Comparison of neuroglobin expression in the retina of Tibetan sheep and domestic sheep*

XIAOHUA DU¹, XIA LIU², JAMES BLACKAR MAWOLO²

¹College of Veterinary Medicine, Gansu Agricultural University,

²College of Life Science and Technology,
Lanzhou City, Gansu Province, 730070, People's Republic of China

Received 27.07.2020

Accepted 11.11.2020

Du X., Liu X., Mawolo J. B.

Comparison of neuroglobin expression in the retina of Tibetan sheep and domestic sheep

Summary

Neuroglobin (Ngb) is a member of the globin member that is mainly expressed in the brain and retina. The expression in the retina is greater than it is in the brain because of the high demand for oxygen by the retina. This protein reversibly binds oxygen and is involved in oxygen transport or protection against oxidative stress. The study aims to explore the pattern of Ngb expression in Tibetan sheep (*Ovis ammon or argali*) living in a plateau region and domestic sheep (*Ovis aries*) dwelling on low land. The study also provides detailed insight into the morphological characteristics of Ngb distribution closely related to oxygen metabolism in the retina of Tibetan and domestic sheep. Immunohistochemical staining procedures were performed to detect the Ngb protein expression in the retinae of both sheep, while the Inter's integrated performance primitives (IPP) semi-quantitative analysis was employed to compare the quantities of Ngb expressed in the retinae of the two types of sheep. The outer nuclear layer and the outer segment of the photoreceptor showed a negative Ngb expression, while a weak positive expression was observed in the inner core layer and ganglion cell layer, while a strong positive expression was found in the other layers. The independent T-test revealed that the expression intensity in the nerve fiber layer, inner and outer plexus layers, inner segment of the photoreceptor, and superior pigment cortex were significantly higher in the Tibetan sheep as compared to the domestic sheep. The overall Ngb expression in the Tibetan sheep was higher than the domestic sheep. The study emphasized that the different Ngb expression in the Tibetan and domestic sheep may influence their adaptation to the respective environments. The stronger Ngb expression in the retina of Tibetan sheep may indicate that Ngb is closely related to the oxygen metabolism of these animals and has a function during oxygen transport.

Keywords: immunohistochemistry, neuroglobin, retina, sheep, oxygen

Neuroglobin (Ngb) is a kind of protein that has a high affinity for oxygen and is mainly distributed in the central nervous system of animals (2). This protein can bind oxygen reversibly, and promote the metabolism of oxygen and survival of nerve cells under the condition of hypoxia and ischemia (1-4). Research has revealed that Ngb is expressed in multiple tissues, especially the retina tissues where the concentration of expression is 100 times greater than the brain tissues (5). Exhibiting visual process, the vertebrate retina structure consumes a large amount of oxygen making the retina the highest oxygen-consuming tissue (6-8), which has significant functions in the process of metabolism. Hundahl et al. reported that Ngb is expressed

in the murine retina, mainly in the ganglion cell and the inner nuclear layers co-expressing melanopsin and tyrosine hydroxylase (9) while Schmidt et al. (16) examined the vascular retinae of the mouse and reported that Ngb was highly expressed in the inner segments of photoreceptor cells, the outer and inner plexiform layers, and the ganglion cell layer. These regions consume more oxygen so the expression of Ngb was higher as compared to other tissues. A study conducted by Ostojic et al. revealed that Ngb was observed in the ganglion cell layer, inner nuclear layer, inner and outer plexiform layers, and retinal pigment epithelium in the human retina (13). Despite these reported results, limited references report Ngb expression in the retinae of Tibetan and domestic sheep. As one of three original breeding animals in China, Tibetan sheep have been living in the high-altitude Hezuo and Qinghai-Tibet

* The research project was funded by the National Natural Science Foundation of China (Grant No. 31760305). The funders had no role in the study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Plateau environment for a long period and have adapted to a high temperature and low oxygen environment. The current study performed immuno-histochemical staining procedures (SP) and inter's integrated performance primitives (IPP) semi-quantitative analysis to detect Ngb expression and compared the expression pattern in the retinae of both sheep. The study also provided Ngb morphological data and detailed insight into the complex physiological mechanism.

Material and methods

Animals and setting. All Laboratory proceedings were reviewed and approved by the Animal Ethics and Welfare Committee of Gansu Agricultural University (GAU-AEWC-2019-109). A total of 10 healthy sheep, 5 Tibetan sheep (6 years), and 5 domestic sheep (5 years) were used in the study. The sheep specimens were extracted within one (1) week. The Tibetan samples were collected for four (4) days, while domestic sheep samples were collected within three (3) days. Animals were retrieved one after another from their housing area and tested before taken to the slaughterhouse. Specimens were collected in the morning and evening hours during July. All sheep involved in this study were taken from the Hezuo and Lanzhou cities in the Gansu Province of China. The Hezuo city is regarded as the administrative seat of the Tibetan Autonomous Prefecture in Gansu Province of Western China. With an elevation of approximately 3,000 meters (9,800 ft), The city has an alpine subarctic climate, extremely cold, dry winters, and mild summers. The monthly mean temperature in January, which is the coldest month, is -9.9°C (14.2°F), the warmest month is September with 12.8°C (55.0°F). The annual mean is 2.40°C (36.3°F). Most of the annual precipitation occurs between May to September. The monthly sunshine ranges from 44%-1% during June and September. In December, the annual bright sunshine is 2,370 hours. Lanzhou city is known for its yellow river and situated in the temperate zone with a semi-arid climate during hot summer and extremely cold and dry winter. The average monthly temperature ranges from -5.3°C (22.5°F) in January to 22.4°C (72.3°F) in July.

Samples extraction and preparation. All animals were fed, rested, and retrieved one at a time from their living area. After slaughter, the eyeballs were quickly removed and placed in Davidson's fixation solution for 24 hours and stored at 4°C . The sections were rinsed three times (five minutes each) in PBS (0.01 mol/L , $\text{pH} = 7.2$), treated with 0.125 trypsin antigen (A100458, Shanghai, China) repair for 30 minutes, and then rinsed in PBS. Each slice was incubated for ten minutes at 20°C room temperature with a drop of 30 mL/L hydrogen peroxide blocking solution and then rinsed in PBS. Normal rabbit serum was added and the sections were incubated at room temperature for 15 minutes. A volume of the corresponding primary antibody was added, and the sections were incubated at 37°C for two hours, followed by three washes with PBS. Next, PBS was removed, and the corresponding second antibody was added for 15 minutes at 37°C before rinsing the sec-

tions three times with PBS and subsequent immersion in PBS. A drop of Streptomyces biotin peroxidase solution was added to each slice. This was incubated at 37°C for 15 minutes before rinsing the sections with PBS. PBS was then removed and DAB mixed with PBS was added. The color was observed and controlled under a microscope. Distilled water was added to terminate the staining. The sections were then re-dyed with hematoxylin, dehydrated, encapsulated with transparent and neutral gum, photographed, and finally analyzed under a microscope. The negative control sections were stained with PBS without the primary antibody. The Nikon Eclipse 80i micro camera system was employed to capture specimen images with consent shooting parameters and the Image-Pro Plus 6.0 was introduced to acquire, count, measure, and classify the optical density of each retina layer. The Trypsin (A100458) and rabbit anti-Ngb polyclonal antibody (D221024) were purchased from Shanghai sangon biological Co. LTD while the immunohistochemical staining kit (sp-0041) and DAB color rendering kit (DA1015) were purchased from Beijing solabao Technology Co. LTD.

Immunohistochemical coloration. Streptavidin Peroxidase (SP) method was used for immunohistochemical staining while PBS was used as a negative control instead of the primary antibody. 100 I DAB color rendering solution was added to each section and observed under the optical microscope (Olympus BX51) to control color rendering. The color rendering period for each section was consistent. After the reaction was stopped by distilled water, the slices were re-dyed, dehydrated, and transparent by hematoxylin and sealed.

Origin of neuroglobin antibody. The Ngb antibody was raised in rabbits and species reactive in humans, mice, and rats. The Immunogen of this protein was the fusion of the protein of human Ngb while the Immunogen species is the *Homo sapiens* (human). It is non-conjugated with the isotype of the IgG and antigen affinity purification method. Its concentration was $100\text{ }\mu\text{l}$ (Catalog # AM4302) and the buffer was -20°C , $\text{Ph } 7.4$ PBS, 0.05% NaN_3 , 40% Glycerol from liquid, and tested in IHC.

Expression intensity measurement. We employed a color deconvolution technique to unmix the pure DAB, hematoxylin stained areas leaving a complimentary image. The pixel intensities of separated DAB or hematoxylin images range from 0 to 200. Value 0 represents the darkest shade of the color while 200 represent the lightest shade of the color in the image. In order to assign an automated score by judging the pure DAB staining pattern, a histogram profile of every image i.e. the number of pixels of a specific intensity value vs. their respective intensity was raised using imageJ standard program feature. In the histogram profile, we categorized pixel intensity ranges from 0-60 for a score value of 3+, 61-120 for 2+, 121-170 for 1+ and 171-230 for 0. We excluded the pixel intensity values above 200 as the abundant fatty tissues in the retina tissue sections were found to contribute in the range of 20-200. A macro was developed and plugged in the ImageJ program to receive automated counting of the numbers of pixels in the four different intensity zones.

Statistical analysis. Data analysis was performed using Statistical Package Social Sciences (SPSS) 8.0 (1998) to conduct one-way ANOVA, multiple comparisons (Tamhane ST2 method), and the Independent Sample T-test was employed to evaluate the significance level. $P < 0.05$ was considered significant.

Results and discussion

Expression of Ngb in the retina of Tibetan sheep and domestic sheep. The immunohistochemical results showed that Ngb was distributed in the retinae of Tibetan and domestic sheep, and their distribution patterns are identical (Fig. 1A, 1B). Ngb positive expression mainly exists in the inner membrane, retinal nerve fiber layer, ganglion cell layer, inner plexiform layer, kernel layer, outer plexiform layer, outside membrane, photoreceptor inner segment, and pigment in the cortex. The outer nuclear layer and photoreceptor outer segments did not show Ngb positive expression (Fig. 1C, 1D). Table 1 recorded the expression intensity found in the retina tissues. The ganglion cell axons penetrate the optic nerve fiber layer, and there exist a large number of Ngb positive coloration. Ngb expression in the nodal cell layer was mainly located in the cytoplasm, and the positive substances appeared in the nodal cell bodies of different sizes, but no positive expression was observed in the nucleus (Fig. 1E, 1F). Ngb expression in the inner plexus was mainly located in the axons and dendrites of bipolar cells. Cell bodies of various types are common in the kernel layer, and Ngb positive color is mainly located in the cytoplasm of each cell, while no positive expression is found in the nucleus (Fig. 1G, 1H). The expression of Ngb in the outer plexus was situated in the axons of photoreceptor cells, dendrites of bipolar cells, horizontal cells, and protuberances of cells without a long process. Ngb expression found in the pigment epithelial layer was observed in the cytoplasm of pigment epithelial cells (Fig. 1I).

Immunohistochemical analysis. The semi-quantitative results showed that the optic nerve fiber

layer, inner and outer plexiform layer, photoreceptor inner segment, and pigments in the cortex are strongly positive (++), while the ganglion cells layer and kernel layer are weak positive (+) and the outer nuclear layer and photoreceptor outer phase were negatively expressed (–), ($P < 0.05$).

As discovered by Burmester et al. (2), Ngb is the third oxygenated globulin protein found in mammals, which mainly exists in the central nervous system. This monomer protein has a high affinity for oxygen elements, similar to the first two globulins, hemoglobin (Hb) and myoglobin (Mb). Because of its ability to reversibly bind oxygen, researchers have suggested that Ngb has key functions in the energy metabolism of the nervous system and the compensatory function of nerve cells in hypoxic environments. Additionally, several studies have been conducted on the Ngb expression patterns, physiological functions, and distribution in various animal tissues. At present, references have shown that Ngb expression is found in the human, rat, poultry, and zebrafish retinae (6, 12-15), and the concentration is mainly located in the nodal cell layer, plexus layer, and the ellipsoid region of the segment within the photoreceptor. During the localization of Ngb sub-cells, a large portion of expression was found in pericytes of the inner and outer nuclear layer of the rats and other mammal retinae (10). The terminals in the plexus layer, which contained the aggregation area of mitochondria indicate that Ngb was closely related to the oxygen metabolism of nerve cells (13).

The current study showed similarities and comparative differences to these reported results. Except for the photoreceptor outer segments and an outer nuclear layer that showed negative expression, Ngb was positively expressed in all other layers of the Tibetan and domestic sheep retinae. The comparative differences between the present reports and published data might be the different experimental procedures and species used. The strong positive expression of Ngb in the Tibetan and domestic sheep retinae suggest that Ngb may play an important function in the mechanism of an

Tab. 1. Comparison of Ngb expression between Tibetan sheep and domestic sheep retinae

Retina cell layers	Tibetan sheep	Expression intensity	Domestic sheep	Expression intensity
Nerve fiber layer (NFL)	$0.109 \pm 0.011^{**\Delta}$	++	$0.084 \pm 0.021^{**\Delta}$	++
Ganglion cell layer (GCL0)	$0.027 \pm 0.016^{**}$	+	$0.032 \pm 0.011^{**}$	+
Inner plexiform layer (IPL)	$0.069 \pm 0.012^{**\Delta}$	++	$0.052 \pm 0.009^{**\Delta}$	++
Inner nuclear layer (INL)	$0.018 \pm 0.003^{**}$	+	$0.014 \pm 0.002^{**}$	+
Outer plexiform layer (OPL)	$0.064 \pm 0.009^{**\Delta\Box}$	++	$0.044 \pm 0.005^{**\Delta\Box}$	++
Outer nuclear layer (ONL0)	$0.002 \pm 0.001^*$	–	$0.002 \pm 0.001^*$	–
Photoreceptor inner segments (IS)	$0.126 \pm 0.030^{**\Delta\Box}$	++	$0.062 \pm 0.008^{**\Delta\Box}$	++
Photoreceptor outer segments (OS)	$0.003 \pm 0.002^*$	–	$0.004 \pm 0.001^*$	–
Pigmented epithelial layer (PEL)	$0.184 \pm 0.035^{**\Delta}$	++	$0.129 \pm 0.038^{**\Delta}$	++

Explanations: * compared with the negative control group, $P > 0.05$; ** compared with negative control group $P < 0.05$; Δ intra-group comparisons of the positive control group $P < 0.05$; $\Box$ comparison between Tibetan sheep and sheep positive control group

action potential. Most nutrients of the mammals' retinal are provided by the central retinal blood vessels and circulation of choroid blood capillary. Oxygen does not directly reach the retinal photoreceptor cell layer, so

they diffuse through the pigment in the cortex and photosensitive space (9-10). Ngb is suggested to play key functions in the promotion of respiratory chain transfer or short oxygen storage. In each cell layer with strong

Fig. 1A-I. Expression of neuroglobin in the retina of Tibetan sheep and domestic sheep

Fig. 1A. Expression of Ngb on the wall of the Tibetan sheep eye. Immunohistochemical staining, scale = 200 μm. R: retina, C: choroid, S: sclera

Fig. 1B. Ngb expression in the retina of Tibetan sheep. Immunohistochemical staining, scale = 20 μm. NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, IS: the internal segment of photoreceptor, OS: the outer segment of photoreceptor, PEL: pigment superior cortex

Fig. 1C. Expression of Ngb in Tibetan sheep optic disc. Immunohistochemical color rendering, scale = 20 μm. ↑: show the optic nerve

Fig. 1D. Ngb expression on the ocular wall of sheep. Immunohistochemical staining, scale = 100 μm, R: retina, S: sclera

Fig. 1E. Expression of Ngb in the sheep retina. Immunohistochemical color rendering, scale = 20 μm, NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, IS: the internal segment of photoreceptor, OS: the outer segment of photoreceptor, PEL: pigment superior cortex

Fig. 1F. Ngb expression in sheep optic disc. Immunohistochemical color rendering, scale = 20 μm. ↑: show the optic nerve

Fig. 1G. Structure of Tibetan sheep retina. H&E staining scale = 20 μm, ILM: inner boundary membrane, NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, OLM: external membrane, RCL: rod and cone layer, PEL: pigment superior cortex

Fig. 1H. Structure of sheep retina. H&E staining scale = 20 μm, ILM: inner boundary membrane, NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, OLM: external membrane, RCL: rod and cone layer, PEL: pigment superior cortex

Fig. 1I. Expression of Ngb in the retina of Tibetan sheep. The negative control, scale = 20 μm. ILM: inner boundary membrane, NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, OLM: external membrane, IS: the internal segment of photoreceptor, OS: the outer segment of photoreceptor, PEL: pigment superior cortex

Ngb positive expression, optic ganglion cells serve as the starting point for receiving integrated visual signals from the retina (16), and the upward formation of the nerve fiber layer requires the maintenance of dark current to achieve signal transmission. Downlink requires receiving signal input from the inner and outer plexus layer through bipolar cells and pod-free cells to form a large number of synaptic structures (10).

However, in the phase of photoreceptor, photoreceptor cells need to complete the physiological function of visual formation through complex signal conversion, and the completion of these processes requires a large amount of energy consumption. The strong positive expression of Ngb reveals its close relationship with the physiological activity of energy metabolism. Tibetan sheep belong to plateau animals, which are tolerant of high altitudes and a low oxygen environment for a long period. Under normal physiological conditions, the utilization rate of oxygen is higher in the Tibetan sheep than the domestic sheep and Ngb performs an important function in the distribution pattern between both sheep retinas. The experimental results in the current study showed that strong positive Ngb expression in the Tibetan sheep was significantly higher than the domestic sheep in each cell layer and aid in the promotion of oxygen metabolism. This report was consistent with Yan et al. (19) studies which reported that strong positive Ngb expression promotes oxygen metabolism.

A report about the turtle's brain showed that Ngb expression in the retina of a hypoxic environment surge to slow decline (18). The result further stated that due to the experimental treatment of hypoxia and hypoxia-reoxygenation in the turtle brain, Ngb expression could significantly increase. The different experimental species showed that hypoxic conditions could directly lead to an increase of Ngb expression, whether in plateau animals living in a long-term hypoxic environment or experimental animal models of hypoxic conditions. Despite the significant differences, which to some extent reflect the physiological level in Tibetan and domestic sheep.

It is also worth mentioning that the results of Ngb in each positive expression layer of the retina were higher in the Tibetan sheep than the domestic sheep. Researchers determined the large presence of Ngb in the retina of humans and dogs with the use of fluorescent immunity and western blot tests (10-12). The results led to speculation that Ngb has an important function in scavenging reactive oxygen radicals, transporting oxygen, protective effect on retinal ischemia, and hypoxia-related diseases. Lechaue et al. (6) also performed eyes vitreous injection of Ngb expression in the rat inhibitors and reported that this led to the sharp decline of Ngb in the rabbit retina and respiratory chain complex I and III inactivations, optic ganglion cells death, and visual damage. This emphasized that Ngb is involved in maintaining the integrity of the respira-

tory chain mitochondrial protein. A study reported by Chan et al. showed that the rat model of Ngb excessive expression can reduce retinal cell apoptosis, maintain retinal integrity and have an obvious protective effect (2), while another reference explained the expression of Ngb may be regulated by testosterone and other hormones (19) and have a protective effect on nerve cells (20), but the mechanism of its physiological function remains unclear. Reports from Roberts et al. and Zhang et al., (15, 22) confirmed that although the protein structure of Ngb cannot transport a large amount of oxygen, it does play a role in the transport of oxygen in special structures such as the retina. The current study and these relevant data prove the important role Ngb plays in the retina.

The current research provides morphological (in relationship with anatomy) data of Ngb expression in the retinas of Tibetan and domestic sheep and further explores the diverse Ngb expression among animals at different altitudes. The high expression of Ngb in the retina of Tibetan sheep and sheep is similar to that of other species, proving its importance in life activities, especially close functions to oxygen metabolism of animals under a natural selection of high altitude. Furthermore; the results set a theoretical foundation for further insight into Ngb role in specific physiological mechanisms.

References

- Blank M., Kiger L., Thielebein A., Gerlach F., Hankeln T., Marden M. C., Burmester T.: Oxygen supply from the Bird's eye perspective. *J. Biol. Chem.* 2011, 30, 26507-26515, doi: 10.1074/jbc.M111.224634.
- Burmester T., Weich B., Reinhardt S., Hankeln T.: A vertebrate globin expressed in the brain. *Nature* 2000, 407, 520-523, doi: 10.1038/35035093.
- Chan A., Saraswathy S., Rehak M., Ueki M., Rao N. A.: Neuroglobin protection in retinal ischemia. *Investig. Ophthalmol. Vis. Sci.* 2012, 53, 704-711, doi: 10.1167/iovs.11-7408.
- Guidolin D., Tortorella C., Marcoli M., Agnati L. F.: Neuroglobin, a factor playing for nerve cell survival. *J. Mol. Sci.* 2016, 10, 1813-1817, doi: 10.3390/ijms17111817.
- Hundahl C. A., Fahrenkrug J., Luuk H., Hay-Schmidt A., Hannibal J.: Restricted expression of neuroglobin in the mouse retina and co-localization with melanopsin and tyrosine hydroxylase. *Biochem. Biophys. Res. Commun.* 2012, 425, 100-106, doi: 10.3390/cells1041133.
- Lechaue C., Augustin S., Cwerman-Thibault H., Bouatia A., Forster V., Celier C., Rustin P., Mardin M. C., Sahel J. A., Corral-Debrinski M.: Neuroglobin involvement in respiratory chain function and retinal ganglion cell integrity. *Biophys. Acta* 2012, 12, 2261-2273, doi: 10.1016/j.bbamer.2012.09.009.
- Lechaue C., Rezaei H., Celier C., Kiger L., Corral-Debrinski M., Noinville S., Chauvierre C., Hamdane D., Pato C., Marden M. C.: Neuroglobin and prion cellular localization: investigation of a potential interaction. *Mol. Biol.* 2009, 388, 68-977, doi: 10.1016/j.jmb.2009.03.047.
- Li Y., Liu H., Tong Y.: Immunohistochemical study on the distribution of brain globin in rat eyeball. *Ophthalmic Res.* 2006, 24, 461-464, doi: 10.1016/S0006-8993(01)03360-1.
- Liu J., Yu Z., Guo S., Lee S. R., Xing C., Zhang C., Gao Y., Nicholls D. G., Lo E. H., Wang X.: Effects of neuroglobin overexpression on mitochondrial function and oxidative stress following hypoxia/reoxygenation in cultured neurons. *Neurosci. Res.* 2009, 87, 164-170, doi: 10.1002/JNR.21826.
- Milton S. L., Nayak G., Lutz P. L., Prentice H. M.: Gene transcription of neuroglobin is upregulated by hypoxia and anoxia in the brain of the anoxia-tolerant turtle *Trachemys scripta*. *J. Biomed. Sci.* 2006, 4, 509-514, doi: 10.1007/s11373-006-9084-8.
- Morozov A. N., Roach J. P., Kotzer M., Chatfield C. C.: A possible mechanism for redox control of human neuroglobin activity. *J. Am. Chem. Soc.* 2014, 54, 1997-2003, doi:10.1021/C15002108.

12. Ostojic J., Grozdanic S. D., Syed N. A., Hargrove M. S., Trent J. T., Kuehn M. H., Kwon Y. H., Kardon R. H., Sakaguchi D. S.: Neuroglobin and cytoglobin distribution in the anterior eye segment: a comparative immunohistochemical study. *J. Histochem. Cytochem.* 2008b, 56: 863-872. DOI:10.1369/jhc.2008.951392.
13. Ostojic J., Grozdanic S. D., Syed N. A., Hargrove M. S., Trent J. T., Kuehn M. H., Kwon Y. H., Kardon R. H., Sakaguchi D. S.: Patterns of distribution of oxygen-binding globins, neuroglobin and cytoglobin in the human retina. *Arch. Ophthalmol.* 2008a, 126, 1530-1536, doi: 10.1001/archophth.126.11.1530.
14. Ostojic J., Sakaguchi D. S., de Lathouder Y., Hargrove M. S., Trent J. T., Kwon Y. H., Kardon R. H., Kuehn M. H., Belts D. M., Grozdanic S.: Neuroglobin and cytoglobin: oxygen-binding proteins in retinal neurons. *Invest Ophthalmol. Vis. Sci.* 2006, 47, 1016-1023, doi: 10.1167/IOVS.05-0465.
15. Roberts P. A., Gaffney E. A., Luthert P. J., Foss A. J. E., Bryne H. M.: Retinal oxygen distribution and the role of neuroglobin. *Math. Biol.* 2016, 73, 1-38, doi:10.1007/S00285-015-0931-y.
16. Schmidt M., Giessel A., Laufs T., Hankeln T., Wolfrum U., Burmester T.: How does the eye breathe? Evidence for neuroglobin-mediated oxygen supply in the mammalian retina. *J. Biol. Chem.* 2003, 278, 1932-1935, doi: 10.1074/jbc.M209909200.
17. Toro-Urrego N., Garcia-Segura L. M., Echeverría V., Barreto G. E.: Testosterone protects mitochondrial function and regulates neuroglobin expression in astrocytic cells exposed to glucose deprivation. *Front Aging Neurosci.* 2016, 8, 152, doi: 10.3389/fnagi.2016.00152.
18. Tsacopoulos M., Poitry-Yamate C. L., MacLeish P. R., Poitry S.: Trafficking of molecules and metabolic signals in the retina. *Prog. Retin Eye Res.* 1998, 17, 429-442, doi: 10.1016/S1350-9462(98)00010-x.
19. Yan Z. G., Yue H. Y., Duan W. J.: Effects of high altitude oxygen on the expression of retinal myoglobin in rabbits. *Prog. Retin. Eye Res.* 2010, 30, 618-621, doi: 10.1242/jeb.02243.
20. Yang Y., Liu X., Gao X.: Distribution of brain globin in sheep retinas. *J. Anat.* 2015, 3, 275-278 (In Chinese).
21. Yu D. Y., Cringle S. J.: Oxygen distribution and consumption within the retina in vascularized and avascular retinas and animal models of retinal disease. *Prog. Retin. Eye Res.* 2001, 20, 175-208, doi:10.1016/S1350-9462(00)00027-6.
22. Zhang W.: Neural mechanism of vision formation. *Ophthalmic Res.* 2002, 20, 472-474, doi: 10.1016/S1350-6794(00)00027-6.

Corresponding author: Dr. Xia Liu, Anning District, Lanzhou City, Gansu Province, 730070, People's Republic of China; e-mail: drdugau2019@gmail.com (XL)