# Comparison of Neuroglobin Expression in the Retina of Tibetan Sheep (*Ovisammon or argali*) and Sheep (*Ovis aries*)

Xiaohua DU[1]

Xia LIU[2]*

James Blackar MAWOLO[2]

ORCID: 0000-0001-9604-68601, ORCID: 0000-0003-1273-81012, ORCID: 0000-001-9984-44752

[1]Affiliation: College of Veterinary Medicine,
Gansu Agricultural University, Lanzhou City, Gansu Province,
730070, People's Republic of China

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

[3]Affiliation: College of Life Science and Technology, Lanzhou City,
Gansu Province,730070, People's Republic of China

*Corresponding Author:
Xia Liu
Anning District, Lanzhou City, Gansu Province, 73000, People's Republic of China
Email address: drdugau2019@gmail.com (XL)

**KEY WORDS:** neuroglobin; retina; Tibetan sheep; sheep; immunohistochemistry

## ABSTRACT

Neuroglobin (Ngb) is a respiratory protein mainly expressed in the brain and retina. Its expression in the retina is greater than the brain due to 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 goal of this study is to explore the pattern of Ngb expression in the Tibetan sheep (Ovis ammon or argali) living in the plateau region and the sheep (Ovis aries) dwelling on low land. For detail insight, the study explained the morphological characteristics of Ngb distribution closely related to oxygen metabolism in the retina of Tibetan sheep and sheep. Immunohistochemistry staining procedures were performed to detect the Ngb protein expression in the retinae of Tibetan sheep and sheep while Inter's integrated performance primitives (IPP) semi-quantitative analysis was employed to compare the quantity of Ngb expression in the retinae of both sheep. The outer nuclear layer and outer segment of the photoreceptor showed negative Ngb expression while weak positive expression was observed in the inner core layer and ganglion cell layer, and strong positive expression was found in other layers. The independent T-test reported that the expression intensity in the nerve fiber layer, inner and outer plexus layer, inner segment of photoreceptor, and superior pigment cortex in the Tibetan sheep was significantly higher than that of the sheep. Other differences were not significant. The trend of Ngb distribution in the retinae of Tibetan sheep and sheep was comparatively similar despite these animals living in different altitude

environment. The overall intensity of this protein in the retina of the Tibetan sheep was higher than the sheep. The study emphasized that the different Ngb expression in the Tibetan sheep and sheep may be closely related to the adaptation to their respective environment. The stronger Ngb expression in the retina of Tibetan sheep may indicate that Ngb is closely related to the oxygen metabolism of the animals under natural selection and plays a role in the metabolism of oxygen.

## INTRODUCTION

Neuroglobin (Ngb) is a kind of protein that has a high affinity for oxygen and mainly distributed in the central nervous system of animals (Burmester et al., 2000). This protein can bind oxygen reversibly, and promote the metabolism of oxygen and survival of nerve cells under the condition of hypoxia and ischemia (Burmester et al., 2000; Liu et al., 2009; Chan et al., 2012; Morozov et al., 2014). Except for Ngb expression in the central nervous system, studies have shown that it is also expressed in multiple tissues, especially the retina tissues where the concentration of expression is 100 times greater than the brain tissues (Ostojic et al., 2006). During the visual production process, the vertebrate retina structure consumes a large amount of oxygen, making retina the highest oxygen-consuming tissue (Tsacopoulos et al., 1998; Yu & Cringle, 2001; Blank et al., 2011), which plays an important role in the process of metabolism. Reports on the murine retina found the expression of Ngb in few neurons of the ganglion cell and the inner nuclear layers co-expressing melanopsin and tyrosine hydroxylase (Hundahl et al., 2012), while Schmidt et al., (2003) examined the vascular retinae of the mouse and reported that Ngb was highly expressed in the inner segments of photoreceptor cells, outer and inner plexiform layers, and the ganglion cell layer. These are the regions that consume higher oxygen and the levels of Ngb expression found in these regions are higher. Another study emphasized 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 (Ostojic et al., 2008). The expression of Ngb and Cyb found in these regions are comparatively similar. Despite all of these reported results, there still exist limited references reporting Ngb expression in the retinae of Tibetan sheep and sheep. As one of three original breeding animals in China, Tibetan sheep have been living in high-altitude Hezuo and Qinghai-Tibet Plateau environment for a long period, and have adapted to a high temperature and low oxygen environment. The current study performed the immunohistochemical Staining Procedures (SP) method and Inter's integrated performance primitives (IPP) semi-quantitative analysis to detect Ngb expression and compared the expression pattern in the retinae. The enrich vertebrate retinae express Ngb morphological data and provide insight about the complex physiological mechanism of this protein.

## MATERIALS AND METHODS

### Animals and Setting.

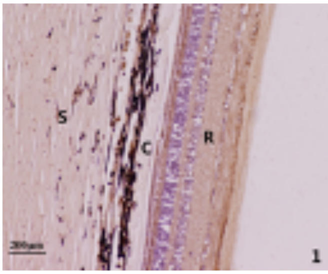
All procedures used in this study were reviewed and approved by the Animal Ethics and Welfare Committee of Gansu Agricultural University (GAU-AEW-2019-0023). A total of 10 healthy sheep, 5 Tibetan sheep (6 years) and 5 sheep (5 years) were used in this study. The Tibetan sheep and sheep specimens were collected at separate times. The specimens were extracted within 1 week. All Tibetan samples were collected for 4 days, while sheep samples were collected within 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 the month of July. All sheep involved in this study were taken from the Hezuo and Lanzhou cities in Gansu Province, the People's Republic of China.

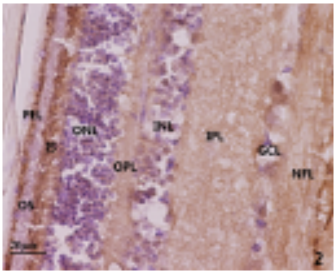
The Hezuo city is regarded as the administrative seat of the Tibetan Autonomous Prefecture in Gansu Province of Western China. With an elevation of approximately 3,000 meters (9,800 ft), The city has an

***Figure 1.*** *Expression of Ngb on the wall of the Tibetan sheep eye. Immunohistochemical staining, scale =100m. R: retina, C: choroid, S: sclera.*

***Figure 2.*** *Ngb expression in the retina of Tibetan sheep. Immunohistochemical staining, scale =20°m. NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, IS: internal segment of photoreceptor, OS: outer segment of photoreceptor, PEL: pigment superior cortex.*

***Figure 3.*** *Expression of Ngb in Tibetan sheep optic disc. Immunohistochemical color rendering, scale =20m.↑: show the optic nerve.*



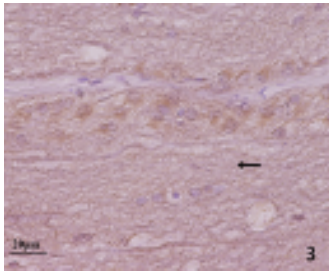
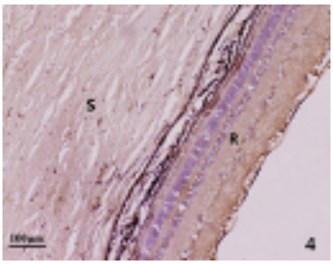
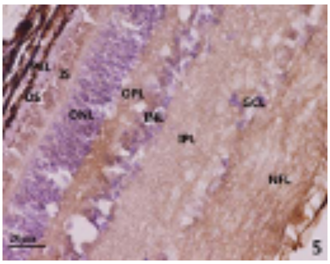



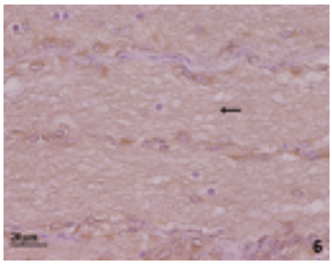

***Figure 4.*** *Ngb expression on the ocular wall of sheep. Immunohistochemical staining, scale =100m, R: retina, S: sclera.*

***Figure 5.*** *Expression of Ngb in the sheep retina. Immunohistochemical color rendering, scale =20m, NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, IS: internal segment of photoreceptor, OS: outer segment of photoreceptor, PEL: pigment superior cortex.*

***Figure 6.*** *Ngb expression in sheep optic disc. Immunohistochemical color rendering, scale =20m.↑: show the optic nerve.*




alpine subarctic climate, extremely cold, dry winters, and mild summers. The monthly mean temperature is in January, which is the coldest month with −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% - 71% 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.

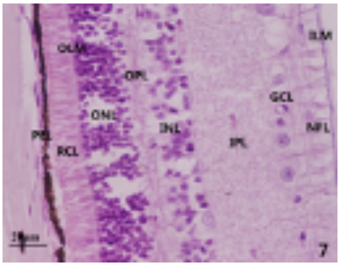
**Samples Extraction and Preparation.**

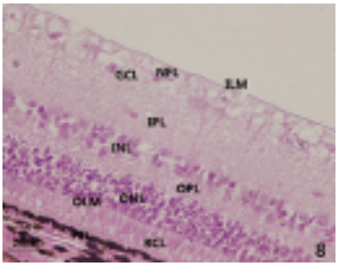
Before beeing sacrificed, all animals were fed, rested, and retrieved one at a time from their living area. After slaughter, the eyeballs were quickly removed and placed in Davidson’s fixation solution for 24 hours and stored at 4°C. The perfusion-fixed retinae were placed on disinfected glass slides and non-specific binding sides were blocked at room temperature for an hour with 1%

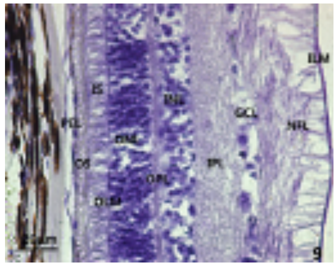
***Figure 7.*** *Structure of Tibetan sheep retina. H&E staining scale =20nm, ILM: inner boundary membrane, NFL: optic fiber layer, GCL: ganglion cell layer, IPL: intranet layer, INL: kernel layer, OPL: outer network layer, ONL: outer nuclear layer, OLM: external membrane, RCL: rod and cone layer, PEL: pigment superior cortex.*

***Figure 8.*** *Structure of sheep retina. H&E staining scale =20nm, 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.*

***Figure 9.*** *Expression of Ngb in the retina of Tibetan sheep. Negative control, scale =20nm. 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: internal segment of photoreceptor, OS: outer segment of photoreceptor, PEL: pigment superior cortex.*







Bovine serum albumin (BSA, Guangzhou, China) in Phosphate-buffered saline (PBS, Guangzhou, China).

These sections were incubated with a rabbit anti-Ngb polyclonal antibody (D221024, Shanghai, China) for one night at 4°C. These sections were rinsed in PBS and incubated at room temperature for 80 minutes. The sections were re-washed and embedded in Trypsin (A100458, Shanghai, China). Later, these sections were embedded into paraffin sections and examined under an optical microscope (Olympus BX51). 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 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 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. One hundred l 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.

**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 significant level. $P < 0.05$ was considered significant.

## RESULTS

**Expression of Ngb in the Retina of Tibetan Sheep and Sheep**.

Immunohistochemical results showed that Ngb was distributed in the retinae of Tibetan sheep and sheep, and their distribution

***Table 1.*** *Comparison of Ngb expression between Tibetan sheep and sheep retinae*

| Retina cell layers | Tibetan Sheep MD | Expression intensity | MD Sheep MD | Expression intensity |
|---|---|---|---|---|
| Nerve fiber layer (NFL) | 0.109±0.011**Δ | ++ | 0.084±0.021**Δ | ++ |
| Ganglion cell layer (GCL) | 0.027±0.016** | + | 0.032±0.011** | + |
| Inner plexiform layer (IPL) | 0.069±0.012**Δ | ++ | 0.052±0.009**Δ | ++ |
| Inner nuclear layer (INL) | 0.018±0.003** | + | 0.014±0.002** | + |
| Outer plexiform layer (OPL) | 0.064±0.009**Δ□ | ++ | 0.044±0.005**Δ□ | ++ |
| Outer nuclear layer (ONL) | 0.002±0.001* | – | 0.002±0.001* | – |
| Photoreceptor inner segments (IS) | 0.126±0.030**Δ□ | ++ | 0.062±0.008**Δ□ | ++ |
| Photoreceptor outer segments (OS) | 0.003±0.002* | – | 0.004±0.001* | – |
| Pigmented epithelial layer | 0.184±0.035**Δ | ++ | 0.129±0.038**Δ | ++ |

*Note: * compared with negative control group, P>0.05;**compared with negative control group, P<0.05;  intra-group comparisons of the positive control group, P<0.05; □ Comparison between Tibetan sheep and sheep positive control group.*

patterns are identical (Figure 1, 4). 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 (Figure 2, 5). In each positive expression cell layer, 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.

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. 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.

**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).

## DISCUSSION

Discovered by Burmester et al., (2000), 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 may play an important role in the energy metabolism of the nervous system and the compensatory function of nerve cells in the hypoxic environments. Therefore, several studies have been conducted on 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 (Li et al., 2006; Ostojic et al., 2008a; Ostojic et al., 2008b; Lechauve et al., 2009; Yang et al., 2015), 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 (Schmidt et al., 2003). 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 (Ostojic et al., 2008a). The current study showed some similarities and comparative differences to these reported results. Except for the photoreceptor outer segments and an outer nuclear layer that show negative expression, Ngb was positively expressed in all other layers of the Tibetan sheep and 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 sheep and sheep retinae suggest that Ngb may play an important function in the mechanism of an 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 (Schmidt et al., 2003; Hundahl et al., 2012). Ngb is suggested to play key functions in the promotion of respiratory chain transfer or short oxygen storage. In each cell layer with strong Ngb positive expression, optic ganglion cells serve as the starting point for receiving integrated visual signals from the retina (Zhang, 2002), and the upward formation of 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 (Schmidt et al., 2003). 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 to high altitude and low oxygen environment for a long period. Under normal physiological conditions, the utilization rate of oxygen is higher in the Tibetan sheep than the sheep, and Ngb performs an important function in the distribution pattern between both sheep retinae. The experimental results in the current study showed that strong positive Ngb expression in the Tibetan sheep was significantly higher than the sheep in each cell layer, and aid in the promotion of oxygen metabolism. This report was consistent with Yan et al. (2010) studies that 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 (Milton et al., 2006). 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 sheep and 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 sheep. (Schmidt et al., 2003; Ostojic et al., 2006; Ostojic et al., 2008a) determined the large presence of Ngb in the retina of humans and dogs with the use of fluorescent immunity and western blot tests. The results speculated that Ngb has an important function in scavenging reactive oxygen radicals, transporting oxygen, protective effect on retinal ischemia, and hypoxia-related diseases. Lechauve et al., (2012) 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 respiratory chain mitochondrial protein.

Rat research showed that the rat model of Ngb excessive expression can reduce retinal cell apoptosis, maintain retinal integrity, and have an obvious protective effect (Chan et al., 2012), while another reference explained the expression of Ngb may be regulated by testosterone and other hormones (Toro-Urrego et al., 2016), and have a protective effect on nerve cells (Guidolin et al., 2016), but the mechanism of its physiological function remains unclear. Roberts et al., (2016) 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.

## CONCLUSION

The current research provides morphological (in relationship with anatomy) data of Ngb expression in the retinae of Tibetan sheep and sheep and further explore 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 a further insight of Ngb role in specific physiological mechanisms.

**Acknowledgements**: The authors are very grateful to the Authority of Hezuo city for the numerous assistances during the samples collection. This research could be impossible without the assistances rendered.

## REFERENCES

1. Blank M., Kiger L., Thielebein A., Gerlach F., Hankeln T., Marden M.C., Burmester T. (2011): Oxygen supply from the Bird's eye perspective [J]. *The journal of biological chemistry*, 286 (30): 26507-26515. DOI:10.1074/jbc.M111.224634
2. Burmester T., Weich B., Reinhardt S., Hankeln T. (2000): A vertebrate globin expressed in the brain [J]. *Nature*, 407(6803): 520-523. DOI:10.1038/35035093
3. Chan A., Saraswathy S., Rehak M., Ueki M., Rao N.A. (2012): Neuroglobin protection in retinal ischemia [J]. *Investigative ophthalmology & visual science*, 53(2): 704-711.DOI:10.1167/iovs.11-7408
4. Guidolin D., Tortorella C., Marcoli M., Agnati L.F. (2016): Neuroglobin, a factor playing for nerve cell survival [J]. *Molecular sciences*, 10: 17-1817. DOI:10.3390/ijms17111817
5 Hundahl C.A., Fahrenkrug J., Luuk H., Hay-Schmidt A., Hannibal J. (2012): Restricted expression of neuroglobin in the mouse retina and co-localization with melanopsin and tyrosine hydroxylase. *Biochemical and biophysical research communications*, 425 100–106.DOI: 10.3390/cells1041133
6 Lechauve C., Rezaei H., Celier C., Kiger L., Corral-Debrinki M., Noinville S., Chauvierre C., Hamdane D., Pato C., Marden M.C. (2009): Neuroglobin and prion cellular localization: investigation of a potential interaction [J]. *Mol biol*, 388: 68-977.DOI: 10.1016/j.jmb.2009.03.047

7 Lechauve C., Augustin S., Cwerman-Thibault H., Bouatia A., Forster V., Celier C., Rustin P., Mardin M.C., Sahel J.A., Corral-Debrinski M. (2012): Neuroglobin involvement in respiratory chain function and retinal ganglion cell integrity [J]. *Brioches biophys acta*, 1823(12): 2261-2273. DOI:10.1016/j.bbamcr.2012.09.009

8 Li Y., Liu H., Tong Y. (2006): Immunohistochemical study on the distribution of brain globin in rat eyeball [J]. *Ophthalmic research*, 24(5): 461-464. DOI:10.1016/S0006-8993(01)03360-1

9 Liu J., Yu Z., Guo S., Lee S.R., Xing C., Zhang C., Gao Y., Nicholls D.G., Lo E.H., Wang X. (2009): Effects of neuroglobin overexpression on mitochondrial function and oxidative stress following hypoxia/reoxygenation in cultured neurons [J]. *Neurosci Res*, 87: 164-170.DOI:10.1002/JNR.21826

10 Milton S.L., Nayak G., Lutz P.L., Prentice H.M. (2006): Gene transcription of neuroglobin is upregulated by hypoxia and anoxia in the brain of the anoxia-tolerant turtle trachemys scripta [J]. *J. Biomed Sci*, 13(4): 509-514.DOI:10.1007/s11373-006-9084-8

11 Morozov A.N., Roach J.P., Kotzer M., Chatfield C.C. (2014): A possible mechanism for redox control of human neuroglobin activity [J]. *American chemical society*, 54: 1997-2003.DOI:10.1021/CI5002108

12 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. (2006): Neuroglobin and cytoglobin: oxygen-binding proteins in retinal neurons [J]. *Invest ophthalmol vis sci*, 47(3): 1016-1023.DOI:10.1167/IOVS.05-0465

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. (2008a): Patterns of distribution of oxygen-binding globins, neuroglobin and cytoglobin in human retina [J]. *Arch ophthalmol*, 126(11): 1530-1536.DOI: 10.1001/archopht.126.11.1530

14 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. (2008b): Neuroglobin and cytoglobin distribution in the anterior eye segment: a comparative immunohistochemical study [J]. *J. Histochem. Cytochem,* 56: 863-872.DOI:10.1369/jhc.2008.951392

15 Roberts P.A., Gaffney E.A., Luthert P.J., Foss A.J.E., Bryne H.M. (2016): Retinal oxygen distribution and the role of neuroglobin [J]. *Math biol*, 73: 1-38.DOI:10.1007/S00285-015-0931-y

16 Schmidt M., Giessl A., Laufs T., Hankeln T., Wolfrum U., Burmester T. (2003): How does the eye breathe? Evidence for neuroglobin-mediated oxygen supply in the mammalian retina [J]. *J Biol Chem*, 278(3): 1932-1935.DOI:10.1074/jbc.M209909200

17 Toro-Urrego N., Garcia-Segura L.M., Echeverrìa V., Barreto G.E. (2016): Testosterone protects mitochondrial function and regulates neuroglobin expression in astrocytic cells exposed to glucose deprivation [J]. *Front Aging Neurosc*i, 8: 152. DOI:10.3389/fnagi.2016.00152

18 Tsacopoulos M., Poitry-Yamate C.L., MacLeish P.R., Poitry S. (1998): Trafficking of molecules and metabolic signals in the retina [J]. *Prog retin eye res*, 17(3): 429-442.DOI:10.1016/S1350-9462(98)00010-x

19 Yan Z.G., Yue H.Y., Duan W.J. (2010): Effects of high altitude oxygen on the expression of retinal myoglobin in rabbits [J]. *New progress in ophthalmology*, 30(7): 618-621.DOI:10.1242/jeb.02243

20 Yang Y., Liu X., Gao X. (2015): Distribution of brain globin in sheep retinas [J]. *Journal of anatomy*, 3: 275-278.

21 Yu D.Y., Cringle S. J. (2001): Oxygen distribution and consumption within the retina in vascularized and avascular retinas and in animal models of retinal disease [J]. *Prog retina eye res*, 20: 175-208. DOI:10.1016/S1350-9462(00)00027-6

22 Zhang wei (2002): Neural mechanism of vision formation [J]. *Ophthalmic research*, 2002, 20(5): 472-474.DOI: 10.1016/S1350-6794(00)00027-6