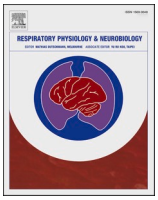
Respiratory Physiology & Neurobiology 313 (2023) 104063

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol



Aging and the carotid body: A scoping review

Camillo Di Giulio [a,*], Susi Zara [b], Andrea Mazzatenta [a], Vittore Verratti [c], Andrea Porzionato [d], Amelia Cataldi [b], Mieczyslaw Pokorski [e]

[a] Department of Neurosciences Imaging and Clinical Sciences, Italy
[b] Department of Pharmacy, Italy
[c] Department of Psychological Sciences, Health, and Territory, University of Chieti-Pescara, Chieti, Italy
[d] Section of Human Anatomy, Department of Neuroscience, University of Padova, Padova, Italy,
[e] Institutte of Health Sciences, Opole University, Opole, Poland

ARTICLE INFO

Edited by Professor Mieczyslaw Pokorski

Keywords:
Aging
Glomus cells
Hypoxia
Mitochondria
Oxidative status
Ventilation

ABSTRACT

The carotid body (CB) is a neuroepithelial tissue consisting of O_2-sensitive glomus cells that constantly scan the arterial blood for O_2 and generate a discharge as an inverse function of O_2 content. Aging is a cumulative result of decreased O_2 supply paralleled by a decreased O_2 tissue demand and oxidative damage to cells derived from aerobic metabolism. Here we studied how CB affects the aging process. This is a study of CB ultrastructural morphometry and immunohistochemical expression of proteins underlying CB responsiveness. The study was based on human CBs obtained from cadavers of people who died due to traumatic events in young and old age. The study was supplemented by investigations of CBs obtained from young and old rats subjected to chronic normoxic and hypoxic conditions. We found changes in the old normoxic CBs akin to the effects of chronic hypoxia such as enhanced extracellular matrix, reduced synaptic contacts between glomus cells, fewer glomus cells, secretory vesicles, and mitochondria. These changes were accompanied by enhanced expressions of hypoxia-inducible factor one-alpha (HIF-1α), vascular endothelial growth factor (VEGF), and nitric oxide synthase (NOS2). We conclude that hypoxia and aging share a common background consisting of deficient O_2 tissue supply, mitochondrial dysfunction, and a limited ability to deal with increased cellular oxidative stress. Aging leads to adaptative reductions in CB responsiveness to hypoxia shifting the chemosensory setpoint upward. We submit that the attenuated CB sensitivity at old age may be tantamount to "physiological denervation" leading to a gradual loss of the chemosensing role in the prevention of tissue hypoxia by increasing lung ventilation.

1. Introduction

The survival of metazoan organisms depends on O_2 delivery and utilization to maintain a balance between the energy generation and production of toxic oxidant molecular species (Finkel and Holbrook, 2000). Molecular oxygen is vital for cellular processes because of its role in ATP production via oxidative mitochondrial phosphorylation. O_2 sensing is a property of all mammalian tissues. Cells maintain PO_2 within a narrow range due to the respiratory and cardiocirculatory O_2 transport. This function demands the O_2 be strategically sensed and its supply closely controlled. The carotid body (CB), a paired chemosensory organ located at the bifurcation of the common carotid artery, instantly senses arterial partial pressure of O_2 and passes afferent signals to the brainstem (Mokashi et al., 2021). The organ is key for the control of tissue "milieu intérieur" and thus body homeostasis. Ivan P. Pavlov postulated at the end of the 19th century, decades before the discovery of CB, the presence of a special type of neural organ having chemosensory properties (Pavlov, 1951). In 1926, Corneille J. F. Heymans, a Belgian physiologist, produced convincing proof of the presence of chemoreceptors in the aortic arch and shortly followed with similar chemoreceptors in the carotid sinus area; a later Nobel laureate for the discoveries (Heymans and Heymans, 1927).

Aging is characterized by reductions in adaptative responses to metabolic requirements, O_2 tissue supply, and activities of several metabolic factors and enzymes (Katschinski, 2006). Cell growth, differentiation, aging, and death are generally related to a series of factors including O_2 consumption, intracellular pH, free radical production, and O_2 tissue supply, all of which adversely affect physiological functions

* Correspondence to: Department of Neurosciences Imaging and Clinical Sciences of the "G. d'Annunzio" University in Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy.
E-mail address: digiulio@unich.it (C. Di Giulio).

https://doi.org/10.1016/j.resp.2023.104063
Received 9 March 2023; Accepted 16 April 2023
Available online 17 April 2023

and expression of enzyme proteins (Krivoruchko, Storey, 2010). A presumptive role of CB in biomolecular control prompted us to examine the organ's function in advanced age CB (Pokorski et al., 2004; Di Giulio et al., 2003). Here we tested the age-dependent expression of three essential molecules engaged in the process of aging: hypoxia-inducible factor one-alpha (HIF-1α), vascular endothelial growth factor (VEGF), and nitric oxide synthase (NOS2) - a constitutively neural expressed protein. We tested the hypothesis that if aging depended on O_2 consumption during the lifespan, then the adaptive responses would be reflected in the content of these neuroactive molecules in the CB, a sensor of hypoxia and a regulator of O_2 supply through lung hyperventilation.

2. Methods

This is a morphometric and immunohistochemical investigation of CBs which consisted of two separate ramifications: human and animal. Human CBs were sampled at autopsies from six young, aged 28 ± 3 years, and six old subjects, aged 74 ± 3 years. All subjects were free of chronic pulmonary or cardiovascular diseases and died of sudden trauma. Autopsies were performed 18–78 h after death. Specimens consisted of 20-mm long fragments of the common carotid, internal, and external carotid arteries dissected about the bifurcation area.

Animal CBs were obtained from Wistar rats weighing 200–250 g. The rats were divided into two age groups of 3 and 24 months of age, six rats each, implicitly corresponding to the young and old ages of dead persons from whom CBs were taken. Either group was studied in chronic normoxia (21% O_2) taken as control and chronic intermittent hypoxia (10–12% of inspired O_2 alternating with 21% O_2, 12/12 h daily for 12 days) performed in a plexiglass chamber. At the end of exposure, rats were anesthetized with Nembutal at a dose of 30 mg/kg, i.p., carotid bifurcation areas were exposed for CBs dissection.

2.1. Electron transmission microscopy

Rats were perfused through the left heart with 2.5% glutaraldehyde in phosphate-buffered saline (PBS), pH 7.4, 320 mOsm, and CBs were dissected and postfixed in the same fixative. The material was dehydrated in a series of increasing ethanol concentrations, propylene oxide, and embedded in epoxy resin. Ultrathin sections of 50 nm thickness were cut on a cryotome (Reichert-Jung Frigocut 2800), mounted on 200 mesh copper grids, and examined under a transmission electron microscope (Jeol 1200EX; Tokyo, Japan).

2.2. Immunohistochemistry and light microscopy

Human and animal CBs preparations were elaborated in like manner. Briefly, CBs were immersed overnight in ice-cold 4% paraformaldehyde in 0.1 M PBS. The material was rinsed in 15% sucrose PBS for 1 h, stored at 4 °C in 30% sucrose in PBS for 2 h, and cut on a cryotome in 10 μm thick sections. The specimens were thaw-mounted onto slides, fixed by immersion in acetone at 4 °C for 5 min, air-dried, and stored at 4 °C until further use.

For the detection of HIF-1, VEGF, and NOS2 proteins, sections were preincubated in PBS for 5 min and then in the presence of rat anti-VEGF and anti-NOS2 antibodies (primary antibodies dilution 1:100) for 30 min at 37 °C. Sections were then washed twice in PBS for 5 min and Tris-HCl buffer of pH 7.6 for 10 min, followed by the incubation with specific biotinylated secondary antibodies and the avidin-biotin-peroxidase complex (Santa Cruz Biotechnology, CA). Peroxidase was developed using diaminobenzidine chromogen (DAB) and nuclei were hematoxylin counterstained. Negative controls were performed by omitting the primary antibodies. Samples were examined under light microscopy (Leica DM 4000 with DFC 320 camera) for digitized image analysis using the QWin Plus 3.5 software (Leica Cambridge Ltd, Cambridge, UK). The expressions of proteins were quantified as the immunoreaction product intensities, histogram-processed using stereological measurements with the Bioquant system connected to a microcomputer through a digitizing tablet and statistically elaborated as outlined below. The quantification of the immunohistochemical reaction yield was carried out.

2.3. Statistical evaluation

The intensities of immunoreaction products were averaged from 12 to 15 photomicrographs per CB group. The mean values of morphometric cellular measurements were calculated. The units of measure for mitochondrial areas were pixels. Differences in the morphometric and immunohistochemical variables between the young and old CBs and the normoxic and hypoxic conditions were assessed using an unpaired t-test. Additionally, experimental data for each group were analyzed using one-way ANOVA. A p-value < 0.05 defined statistically significant differences.

3. Results

Age-related changes in glomus cells were associated with an increased cytoplasm/mitochondria volume ratio. The numbers of the cells, the intracellular dense-core secretory vesicles containing neuroactive substances, and mitochondria decreased with age. Likewise, the lobular size of CB glomic tissue, the cellular volume, and synaptic contact densities. Progressive proliferation of sustentacular glia-like cells was noticed. There also were enlarged endoplasmic reticulum areas and lipofuscin accumulation in the old CBs. Concomitantly, the extracellular matrix and fibronectin increased (Fig. 1). All these changes suggest that old CBs became less responsive to hypoxia.

VEFG and HIF-positive areas were significantly reduced whereas NOS2 expression did not differ significantly in the old versus young CBs (Figs. 2–3–4). Moreover, hyperplasic responses of glomus cells during chronic intermittent hypoxia as well as hypoxic increases in the expression of HIF-1α, VEGF, NOS2 were less evident in the old versus young CBs. Intermittent hypoxia showed changes in CB parenchyma akin to those present in old CBs, i.e., increases in weight, connective tissue, blood vessels, and the cytoplasm/ mitochondria ratio.

4. Discussion

The major findings of this study were that CBs underwent distinct changes with advancing old age. Morphologically, at the ultrastructural level, notable changes consisted of the general hyperplastic trend for CB parenchyma due to the proliferation of connective matrix, but with the smaller lobular appearance of functional elements, i.e., the glomus cells, increased cytoplasm/mitochondria ratio, and reductions in the number of glomus cells, degeneration of mitochondrial content, fewer synaptic junctions between cells, and the accumulation of lipofuscin residues, all of which are important pathogenetic cues for age-related tissue degeneration and increased oxidative burden. Immunohistochemically, expressions of the signaling proteins of HIF-1α, VEGF, and NOS2 were less in the old than young CBs in the normoxic condition. Further, these proteins increased in content in chronic hypoxia, but the increases were dampened in the old CBs.

The present study shows that CB undergoes regressive changes with advancing age which are similar in humans and animals. These changes are liable to shift the functional setpoint of the organ upward concerning chemosensory sensitivity. Interestingly, chronic hypoxia, generally, affects young CBs the way old age does per se. There are, however, differences in ultrastructure and function. When the former grossly followed suit in both hypoxia and aging, the latter concerning the expression of signaling proteins in glomus cells was only dampened in response to hypoxia in old CBs. The functional status of the old CB resembles what may be called "physiological denervation" resulting in a gradual loss of the homeostatic role the CB is naturally endowed with, i.e., hypoxic lung hyperventilation. Further, the corollary is that the

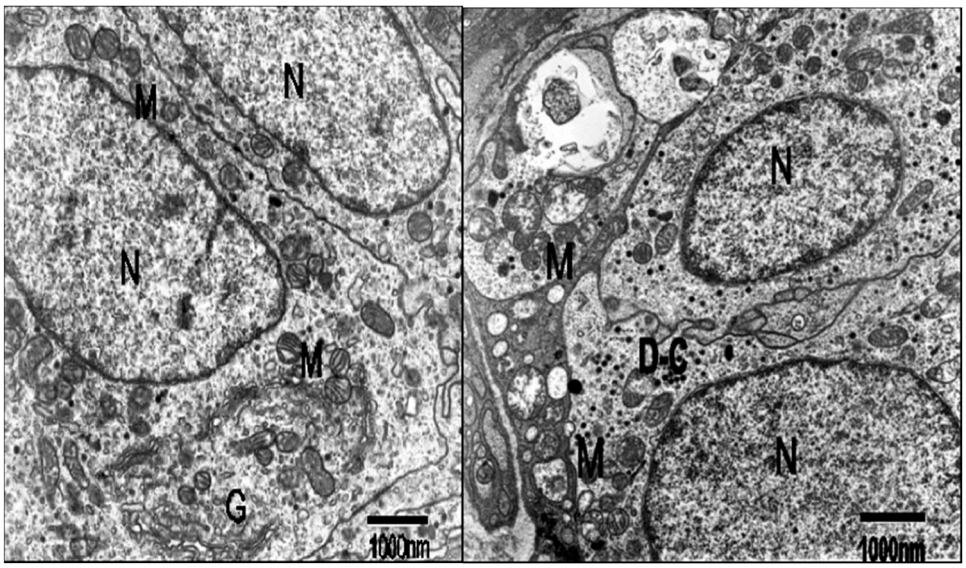


Fig. 1. Photomicrographs of young and old carotid bodies M, Mitochondria; D-C, dense-cored secretory vesicles; N, Nucleus; G, Golgi apparatus.

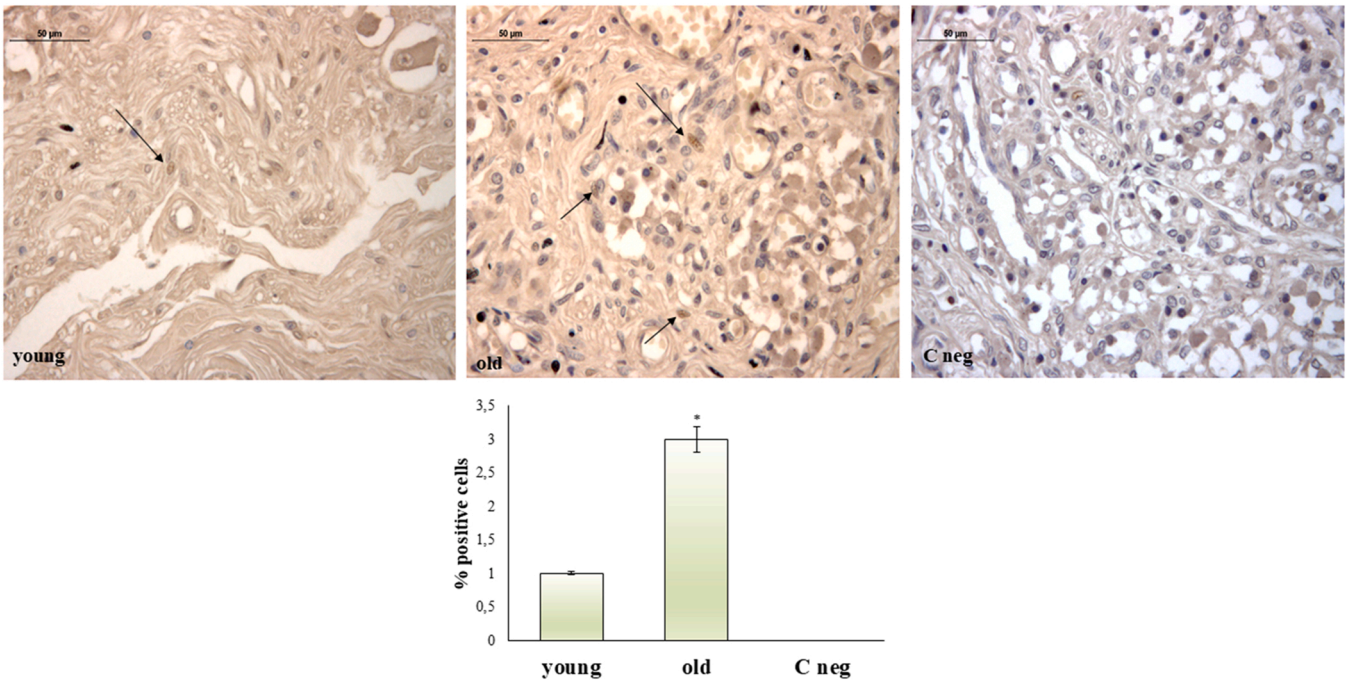


Fig. 2. Immunohistochemical detection of HIF-1α expression in human carotid bodies of young and old human carotid bodies; C neg: negative control; the graph represents HIF-1α positive nuclei marked with arrows. The graph represents densitometric analysis (mean % ± SD) determined by direct visual count of ten fields for each of three slides. Bars in photomicrographs - magnification 50 μm; *old vs young $p < 0.05$.

dampening of the hypoxic ventilatory response during aging is liable to originate at the same stimulus transduction site in CB where the lower sensitivity to hypoxia originates in chronic hyperoxia (Di Giulio et al., 2005; Pokorski and Antosiewicz, 2010). Interestingly, some changes like reductions in glomus cells' synaptic junctions might be a self-protective mechanism through which cells buffer themselves against the accumulation of ROS with age. Moreover, during chronic hypoxia, CB hypertrophy is less evident in the old than young CBs, a likely result of less release of growth factors in the former. Therefore, hypoxia and aging seem to share a similar phenotype of some but not all CB pathophysiologic responses.

Aging, a physiological part of life, is a cumulative result of oxidative damage to cells which derives from aerobic metabolism (Finkel and Holbrook, 2000, Wikens 2001). Several theories have been proposed like the "mitochondrial" theory of aging related to cellular oxidative damage and the accumulation of mutations in mitochondrial DNA caused by ROS (Harman, 2001). In aerobic organisms, more than 90% of the O_2 supply is used by cellular mitochondria, with ROS being normal metabolic byproducts. Cells have a variety of enzymatic and non-enzymatic defenses to convert ROS to less toxic species and maintain them at a harmless level. ROS formation depends on the balance between O_2 supply and metabolic demand for it, more than the sheer level of O_2. This balance is referred to as "constant oxygen concentration". ROS are alarm signals which modulate the gene expression of aging. This expression is also modulated by a decreased mitochondrial mass and function, a prime factor of the aging process, which we found

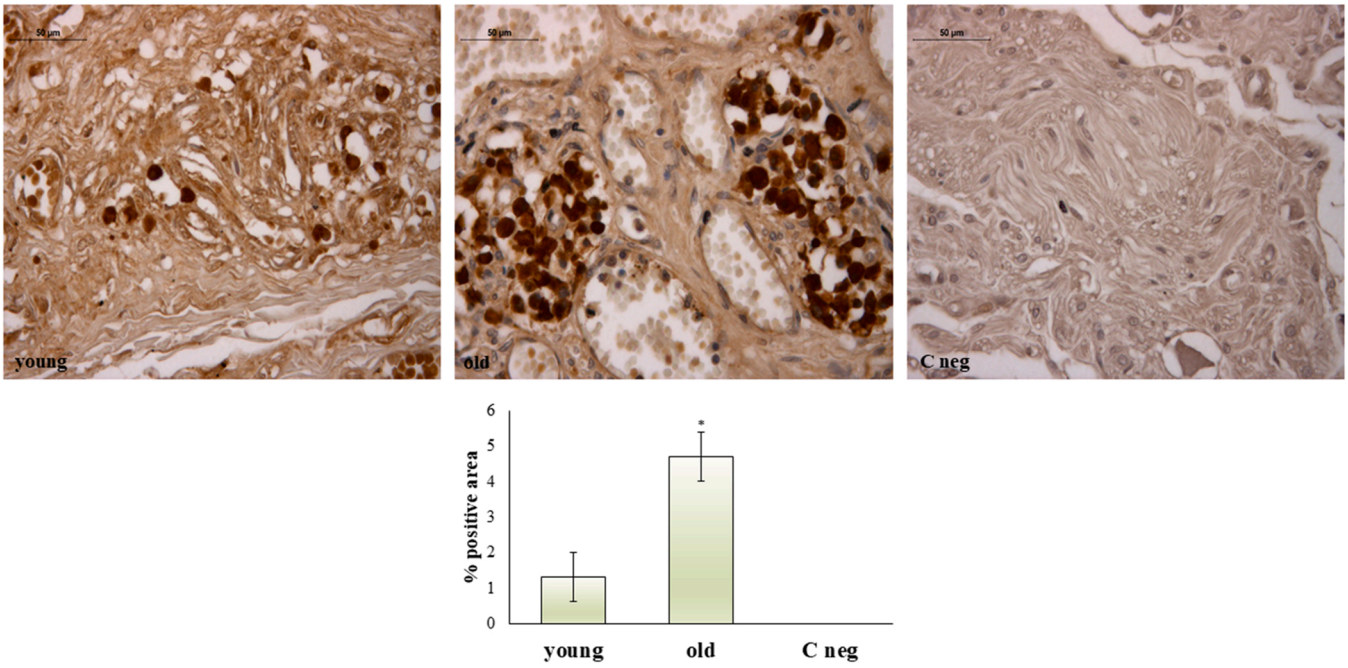


Fig. 3. Immunohistochemical detection of VEGF expression in human carotid bodies of young and old human carotid bodies; C neg: negative control; The graph represents densitometric analysis of VEGF positive area (mean % ± SD) determined by direct visual count of ten fields for each of three slides *old vs young $p < 0.05$. Bars in photomicrographs - magnification 50 µm.

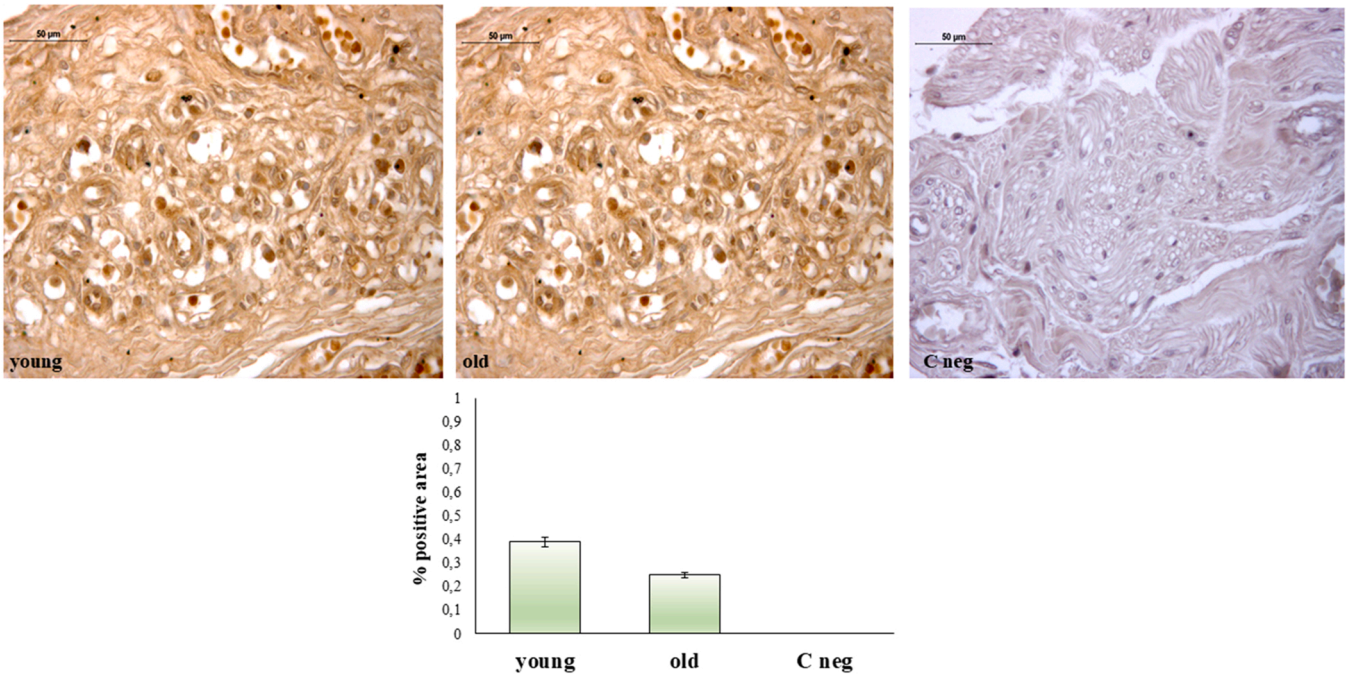


Fig. 4. Immunohistochemical detection of NOS2 expression in young and old carotid bodies; C neg: negative control; The graph represents densitometric analysis of NOS2 positive area (mean % ± SD) determined by direct visual count of ten fields for each of three slides. Bars in photomicrographs - magnification 50 µm.

in this study as an adaptive response to the lower O_2 tissue supply. Hypoxia per se modulates mitochondrial activity, decreasing O_2 consumption which, in turn, affects gene expression and aging.

Ultrastructural rearrangements in CB parenchyma with enhanced extracellular matrix during aging restrictively modify the O_2 diffusion pathway to mitochondria. Chronic hypoxia reduces maximum oxygen consumption, mitochondrial volume, and lactic acid capacity, the features typical for high altitude and aging, which supports the notion of CB undergoing ”physiological denervation’ with aging leading to a reduction in its homeostatic capacity Prolonged hypoxia and aging both lead to the accumulation of lipofuscin granules in muscles (Martinelli et al., 1990; Amicarelli et al., 1999).

Hypoxia seems to share some type of link with aging at different cell sites (Pamenter and Munro, 2019) and may represent an experimental model for studying the aging process. Acute hypoxia increases the chemosensory discharge of CB, which is less evident in old age or

long-lasting hypoxia at a young age (Lahiri et al., 1987). Since chemosensory aging results in hypoxia-like ventilatory adaptation, the prevention of hypoxia could delay or reduce the effects of aging. Recent studies demonstrate age-related changes in HIF-1α, the master regulator for hypoxia-induced gene expression, which might explain a reduced ability to cope with hypoxia in the elderly (Lahiri et al., 2007; Semenza, 1999). The increase in HIF-1α along the life axis suggests that HIF-1α acts as an oxygen sensor for aging. The HIF protein system may link aging and hypoxia facilitating O_2 supply and regulating energy production under limited oxygen conditions. The system also interacts with a series of signaling proteins whose distorted activation contributes to aging-associated ailments (Yeo, 2019).

During aging, HIF-1α mediates transcriptional activation of a network of genes encoding VEGF, NOS2, erythropoietin, and several glycolytic enzymes (Iyer et al., 1998). A decrease in cellular PO_2 induces angiogenesis through VEGF release and HIF-1α accumulation. Increases in HIF-1α, VEGF, and NOS2 are less evident in old compared to young CBs. A PO_2 drop is key for gene expression, but a tight correlation between arterial and tissue PO_2 needs more exploration during aging. A reduction in mitochondria and an increase in fibronectin in CB with aging are consistent with the notion that there is less need for oxygen in old age. Age-related changes, like atheromatosis, lead to CB tissue hypoxia, stimulating the growth of the extracellular matrix (Habeck et al., 1988). Likewise, lipofuscin accumulation with age is consistent with its increase in hypoxia, a kind of general stress response. These changes are accompanied by age-dependent reductions in the release of essential signaling molecules like HIF-1α, VEGF, and NOS2. CB-driven hyperventilation in hypoxia decreases in old rats suggesting the age-dependency of O_2-sensitive mechanisms (Pokorski and Antosiewicz, 2010). A decrease in hypoxic ventilatory responses may also reflect body defenses against cellular damage caused by O_2-related increases in ROS production; the greater the oxidative damage the shorter the life span (Sohal and Orr, 2012, Di Giulio, 2018).

In conclusion, hypoxia and aging share a common background consisting of deficient O_2 tissue supplies, mitochondrial dysfunction, and a limited ability to deal with increased cellular oxidative stress. Aging leads to adaptative reductions in CB responsiveness to stimuli shifting the chemosensory setpoint upward. The attenuated ventilatory response during chronic hypoxia is a regulatory process to reduce the energy cost of CB-driven hyperventilation. CB is a useful experimental model to study the modulatory mechanisms of the aging process correlating high blood flow with cell metabolism.

Ethical approval

All procedures performed in studies involving human participants were in accord with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The collection and handling of biological material were approved by the Ethics Committee of the University of Padova and followed Italian and European legislations.

Conflicts of Interest

The authors declare no conflict of interest concerning this article.

Data Availability

Data will be made available on request.

References

Amicarelli, F., Ragnelli, A.M., Aimola, P., Bonfigli, A., Colafarina, S., Di Ilio, C., Miranda, M., 1999. Age dependent ultrastructural alterations and biochemical response of rat skeletal muscle after hypoxic or hyperoxic treatments. Biochim. Biophys. Acta 1453, 105–114.

Di Giulio, C., 2018. Ageing of the carotid body. J. Physiol. 5 (96), 3021–3027.

Di Giulio, C., Cacchio, M., Bianchi, G., Rapino, C., Di Ilio, C., 2003. Selected contribution: Carotid body as a model for aging studies: is there a link between oxygen and aging? J. Appl. Physiol. 95, 1755–1758.

Di Giulio, C., Cacchio, M., Bianchi, G., Rapino, C., Di Ilio, C., 2005. Oxygen and life span: chronic hypoxia and hyperoxia as models for studying VEGF, NOS and HIF during ageing. Respir. Physiol. Neurobiol. 147, 31–38.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Habeck, J.O., Huckstorf, C., Behm, R., 1988. The paraganglia within the carotid bifurcation regions of young and old spontaneously hypertensive rats (SHR) after exposure to chronic hypobaric hypoxia. Anat. Anz. 165, 45–54.

Harman, D., 2001. Aging: overview. Ann. N. Y. Acad. Sci. 928, 1–21.

Heymans, J.F., Heymans, C., 1927. Sur les modifications directes et sur la régulation réflexe de l'activité du centre respiratoire de la tête isolée du chien. Arch. Int. Pharmacodyn. Ther. 33, 273–372.

Iyer, N.V., Kotch, L.E., Agani, F., Leung, S.W., Laughner, E., Wenger, R.H., Gassmann, M., Gearhart, J.D., Lawler, A.M., Yu, A.Y., Semenza, G.L., 1998. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes 15 12 (2), 149–162.

Katschinski, D.M., 2006. Is there a molecular connection between hypoxia and aging. Exp. Gerontol. 41 (5), 482–484.

Krivoruchko, A., Storey, K.B., 2010. Forever young: mechanisms of natural anoxia tolerance and potential links to longevity. Oxid. Med. Cell. Longev. 3 (3), 186–198.

Lahiri, S., Mulligan, E., Andronikou, S., Shirahata, M., Mokashi, A., 1987. Carotid body chemosensory function in prolonged normobaric hyperoxia in the cat. J. Appl. Physiol. 62, 1924–1931.

Lahiri, S., Antosiewicz, J., Pokorski, M., 2007. A common oxygen sensor regulates the sensory discharge and glomus cell HIF-1α in the rat carotid body. J. Physiol. Pharm. 58, 327–333.

Martinelli, M., Winterhalder, R., Cerretelli, P., Howald, H., Hoppeler, H., 1990. Muscle lipofuscin content and satellite cell volume is increased after high altitude exposure in humans. Experientia 46, 672–676.

Mokashi, A., Roy, A., Baby, M.S., Mulligan, E., Lahiri, S., Di Giulio, C., Pokorski, M., 2021. Role of IP3 receptors in shaping the carotid chemoreceptor response to hypoxia but not to hypercapnia in the rat carotid body: an evidence review. Adv. Exp. Med. Biol. 1289, 1–25. https://doi.org/10.1007/5584. 2020.561.

Pamenter, M.E., Munro, D., 2019. Longevity or hypoxia: who's driving? Aging 11 (16), 5864–5865.

Pavlov, I., 1951. Complete works. Mosc., Leningr. Vol 1, 525.

Pokorski, M., Antosiewicz, J., 2010. Alterations in the hypoxic ventilatory response with advancing age in awake rats. J. Physiol. Pharm. 61, 227–332.

Pokorski, M., Walski, M., Dymecka, A., Marczak, M., 2004. The. Aging carotid body. J. Physiol. Pharmacol. 55, 107–113.

Semenza, G.L., 1999. Ann. Rev. Cell. Dev. Biol. 15, 551–578.

Sohal, R.S., Orr, W.C., 2012. The redox stress hypothesis of aging. Free Radic. Biol. Med. 1 52 (3), 539–555.

Yeo, E.J., 2019. Hypoxia and aging. Exp. Mol. Med. 51 (6), 1–15.