УДК 613.2.038-02:617.735] -092.4:599.233.4
https://doi.org/10.20538/1682-0363-2022-4-98-104

Effects of a high-fat, high-carbohydrate diet on the retina of young and old rats

Logvinov S.V.[1], Mustafina L.R.[1], Kurbatov B.K.[2], Naryzhnaya N.V.[2], Varakuta E.Yu.[1], Potapov A.V.[1]

[1] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Cardiology Research Institute, Tomsk National Research Medical Center (NRMC) of the Russian Academy of Sciences*
111a, Kievskaya Str., Tomsk, 634012, Russian Federation

ABSTRACT

Aim. To study the effect of a high-fat, high-carbohydrate diet on retinal morphology of young and old rats in the experiment.

Materials and methods. The study was carried out on male Wistar rats aged 60 and 450 days at the beginning of the experiment. The animals were divided into 4 groups: group 1 (n = 14) included intact rats aged 150 days at the end of the experiment; group 2 (n = 14) encompassed rats (60 days old) fed with a high-fat, high-carbohydrate diet (HFHCD) for 90 days; group 3 (n = 14) included intact rats (450 days old) receiving a standard diet for 90 days; group 4 (n = 14) included rats (450 days old) fed with HFHCD for 90 days. Immunoassay and histology were used in the work.

Results. HFHCD resulted in an increase in glucose concentration in animals of both age groups. In old animals, it caused a pronounced increase in the content of insulin, TGFβ, and fibronectin in the blood serum, neovascularization of outer retinal layers, as well as karyopyknosis and death of neurosensory cells, leading to destruction of photoreceptors and drastic thinning of the outer nuclear and outer plexiform layers. In young rats fed with HFHCD, no pronounced histologic disorders of the retina were noted.

Conclusion. HFHCD enhances age-related retinal changes in old (450-day-old) rats.

Keywords: retinopathy, age-related changes in the retina, high-fat, high-carbohydrate diet

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Cardiology Research Institute of Tomsk NRMC (Protocol No. 201 of 30.07.2020).

For citation: Logvinov S.V., Mustafina L.R., Kurbatov B.K., Naryzhnaya N.V., Varakuta E.Yu., Potapov A.V. Effects of a high-fat, high-carbohydrate diet on the retina of young and old rats. *Bulletin of Siberian Medicine.* 2022;21(4):98–104. https://doi.org/10.20538/1682-0363-2022-4-98-104.

✉ *Mustafina Liliia R.,* lrmustafina@yandex.ru

Влияние высокоуглеводной высокожировой диеты на сетчатку молодых и старых крыс

Логвинов С.В.[1], Мустафина Л.Р.[1], Курбатов Б.К.[2], Нарыжная Н.В.[2], Варакута Е.Ю.[1], Потапов А.В.[1]

[1] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

[2] Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, г. Томск, ул. Киевская, 111а

РЕЗЮМЕ

Цель: изучить в эксперименте влияние высокоуглеводной высокожировой диеты на сетчатку в молодом и старческом возрасте.

Материалы и методы. Исследование проводили на самцах крыс линии Вистар в возрасте 60 и 450 сут в начале эксперимента. Животных распределяли на четыре группы: 1-я (n = 14) – интактные крысы в возрасте 150 сут на момент окончания исследования; 2-я (n = 14) – 150-суточные крысы на момент окончания 90 сут высокоуглеводной высокожировой диеты (ВУВЖД); 3-я (n = 14) – интактные крысы 540-суточного возраста на момент окончания исследования; 4-я (n = 14) – 540-суточные крысы после окончания 90 сут ВУВЖД. В работе использовали иммуноферментный и гистологический методы исследования.

Результаты. ВУВЖД приводила к повышению концентрации глюкозы у животных обеих возрастных групп, а у старых животных вызывала выраженное увеличение содержания инсулина, TGFβ и фибронектина в сыворотке крови, неоваскуляризацию наружных слоев сетчатки, кариопикноз и массовую гибель нейросенсорных клеток, влекущую за собой разрушение слоя палочек и колбочек, резкое истончение наружного ядерного и наружного сетчатого слоев. У молодых крыс, содержавшихся на ВУВЖД, не было отмечено выраженных гистологических нарушений сетчатки.

Заключение. ВУВЖД усиливает возрастные изменения сетчатки у старых (450-суточных) крыс.

Ключевые слова: ретинопатия, возрастные изменения сетчатки, высокоуглеводная высокожировая диета

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Исследование одобрено этическим комитетом НИИ кардиологии Томского НИМЦ (протокол № 201 от 30.07.2020).

Для цитирования: Логвинов С.В., Мустафина Л.Р., Курбатов Б.К., Нарыжная Н.В., Варакута Е.Ю., Потапов А.В. Влияние высокоуглеводной высокожировой диеты на сетчатку молодых и старых крыс. *Бюллетень сибирской медицины.* 2022;21(4):98–104. https://doi.org/10.20538/1682-0363-2022-4-98-104.

INTRODUCTION

Age-related changes in the retina, in particular age-related macular degeneration, cause vision loss in millions of people around the world. At the same time, excessive consumption of high-calorie food, especially in developed countries, leads to diabetic retinopathy, which is the main cause of blindness in the working age population [1, 2]. The mechanisms of development of senile macular degeneration and diabetic retinopathy have some common features [3]. Numerous studies are devoted to retinal aging [4–6], retinal pathology in metabolic syndrome, and type 2 diabetes [7–9], including models with high-fat and high-carbohydrate diets [3, 10, 11]. However, the impact of high-calorie diets on the development of age-related retinal diseases and the structural basis of retinopathy caused by a high-fat, high-carbohydrate

diet (HFHCD) in various age groups is poorly studied. Knowledge about the association of aging with HFHCD is essential for development of prevention and treatment strategies for age-related macular degeneration and diabetic retinopathy. The aim of the study was to investigate the effect of HFHCD on retinal morphology of young and old rats in the experiment.

MATERIALS AND METHODS

The study was carried out on male Wistar rats aged 60 and 450 days. All procedures were carried out in accordance with the European Parliament Directive 2010/63/ EU and the FASEB statement on the principles for the use of animals in research and education. The following experimental groups were formed: group 1 (n = 14) included intact 150-day-old rats fed with a standard diet for 90 days (from 60 days of age); group 2 (n = 14) included 150-day-old rats fed with HFHCD for 90 days (from 60 days of age); group 3 (n = 14) – intact 540-day-old rats fed with a standard diet for 90 days (from 450 days of age); group 4 (n = 14) – 540-day-old rats fed with HFHCD for 90 days (from 450 days of age).

HFHCD contained 16% proteins, 21% fats, and 46% carbohydrates, including 17% fructose and 0.125% cholesterol. The water was replaced with a 20% fructose solution. Rats of groups 1 and 3 (intact animals) received standard rodent feed (24% proteins, 6% fats, 44% carbohydrates) and pure water *ad libitum*. The animals were removed from the experiment by decapitation with preliminary anesthesia with chloralose (100 mg / kg intraperitoneally).

Before decapitation, blood samples were taken, which were centrifuged (for 15 min at 3,000 rpm). Serum samples were stored in a freezer at –70 °C. Glucose concentration in the blood serum was determined by the enzymatic colorimetric method using B-8054 kits (Vector-Best, Russia). The serum levels of insulin (ab100578, Abcam), fibronectin (ab108850, Abcam), and tissue growth factor beta (TGFβ) (ab119558, Abcam) were determined by the enzyme immunoassay. Sample measurements were performed using the Infinite 200 PRO microplate reader (Tecan GmbH, Austria). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as the ratio of insulin * glucose / 22.5. For the histologic examination, the eyeballs were fixed in a 10% buffered formalin solution (BioVitrum LLC, Russia) and embedded in paraffin according to the standard method. Sections of the posterior wall of the eyes were stained with hematoxylin and eosin (BioVitrum LLC, Russia).

Micropreparations were viewed and photographed using the AxioStar Plus light microscope (Carl Zeiss, Germany) at 400x and 1,000x magnification. In 10 random fields of vision in sections for each retina, nuclei undergoing degeneration (%) were counted in the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GL). In the ONL and INL, rows of nuclei were counted.

Statistical data processing was performed using the Statistica 13.0 software (StatSoft Inc., USA). The data obtained were tested for normality of distribution using the Shapiro – Wilk test. Normally distributed data were presented as the mean and the standard error of the mean ($M \pm SEM$). Not normally distributed data were presented as the median and the interquartile range Me (Q_1; Q_3). Homogeneity of multivariate dispersions was tested using the Levene's test. When comparing several independent samples of quantitative data, a two-way ANOVA was used, followed by the Bonferroni correction for normally distributed variables. The nonparametric Kruskal – Wallis test was applied to compare samples with non-normal distribution. The threshold value of the significance level p was equal to 0.05.

RESULTS

Feeding the animals with HFHCD resulted in an increase in the concentration of glucose in the blood serum in the rats of both age groups (Table 1), as well as in a rise in the HOMA-IR values. However, only in old animals, HFHCD affected insulin levels, which led to a more pronounced increase in HOMA-IR values than in young rats (Table 1). Prescription of HFHCD contributed to an increase in the levels of TGFβ and fibronectin in the animals in group 4.

The histologic examination showed that the retinas in the rats of groups 1 and 2 had normal structural architecture of retinal layers (Figure, *a*). However, a small number of nuclei undergoing degeneration were detected in the ONL; they were characterized by diffuse hyperchromic staining and wrinkling (Figure, *b*).

In the retinas of rats in group 3, nuclei in the ONL were rarefied; in areas with the absence of nuclei, they were replaced by radial glial cell processes. Some of the outer and inner segments of the rods and cones were fragmented. In the subretinal space, fragments of the external processes of rods and cones were found, as well as small nuclei undergoing

degeneration, possibly displaced here from the ONL. Single larger nuclei in the subretinal space belonged to macrophages. Karyopyknosis was detected in the pigmented layer (PL) (Figure, *c*).

Degenerating cells were found not only in the ONL, but also in the INL and GL (Figure, *d*). The most pronounced changes were noted in the retinas of rats in group 4. Thus, the ONL was almost completely destroyed and contained a few nuclei arranged in one incomplete row. Most of these nuclei were also undergoing degeneration and in some areas were displaced close to the PL, since the layer of rods and cones was completely destroyed. The PL was unevenly altered and often had pyknotic nuclei. In some areas, it was very thin and destroyed. Among the pigmented cells, hemocapillaries were found containing blood cells in the lumen. Hemocapillaries were also detected in the ONL, which indicates neovasculogenesis. In areas with the destroyed pigmented and photoreceptor layers, the nuclei of photoreceptors were attached to the Bruch's membrane. In these areas, the choriocapillaries were few and narrowed. The INL and GL contained degenerating cells (Figure, *e, f*). The quantitative assessment did not reveal significant differences in 150-day-old animals of groups 1 and 2. However, the content of nuclei undergoing degeneration in the ONL in group 2 showed a distinct upward trend compared with group 1 (Table 1).

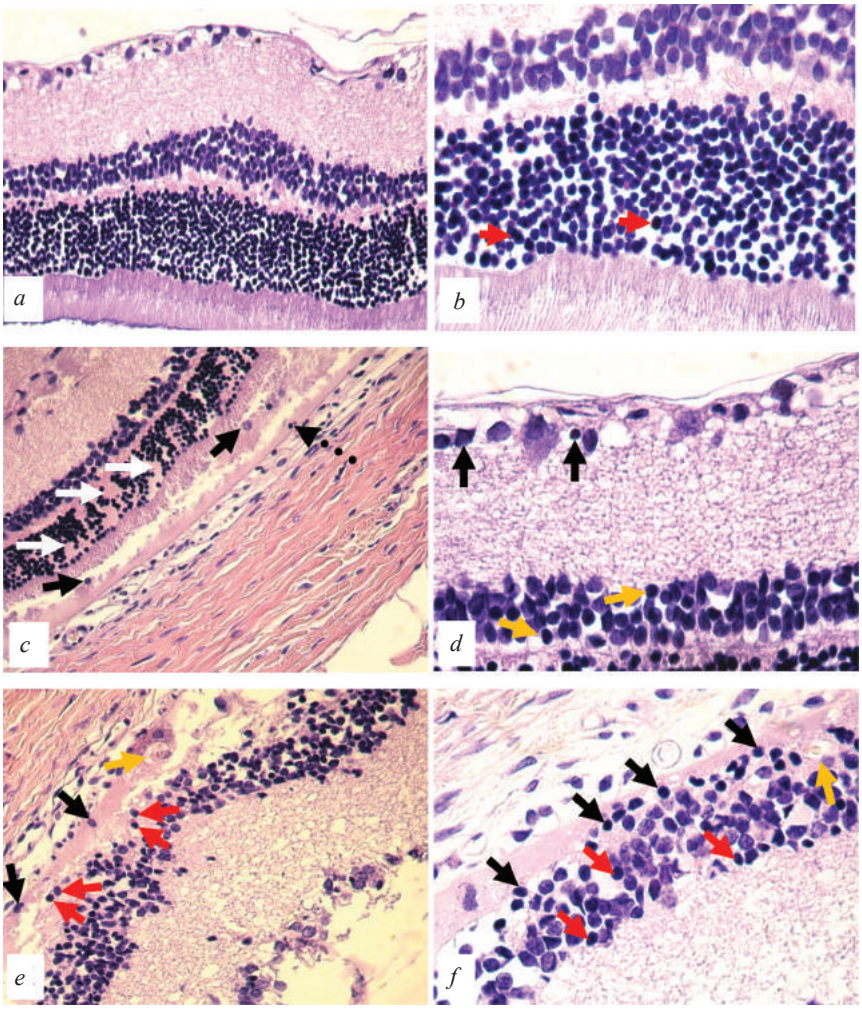

Figure. Histologic changes in the retina of rats associated with age and HFHCD: *a* – usual structural architecture of the retinal layers, group 2; *b* – single nuclei of rods and cones undergoing degeneration (indicated by arrows) in the ONL, group 2; *c* – rarefaction of nuclei in the ONL and their replacement by radial glial cell processes (white arrows), the presence of nuclei in the subretinal space (black arrows), a nucleus undergoing degeneration in the pigmented layer (dashed arrow), group 3; *d* – degenerating cells in the INL (yellow arrows) and the GL (black arrows), group 3; *e* – a blood vessel in the pigmented layer (yellow arrow), pyknosis of pigmented cell nuclei (black arrows), single nuclei undergoing degeneration in the ONL (black arrows) and GL (red arrows), group 4; *f* – nuclei of rods and cones (black arrows), arranged in one incomplete row, close to the pigmented layer, a blood capillary in the subretinal space (yellow arrow), degenerating cells in the INL (red arrows), group 4. Staining with hematoxylin and eosin, x400 (*a*, *c*, *e*); x1,000 (*b*, *d*, *f*)

Table 1

The levels of glucose and insulin in the blood serum of rats of different age fed with HFHCD, *M* ± *SEM*

Parameters	Group 1	Group 2	Group 3	Group 4
Glucose, mM / l	5.4 ± 0.2	7.3 ± 0.2[1]	6.0 ± 0.1	7.7 ± 0.2[1, 3]
Insulin, pM / l	12.2 ± 0.8	18.35 ± 2.57	19.99 ± 2.3	34.7 ± 8.6[1]
HOMA-IR	2.94 ± 0.28	5.97 ± 0.85[1]	5.36 ± 0.61[1]	12.05 ± 3.29[1, 2, 3]
Fibronectin, mg / dl	21.23 ± 1.55	27.58 ± 1.78	29.89 ± 2.38	43.00 ± 3.12[1, 2, 3]
Transforming growth factor β (TGFβ), ng /ml	14.0 ± 3.0	19.1 ± 2.6	35.3 ± 5.2[1]	31.9 ± 4.1[1]

Note: statistical significance of differences with: 1 group 1; 2 group 2; 3 group 3; 4 group 4; two-way ANOVA followed by the Bonferroni correction.

Table 2

Quantitative histologic changes in the retina of albino rats associated with age and HFHCD, *Me* (Q_1; Q_3)

Parameters	Group 1	Group 2	Group 3	Group 4
Nuclei undergoing degeneration in ONL, %	0.25 (0.20; 0.40)	0.80 (0.70; 0.90)	15.05[1] (13.65; 16.65)	88.30[1, 2] (72.80; 92.40)
Number of rows of nuclei in ONL	10.65 (9.70; 11.90)	10.20 (9.30; 10.40)	5.90[1] (4.85; 7.85)	1.40[1, 2] (1.10; 4.90)
Nuclei undergoing degeneration in INL, %	0.15 (0.10; 0.20)	0.10 (0.10; 0.20)	1.95 (1.65; 2.80)	8.10[1, 2] (7.00; 9.30)
Number of rows of nuclei in INL	4.65 (4.20; 4.90)	4.70 (4.60; 5.40)	4.20 (4.05; 4.90)	4.30 (3.70; 5.20)
Ganglion neurons undergoing degeneration	0.50 (0.25; 0.50)	0.75 (0.25; 1.00)	3.00[1] (1.50; 4.00)	9.50[1, 2] (9.00; 11.50)

Note: statistical significance of differences with: 1 group 1; 2 group 2; 3 group 3; 4 group 4; Kruskal – Wallis test.

In rats of group 3, the proportion of degenerating cells in the ONL, INL, and GL significantly increased compared with groups 1 and 2. The most pronounced increase in the proportion of such cells was observed among rods and cones. There was a significant decrease in the number of rows of nuclei in the ONL in group 3 compared with groups 1 and 2. The number of rows of nuclei in the INL did not differ in all 4 groups of animals. In group 4, the trend was similar to that in group 3, but the quantitative changes were significantly more pronounced. A drastic decrease in the number of rows of nuclei in the ONL and an increase in the proportion of nuclei undergoing degeneration among the remaining nuclei were worth noting, which indicates massive death of rods and cones. The proportion of degenerating cells in the INL and GL significantly increased in group 4 compared with group 3, but to a much lesser extent than the proportion of nuclei undergoing degeneration in the ONL.

DISCUSSION

In our opinion, the revealed neovasculogenesis played the most important role in tissue mechanisms of retinopathy in old rats fed with HFHCD. As shown, blood vessels containing blood cells in the lumen appeared in unusual places – among the pigmented cells, in the layer of outer and inner segments, and in the ONL. It is known that invasion of hemocapillaries into layers where they are not present in normal conditions leads to oxidative stress and, as a consequence, death of pigmented and photoreceptor cells, for example, under the combined effect of ionizing radiation and bright light [12]. Under light exposure, new vessels invaded the layer of the outer and inner segments, which was due to expression of VEGF and led to retinal degeneration [13].

C.Toma et al. emphasized the relationship of oxidative stress and neovascularization with changes in choroidal blood flow and degeneration of the pigmented layer and photoreceptor cells in age-related retinopathy [14]. Pathological retinal angiogenesis is associated with expression of VEGF in diabetic retinopathy [15]. The studies carried out by a group of authors [16] showed that HFHCD used in our work causes biochemical disturbances characteristic of the metabolic syndrome.

To date, the pathogenesis of diabetic retinopathy has been characterized in the context of signaling of glucose, insulin, VEGF, and other growth factors, among which TGFβ is of great importance [17]. At an early stage of diabetic retinopathy, TGFβ protects retinal vessels. At later stages, it contributes to the progression of vascular diseases, including proliferative ones [18]. TGFβ is known to control endothelial cell proliferation, cell adhesion, and deposition of the extracellular matrix and plays a key role in the development of diabetic retinopathy [19, 20]. In our study, an increase in the concentration of TGFβ was detected in old rats, which was accompanied

by proliferative changes in the retina in the form of neovasculogenesis in HFHCD. This occurred against the background of an increase in the blood levels of glucose and insulin and a rise in the HOMA-IR value. In old rats fed with HFHCD, the plasma level of fibronectin also increased. It is known that activation of fibronectin in endothelial cells and retinal pericytes is caused by TGFβ, which leads to thickening of capillary basement membranes and impairs permeability of the blood – retina barrier in diabetic retinopathy [21, 22]. Therefore, the changes in the studied growth factors involved in the pathogenesis of retinopathy corresponded to neovasculogenesis and other vascular diseases that play a key role in age-related and HFHCD-related structural retinal abnormalities. This makes determination of these biochemical parameters in the diagnosis and treatment of senile macular degeneration and diabetic retinopathy clinically significant.

CONCLUSION

Our study showed that HFHCD enhances age-related changes in the retina in old rats. Aging and HFHCD exhibit synergism in damaging photoreceptor cells, causing their karyopyknosis and massive death, which results in destruction of the layer of outer and inner segments, drastic thinning of the ONL to 1–2 rows of nuclei, and thinning of the outer retinal layer. Death of rods and cones in the retina is accompanied by focal destructive changes in pigmented cells and a decrease in the number of choriocapillaries in the foci of destruction. HFHCD causes an increase in the serum levels of TGFβ and fibronectin and neovascularization in the outer retinal layers, which, in our opinion, plays a key role in the mechanisms of their destruction. The inner retinal layers were affected to a lesser extent than the photoreceptor and pigmented layers in old rats fed with HFHCD. In young rats, HFHCD did not cause pronounced histologic abnormalities in the retina.

REFERENCES

1. Dai W., Dierschke S.K., Toro A.L., Dennis M.D. Consumption of a high fat diet promotes protein O-GlcNAcylation in mouse retina via NR4A1-dependent GFAT2 expression. *Biochim. Biophys. Acta Mol. Basis Dis.* 2018;1864(12):3568–3576. DOI: 10.1016/j.bbadis.2018.09.006.
2. Hammoum I., Mbarek S., Dellaa A., Dubus E., Baccouche B., Azaiz R. et alStudy of retinal alterations in a high fat diet-induced type ii diabetes rodent: Meriones shawi. *Acta Histochem.* 2017;119(1):1–9. DOI: 10.1016/j.acthis.2016.05.005.
3. Vidal E., Lalarme E., Maire M.-A., Febvret V., Grégoire S., Gambert S. et al. Early impairments in the retina of rats fed with high fructose/high fat diet are associated with glucose metabolism deregulation but not dyslipidaemia. *Sci. Rep.* 2019;9(1):5997. DOI: 10.1038/s41598-019-42528-9.
4. Lee K.S., Lin S., Copland D.A., Dick A.D., Liu J. Cellular senescence in the aging retina and developments of senotherapies for age-related macular degeneration. *J. Neuroinflammation*. 2021;18(1):32. DOI: 10.1186/s12974-021-02088-0.
5. Nag T.C., Wadhwa S. Ultrastructure of the human retina in aging and various pathological states. *Micron*. 2012;43(7):759–781. DOI: 10.1016/j.micron.2012.01.011.
6. Kovács-Valasek A., Etelka Pöstyéni E., Dénes V., Mester A., Sétáló G.Jr., Gábriel R. Age-related alterations of proteins in albino Wistar rat retina. *Cells Tissues Organs*. 2021;210(2): 135–150. DOI: 10.1159/000515447.
7. Karaca C., Karaca Z. Beyond hyperglycemia, evidence for retinal neurodegeneration in metabolic syndrome. *Invest. Ophthalmol. Vis. Sci.* 2018;59(3):1360–1367. DOI: 10.1167/iovs.17-23376.
8. Godisela K.K., Reddy S.S., Kumar C.U., Saravanan N., Reddy P.Y., Jablonski M.M. et al. Impact of obesity with impaired glucose tolerance on retinal degeneration in a rat model of metabolic syndrome. *Mol. Vis.* 2017;23:263–274.
9. Yau P.L., Kim M., Tirsi A., Convit A. Retinal vessel alterations and cerebral white matter microstructural damage in obese adolescents with metabolic syndrome. *JAMA Pediatr*. 2014;168(12):e142815. DOI: 10.1001/jamapediatrics.2014.2815.
10. Thierry M., Pasquis B., Buteau B., Fourgeux C., Dembele D., Leclere L. et al. Early adaptive response of the retina to a pro-diabetogenic diet: Impairment of cone response and gene expression changes in high-fructose fed rats. *Exp. Eye. Res.* 2015;135:37–46. DOI: 10.1016/j.exer.2015.04.012.
11. Paz M.C., Barcelona P.F., Subirada P.V., Ridano M.E., Chiabrando G.A., Castro C. et al. Metabolic syndrome triggered by fructosedDiet impairs neuronal function and vascular integrity in ApoE-KO mouse retinas: Implications of autophagy deficient activation. *Front. Cell Dev. Biol.* 2020;8:573987. DOI: 10.3389/fcell.2020.573987.
12. Logvinov S.V., Potapov A.V. Structural changes to the retina in combined exposure to light and X-ray. *Morfologiia.* 2000;117(1):19–23 (in Russ.).
13. Tisi A., Parete G., Flati V., Maccarone R. Up-regulation of pro-angiogenic pathways and induction of neovascularization by an acute retinal light damage. *Sci. Rep.* 2020;10(1):6376. DOI: 10.1038/s41598-020-63449-y.
14. Toma C., De Cillà S., Palumbo A., Garhwal D.P., Grossini E. Oxidative and Nitrosative Stress in Age-Related Macular Degeneration: A Review of Their Role in Different Stages of Disease. *Antioxidants (Basel)*. 2021;10(5):653. DOI: 10.3390/antiox10050653.
15. Rezzola S., Loda A., Corsini M., Semeraro F., Annese T., Presta M. et al. Angiogenesis-Inflammation Cross Talk in Diabetic Retinopathy: Novel Insights From the Chick Embryo Chorioallantoic Membrane/Human Vitreous Platform. *Front. Immunol.* 2020;11:581288. DOI: 10.3389/fimmu.2020.581288.
16. Birulina J.G., Ivanov V.V., Buyko E.E., Trubacheva O.A., Petrova I.V., Grechishnikova A.Yu., Nosarev A.V., Gusako-

va S.V. Effects of a high-fat, high-carbohydrate diet on blood cells of rats. *Bulletin of Siberian Medicine.* 2021;20(3):6–12 (in Russ.). DOI: 10.20538/1682-0363-2021-3-6-12.
17. Wheeler S.E., Lee N.Y. Emerging roles of transforming growth factor β signaling in diabetic retinopathy. *J. Cell. Physiol.* 2017;232(3):486–489. DOI: 10.1002/jcp.25506.
18. Huang H. Pericyte-endothelial interactions in the retinal microvasculature. *Int. J. Mol. Sci.* 2020;21(19):7413. DOI: 10.3390/ijms21197413.
19. Goumans M.-J., Valdimarsdottir G., Itoh S., Rosendahl A., Sideras P., Ten Dijke P. Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *EMBO J.* 2002;21(7):1743–1753. DOI: 10.1093/emboj/21.7.1743.
20. Gacka M., Adamiec J. The role of transforming growth factor-beta in the pathogenesis of diabetic retinopathy. *Przegl. Lek.* 2006;63(5):296–298. (In Polish).
21. Van Geest R.J., Klaassen I., Vogels I.M., Van Noorden C.J., Schlingemann R.O. Differential TGF-{beta} signaling in retinal vascular cells: a role in diabetic retinopathy? *Invest. Ophthalmol. Vis. Sci.* 2010;51(4):1857–1865. DOI: 10.1167/iovs.09-4181.
22. Chaqour B., Karrasch C. Eyeing the extracellular matrix in vascular development and microvascular diseases and bridging the divide between vascular mechanics and function. *Int. J. Mol. Sci.* 2020;21(10):3487. DOI: 10.3390/ijms21103487.

Acknowledgments

The authors are grateful to Mukhomedzyanov A.V., Gorbunov A.S., and Voronkov N.S. for help in conducting the experiment, as well as to Ivanov V.V. and Birulina Yu.G. for methodological assistance in developing a diet.

Authors contribution

Logvinov S.V. – conception, drafting of the Morphology section of the article, substantiation of the manuscript, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication. Mustafina L.R. – carrying out of the morphologic studies, work on the illustrations, interpretation of the morphologic findings. Kurbatov B.K. – design of the study, carrying out of the studies, statistical analysis and interpretation of the results. Naryzhnaya N.V. – conception and design, carrying out of the studies, statistical processing of the results, drafting of the article, substantiation of the manuscript. Varakuta E.Yu. – carrying out of the morphologic studies, interpretation of the data. Potapov A.V. – conception, drafting of the article, substantiation of the manuscript, critical revision of the manuscript for important intellectual content.

Authors information

Logvinov Sergey V. – Dr. Sci. (Med.), Professor, Head of the Histology, Embryology and Cytology Division, Siberian State Medical University, Tomsk, s_logvinov@mail.ru, https://orcid.org/0000-0002-9876-6957

Mustafina Liliia R. – Dr. Sci. (Med.), Associate Professor, Professor, Histology, Embryology and Cytology Division, Siberian State Medical University, Tomsk, lrmustafina@yandex.ru, https://orcid.org/0000-0003-3526-7875

Kurbatov Boris K. – Junior Researcher, Laboratory for Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, bobersanker@gmail.com, https://orcid.org/0000-0001-9603-822X

Naryzhnaya Natalia V. – Dr. Sci. (Med.), Leading Researcher, Laboratory for Experimental Cardiology, Cardiology Research Institute, Tomsk NRMC, Tomsk, natalynar@yandex.ru, https://orcid.org/0000-0003-2264-1928

Varakuta Elena Yu. – Dr. Sci. (Med.), Associate Professor, Head of the Division of Human Anatomy with a Course in Topographic Anatomy and Operative Surgery, Siberian State Medical University, Tomsk, varelen@rambler.ru, https://orcid.org/0000-0003-3173-5336

Potapov Aleksey V. – Dr. Sci. (Med.), Professor, Histology, Embryology and Cytology Division, Siberian State Medical University, Tomsk, potalex@mail.ru, https://orcid.org/0000-0002-0468-3959

(✉) **Mustafina Liliia R.,** lrmustafina@yandex.ru

Received 19.01.2022;
approved after peer review 25.02.2022;
accepted 09.06.2022