.

The increase in the cross-sectional area of hepatocytes and their nuclei in the experimental group may be a consequence of: cell cycle dysregulation (*e.g.*, activation of the mTOR signaling pathway, which promotes cell hypertrophy); lipid accumulation due to dysfunction of mitochondrial β-oxidation or impaired expression of PPARα (peroxisome proliferator-activated receptor, which regulates lipid metabolism) [70-73].

At the same time, due to the death of hepatocytes, compensatory hypertrophy and cell enlargement are observed due to an age-related increase in cell ploidy [74-78]. In the control group, by 24 months, signs of reparative regeneration (newly formed hepatocytes) were observed, but in the experimental group this process was absent, which is due to the depletion of the pool of hepatocyte precursors and the accumulation of senescent cells secreting proinflammatory cytokines (SASP phenotype), which suppresses regeneration [79, 80].

Swelling of cells due to mitochondrial dysfunction and deterioration of the organ's blood supply, causing hypoxia and edema of hepatocytes, also contribute to an increase in their size [81-83]. It should be noted that in animals of the experimental group, hepatocyte hypertrophy develops significantly faster than in the control group and is expressed more clearly. Such changes are caused by a deficiency of melatonin due to constant illumination [84, 85].

In hepatocytes of rats of both groups by 12 months an increase in the size of the nuclei is noted. At the same time, in the control group a decrease in the size of the nucleus with age is observed, however, in animals of the experimental group the size of the nucleus does not change, which, given the range of sizes of nuclei, is apparently due to compensatory polyploidy [86-88]. The change in NCR, which occurs more rapidly in rats of the experimental group, is due to hypertrophy of hepatocytes and polyploidization of nuclei [89-91]. Chromatin condensation (heterochromatinization) indicates a decrease in transcriptional activity, and deformation of the nuclear membrane is a consequence of the destruction of lamin A/C [92-95].

The decrease in mitochondrial density with age, along with an increase in their size and a decrease in the number

of cristae, indicates an imbalance between mitophagy and mitochondrial biogenesis. This may be due to a decrease in the activity of PGC-1α (the main regulator of mitochondrial biogenesis). A decrease in melatonin content leads to a decrease in SIRT1 deacetylase, which increases the activity of PGC-1α [96, 97]. Accumulation of damaged mitochondria may be observed due to autophagy defects (*e.g.* decreased Parkin/PINK1 protein expression) [98, 99].

The increase in the mitochondrial circularity index in the control group may reflect their swelling due to the opening of the mitochondrial transition pore (MTP), which is characteristic of apoptosis. In the experimental group, this indicator remained low, which may indicate alternative mechanisms of cell death (necroptosis) [100]. The described ultrastructural changes in mitochondria indicate a decrease in ATP synthesis and the accumulation of DNA mutations due to defects in the respiratory chain (complexes I–IV) [101, 102].

The decrease in the number of Golgi complex cisterns and their area in both groups indicates an age-related decrease in the secretory activity of hepatocytes. This may be due to a disruption of calcium homeostasis (excessive accumulation of Ca^{2+} in the cytoplasm leads to fragmentation of the Golgi apparatus) and activation of ER stress due to the accumulation of misfolded proteins, which leads to dilation of the ER cisterns [103, 104].

The conducted study does not touch upon the study of circadian rhythms of mitochondria and the Golgi complex and the relationship with other rhythmic processes, although our previous studies on animals of different ages [63-65, 105-107] show that constant illumination affects the daily rhythms of many processes in the organism of mammals, with most of which the rhythmic work of hepatocytes and their mitochondria is somehow connected, however, the establishment of these connections is a further complex and comprehensive task for future studies.

Conclusions

Thus, the aging of hepatocytes is manifested in accumulation of reactive oxygen species (ROS) due to mitochondrial dysfunction; decreased autophagy (accumulation of damaged organelles); accumulation of mutations: disruption of DNA repair. However, the study shows that the described age-related changes manifest themselves in rats of the experimental group much earlier and in a more pronounced form than in animals of the control group. Thus, these facts allow us to conclude that the effect of constant illumination on the mammalian organism causes accelerated aging of hepatocytes, manifested at the ultrastructural level. Further research should be aimed at studying possible therapeutic strategies, such as modulation of autophagy or the use of antioxidants, to correct the identified disorders.

Declarations

Author Contributions: D.A.A. and M.A.K. conceived the study and designed the experiment; collected the data and performed the analysis; wrote the paper and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The keeping of animals and the experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 18 March 1986). This research was approved by the Bioethical Committee of the Federal State Budgetary Scientific Institution "Avtsyn Research Institute of Human Morphology", protocols No. 38 (10) (14 March 2022).

Data Availability Statement: The data presented in this study are available within the article text, tables, and figures.

Conflicts of Interest: The authors declare no conflicts of interest.

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