The Indonesian Biomedical Journal, Vol.18, No.5, October 2026, p.416-519 Print ISSN: 2085-3297, Online ISSN: 2355-9179

RESEARCH ARTICLE

# Intermittent Exposure to Hypobaric Hypoxia Increases MDA and LC3 Levels and Differentially Affects GSH Levels in Rat Brain Tissue

Riska[1], Ninik Mudjihartini[2,*], Ani Retno Prijanti[2]

[1]Master's Programme in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, Jakarta 10430, Indonesia
[2]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, Jakarta 10430, Indonesia

*Corresponding author. Email: ninikbiokim@gmail.com

Received date: Aug 6, 2026; Revised date: Oct 7, 2026; Accepted date: Oct 13, 2026

## Abstract

**BACKGROUND:** Intermittent hypobaric hypoxia (IHH) induces repeated episodes of reduced oxygen availability that may elicit adaptive responses in the brain. Oxidative stress, antioxidant defences, and autophagy-related processes are interconnected during hypoxic adaptation. However, the relationship among malondialdehyde (MDA), reduced glutathione (GSH), microtubule-associated protein 1 light chain 3 (LC3), and mechanistic target of rapamycin (mTOR) during repeated IHH exposure remains incompletely characterized. This study was conducted to evaluate these biomarkers simultaneously to provide an integrated perspective on the cerebral response to repeated IHH exposure.

**METHODS:** Twenty-five male Sprague-Dawley rats were divided into five groups: a control group and 4 IHH groups subjected to either 1, 2, 3, or 4 exposures, respectively, to a simulated altitude of 25,000 feet for 5 minutes at one-week intervals. Brain tissues were collected one hour after the final IHH exposure, homogenized and centrifuged to obtain tissue supernatants for analysis. MDA and GSH levels were measured using colorimetric assays, while LC3 and mTOR protein levels were assessed using sandwich enzyme-linked immunosorbent assay (ELISA).

**RESULTS:** MDA concentrations were significantly higher in all IHH groups compared with control group ($p<0.05$), with the highest level observed after three exposures. GSH levels were significantly lower in groups receiving one and four exposure(s) compared with control group ($p<0.05$), whereas groups receiving two and three exposures did not differ significantly from the control. LC3 protein levels were significantly higher in all IHH groups than in control group ($p<0.05$), reaching the highest level after three exposures and remaining elevated after four exposures. In contrast, total mTOR protein levels did not differ significantly among the experimental groups ($p>0.05$).

**CONCLUSION:** Repeated IHH exposure was associated with increased oxidative stress and alterations in antioxidant and autophagy-related responses in the rat brain, as indicated by elevated MDA and LC3 expression and fluctuating GSH levels. Total mTOR protein levels remained unchanged in IHH groups, suggesting that repeated IHH may induce dynamic oxidative stress and autophagy responses without detectable changes in total mTOR.

**KEYWORDS:** autophagy, hypoxia, LC3, oxidative stress, mTOR

***Indones Biomed J. 2026; 18(5): 479-87***

## Introduction

Cellular equilibrium is significantly compromised when tissue oxygenation falls below physiological thresholds, triggering diverse pathological responses depending on the duration and severity of the deficit.(1) Environmental oxygen deprivation at elevated altitudes is further compounded by diminished barometric pressure. This phenomenon is designated as hypobaric hypoxia and directly reduces the partial pressure of inspired oxygen.(2,3) This oxygen restricted state poses a profound challenge to



Copyright © 2026 The Prodia Education and Research Institute.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC-BY-NC) License.

metabolic systems, especially the central nervous system. Although the brain accounts for merely 2% of total body mass, it requires approximately 20% of systemic oxygen consumption to maintain normal functional integrity.(4) Intermittent hypobaric hypoxia (IHH), characterized by cyclic exposures to hypobaric conditions punctuated by normoxic recovery phases has gained prominent attention for investigating physiological adaptations under recurrent oxygen deficits. Unlike continuous chronic exposure, IHH can foster cellular conditioning and endogenous neuroprotection, though the precise physiological outcome remains strictly dependent on exposure frequency and interval length.(5)

The central nervous system's heightened susceptibility to oxygen deprivation arises from its demanding bioenergetic needs, restricted capacity for anaerobic metabolism, and high concentration of peroxidizable membrane lipids. Hypoxia driven oxidative stress induces the overproduction of reactive oxygen species (ROS), driving lipid damage characterized by elevated malondialdehyde (MDA) alongside the rapid exhaustion of endogenous reserves like glutathione (GSH).(6) In response, cells initiate autophagy, which is a specialized lysosomal catabolic mechanism designed to eliminate damaged organelles and protein aggregates to preserve cellular stability.(7) This highly conserved intracellular pathway serves as a regulator of cell fate, survival, and proliferation by recycling damaged structural components into reusable metabolic substrates.(8,9)

Microtubule-associated protein 1 light chain 3 (LC3) functions as a key structural component during autophagic activation, recruiting autophagic receptors through specific LC3-interacting regions (LIRs) to deliver targeted ubiquitinated cargo to autophagosomes.(10) Consequently, tracking LC3 expression provides a reliable quantitative index of autophagic activity.(11) Meanwhile, the mammalian target of rapamycin (mTOR) serves as a primary nutrient sensor and central repressor of autophagy under physiological conditions; however, under hypoxic stress, autophagic processes can inversely downregulate mTOR via lysosomal degradation of mTOR complex 1 (mTORC1) constituents.(12) Although previous studies have examined oxidative stress, antioxidant responses, and autophagy-related mechanisms during intermittent hypoxic exposure, these responses have largely been investigated separately. The simultaneous evaluation of MDA, GSH, LC3, and mTOR in the same experimental model remains limited. Therefore, the present study was conducted to evaluate these biomarkers comprehensively to provide an integrated description of oxidative stress, antioxidant status, and autophagy-related responses in rat brain tissue following repeated IHH exposure.

## Methods

### Study Design

An experimental post-test control group design was conducted using 25 male Sprague-Dawley rats (8 weeks of age, average weight of 200 g). The sample size was determined a priori using the Federer formula for experimental studies, resulting in five animals per group. The animals were randomly assigned into five groups: an unexposed control group maintained under normoxic atmospheric conditions (Control), alongside four intervention groups subjected to IHH at increasing frequencies. Specifically, group receiving a single IHH exposure (Group 1), group receiving two IHH exposures (Group 2), group receiving three IHH exposures (Group 3), and group receiving four IHH exposures (Group 4), with each repeated session separated by a one-week interval. Rats in Group 1 were sacrificed one hour after the first exposure. Meanwhile, for rats in Group 2, Group 3, and Group 4 where subsequent exposure sessions were performed at one-week intervals, the animals were sacrificed one hour after their respective final exposure. Control group was maintained until the end of the experimental period and sacrificed approximately four weeks after the Group 1 (Figure 1). All procedures performed was according to the approved ethical clearance.

### Hypobaric Hypoxia Protocol

Hypobaric exposure was conducted at Lakespra Saryanto Air Force Aerophysiology Laboratory. Hypobaric hypoxia was induced using a specialized decompression chamber calibrated to simulate an altitude of 25,000 feet (282,4 mmHg) for 5 minutes per session, with pressure of approximately 37.65 kPa (282.4 mmHg; 11.12 inHg) under standard atmospheric conditions. Animals were sacrificed one hour after final exposure, and brain tissues were collected for analysis. The detailed altitute and times of this protocol was illustrated in Figure 2.

### Tissue Preparation

Tissue homogenization and all subsequent biochemical analyses were performed at the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. Brain tissue samples were collected one hour

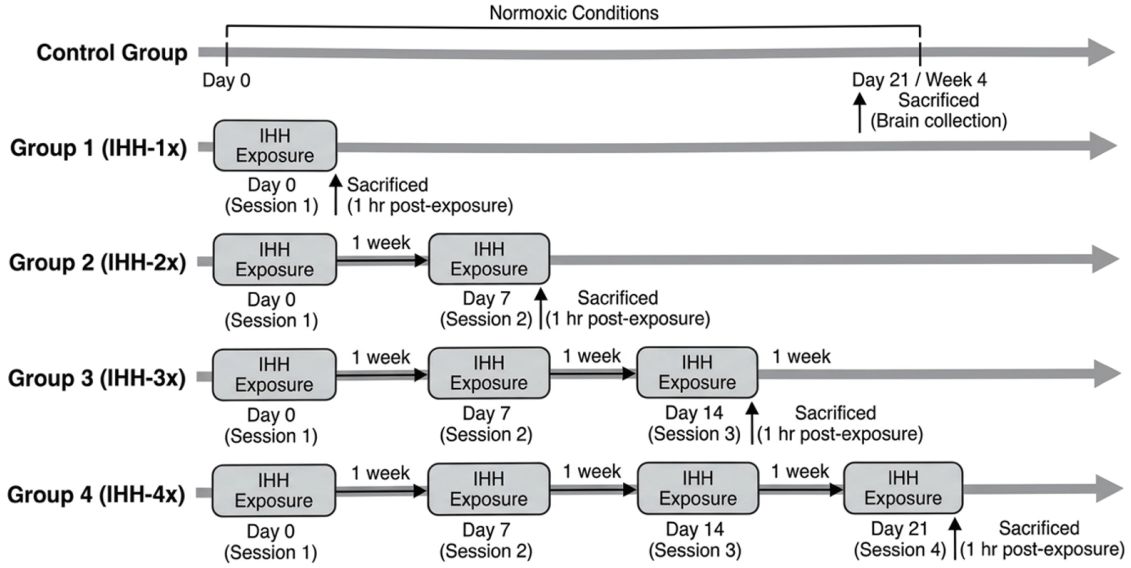



**Figure 1. Experimental protocol and timeline showing intermittent hypobaric hypoxia (IHH) exposure frequency and animal sacrifice schedule.**

after the final IHH exposure. For tissue preparation, 100 mg of brain tissue was homogenized in 1 mL of ice-cold phosphate-buffered saline (PBS) at pH 7.4 using a mechanical homogenizer. The resulting homogenates were centrifuged at 10,000×g for 5 min at 4°C. The cleared supernatants were subsequently collected, aliquoted, and preserved at −80°C for the measurement of MDA, GSH, LC3, and mTOR.

**Measurement of MDA Levels**

Lipid peroxidation in brain homogenates was evaluated by determining MDA levels using the thiobarbituric acid reactive substances (TBARS) spectrophotometric assay. Briefly, a 400 µL volume of tissue supernatant was combined with 200 µL of 20% trichloroacetic acid (TCA) to precipitate proteins, followed by centrifugation at 5,000 rpm for 10 min. A 400 µL aliquot of the resulting supernatant was then reacted with an equal volume (400 µL) of 0.67% thiobarbituric acid (TBA) solution and heated in a 100°C water bath for 10 min. After cooling to room temperature, the optical density of the pink MDA-TBA chromophore was recorded at 530 nm on a spectrophotometer. MDA concentrations were interpolated using a standard linear regression curve (Y = a X + b, where Y = optical density and X = MDA concentration in nmol/mL).(13)

**Measurement of GSH Levels**

Endogenous reduced GSH concentration was quantified using a spectrophotometric 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) assay. Briefly, a 50 µL volume of brain homogenate supernatant was treated with 200 µL of 5% TCA and centrifuged at 3,000×g for 10 min to remove proteins. The cleared supernatant was subsequently diluted with phosphate buffer (pH 8.0) and mixed with 25 µL of 0.01% DTNB. Following a 1-hour incubation period at room temperature, absorbance was measured at 412 nm. Total GSH levels were calculated against a calibration curve generated from known glutathione standards.(14)

**Measurement of LC3 and mTOR Levels**

Quantitative evaluation of LC3 was performed with sandwich enzyme-linked immunosorbent assay (ELISA) using Indolisa® Rat MAP1LC3B ELISA (Cat. No. ID-ER14314; PT Indogen Intertama, Jakarta, Indonesia), which targets MAP1LC3B (LC3B) protein. Meanwhile the evaluation of mTOR protein expression was performed using Indolisa® Rat mTOR ELISA (Cat. No. ID-ER12549; PT

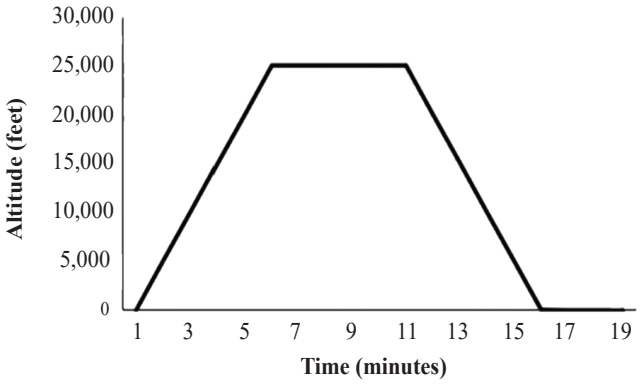



**Figure 2. Hypobaric hypoxia exposure design.**

Indogen Intertama). Supernatants from tissue homogenates were loaded onto pre-coated microplates to allow target antigen capture. Biotin-conjugated detection antibodies specific to LC3 or mTOR were subsequently introduced, followed by the addition of avidin-horseradish peroxidase (HRP) conjugate. Unbound reagents were removed through washing, after which 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added, developing a color intensity directly proportional to target protein concentration. Color development was arrested using 1 M sulfuric acid stop solution, and optical density was recorded at 450 nm on a microplate reader. Sample concentrations were interpolated using standard calibration curves constructed from serial dilutions of purified LC3 and mTOR standards.

### Statistical Methods

Statistical evaluations were conducted using GraphPad Prism version 9.0 (GraphPad Software Inc, San Diego, CA, USA), with statistical significance established at $p<0.05$. Differences among the control and experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test for multiple comparisons. All data were verified for normality and variance homogeneity prior to final analysis.

## Results

### IHH Elevated MDA Concentrations

The cerebral lipid peroxidation levels were determined across all experimental to evaluate hypoxia-induced oxidative damage. Relative to the normoxic control group (0.0990±0.0350 nmol/mg protein), exposure to IHH resulted in statistically significant elevations in brain MDA concentrations across all treatment groups ($p<0.05$). Group 1 marked a significant rise in MDA content (0.255±0.0605 nmol/mg protein), while Group 2 yielded a value of 0.211±0.0429 nmol/mg protein. Peak lipid peroxidation was recorded following Group 3 (0.294±0.0122 nmol/mg protein). In Group 4, MDA levels exhibited a minor attenuation (0.234±0.0426 nmol/mg protein) compared to Group 3, yet remained significantly higher than baseline control values ($p<0.05$) (Figure 3).

### IHH Differentially Affected GSH Concentrations

Evaluation of reduced GSH levels demonstrated frequency-dependent fluctuations across the different hypobaric exposure protocols. Compared to baseline Control group (0.412±0.133 µg/mg protein), animals subjected to Group 1

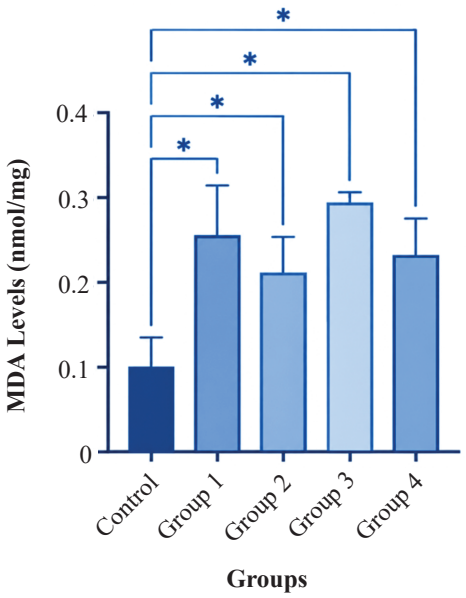



**Figure 3. Brain MDA levels following IHH exposure.** Data are presented as mean±SD (n=5/group). Statistical significance (*$p<0.05$) was determined by one-way ANOVA followed by Bonferroni's post hoc test.

(0.256±0.0513 µg/mg protein) and Group 4 (0.256±0.0385 µg/mg protein) exhibited statistically significant reductions in cerebral GSH content ($p<0.05$). Conversely, Group 2 (0.296±0.0764 µg/mg protein) and Group 3 (0.312±0.0286 µg/mg protein) maintained GSH levels that were not statistically different from the normoxic control ($p>0.05$), reflecting a non-linear modulation of endogenous antioxidant capacity (Figure 4).

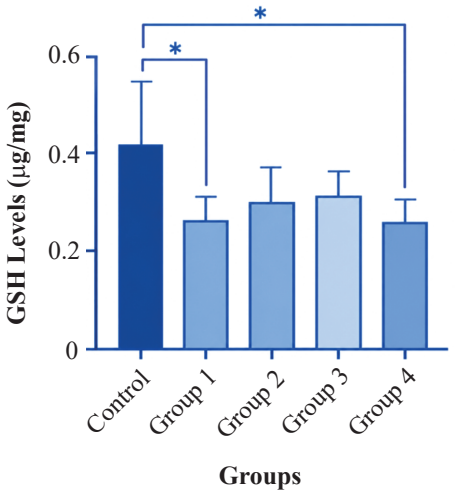



**Figure 4. Brain GSH levels following IHH exposure.** Data are presented as mean±SD (n=5/group). Statistical significance (*$p<0.05$) was determined by one-way ANOVA followed by Bonferroni's post hoc test.

### IHH Increased LC3 Protein Concentrations

Quantitative analysis of LC3 expression revealed significant autophagic activation following hypobaric exposure. Compared to the Control group (2.59±0.7 ng/mg protein), all IHH-treated groups demonstrated statistically significant elevations in cerebral LC3 levels ($p$<0.05). Initial exposure (Group 1) induced a rapid upregulation to 9.132±1.987 ng/mg protein, followed by a moderate elevation in Group 2 (7.44±1.86 ng/mg protein). Maximal LC3 accumulation occurred in Group 3 (12.48±2.89 ng/mg protein) and remained elevated in Group 4 (12.23±2.82 ng/mg protein), suggesting a saturation threshold in autophagic induction after three exposure cycles (Figure 5).

### IHH Did Not Affect the Total mTOR Protein Concentrations

The evaluation of total mTOR protein expression demonstrated stable levels across all experimental conditions, with no statistically significant differences detected between any IHH-exposed group and the Control group (15.65±3.343 pg/mg protein, $p$>0.05). Minor numerical fluctuations were observed, with Group 1 exhibiting 11.49±3.152 pg/mg protein, Group 2 recording 12.61±2.116 pg/mg protein, and Group 4 measuring 12.36±2.101 pg/mg protein. Meanwhile, mTOR expression in Group 3 (15.30±0.5788 pg/mg protein) closely approximated control values (Figure 6). These data confirm that IHHdid not significantly alter total mTOR protein abundance under the evaluated conditions.

## Discussion

The complex interaction among oxidative stress, antioxidant responses, autophagy-related processes, and mTOR signaling provides a broader perspective on the adaptive response of the rat brain following IHH. Functioning as an established model of hypoxic preconditioning, IHH has been proposed to enhance cellular adaptation to repeated oxygen deprivation. Previous studies have shown that repetitive short-duration hypoxic protocols improve cerebral blood perfusion and systemic oxygen saturation, suggesting that intermittent hypoxic exposure may induce adaptive physiological responses rather than persistent tissue injury.(15)

Hypoxia hypobaric stress provokes an overproduction of ROS, initiating oxidative stress. The central nervous system remains exceptionally vulnerable to ROS-mediated lipid degradation owing to its high lipid density, specifically the abundance of polyunsaturated fatty acids within neuronal cell membranes.(6,16,17) Previous reports have established that hypobaric hypoxia causes significant MDA accumulation across both cerebral cortical and cerebellar structures, serving as a classic indicator of oxidative lipid damage.(18,19) Consistent with these observations, all IHH-treated groups in the present study displayed elevated MDA concentrations relative to normoxic controls, indicating increased lipid peroxidation following repeated IHH exposure. MDA concentrations reached the

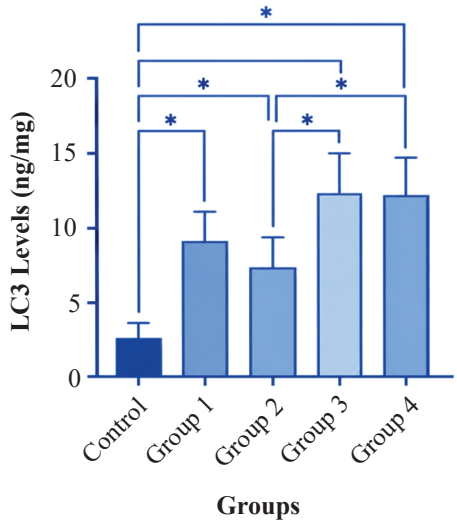



**Figure 5. Brain LC3 levels following IHH exposure.** Data are presented as mean±SD (n=5/group). Statistical significance (*$p$<0.05) was determined by one-way ANOVA followed by Bonferroni's post hoc test.

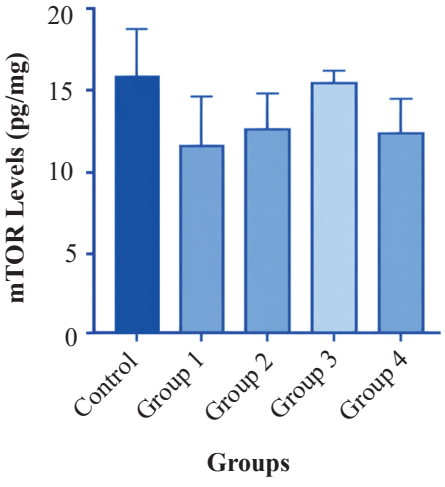



**Figure 6. Brain mTOR levels following IHH exposure.** Data are presented as mean±SD (n=5/group). Statistical significance (*$p$<0.05) was determined by one-way ANOVA followed by Bonferroni's post hoc test.

highest level after three exposure cycles and subsequently declined after four exposures. This non-linear pattern may reflect changes in the balance between oxidative stress and endogenous adaptive responses during repeated exposure. Elevated MDA concentrations have been reported to activate nuclear factor erythroid 2-related factor 2 (Nrf-2) signaling, which subsequently promotes the expression of antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx).(19) Such antioxidant responses may contribute to the regulation of oxidative stress during repeated IHH exposure.

As a vital non-enzymatic endogenous antioxidant, reduced GSH operates in tandem with the enzymatic catalyst GPx to convert hydrogen peroxide ($H_2O_2$) into water ($H_2O$). In the current investigation, cerebral GSH reserves experienced marked depletion in Group 1 and Group 4. This reduction may reflect increased utilization of GSH in response to oxidative stress following hypobaric exposure. Intriguingly, despite the marked difference in exposure frequency, Group 1 and Group 4 displayed nearly identical levels of GSH reduction. This equivalence points to either a distinct threshold in hypoxia-induced oxidative stress or a metabolic recalibration of cellular antioxidant capacity. The sharp decline in GSH observed in Group 1 following a single session aligns with earlier evidence demonstrating that acute oxygen deprivation (*e.g.*, 7% $O_2$ for 6 hours) significantly lowers brain GSH content and alters the GSH/oxidized glutathione (GSSG) redox ratio.(20)

Conversely, GSH concentrations in Group 2 and Group 3 remained close to normoxic baseline values, indicating that IHH disturbs redox balance only at specific exposure frequencies. This physiological profile suggests a compensatory induction of antioxidant defences, wherein two or three exposure sessions stimulate adaptive machinery to preserve GSH availability, attenuate oxidative injury, and support cellular adaptation under oxidative stress. In agreement with these observations, preconditioning models utilizing an IHH regimen (3 h/day at 460 mmHg across 8 days) preserved both cortical and hippocampal GSH pools during subsequent hypoxic challenges.(21) Taken together, these findings reinforce the concept that IHH may induced adaptive responses associated with oxidative stress regulation through integrated mechanisms, encompassing improved cerebrovascular regulation, upregulation of antioxidant and anti-apoptotic cascades (such as Nrf-2, hypoxia-inducible factor (HIF)-1α), mitigation of excitotoxic pathways, and dynamic modulation of autophagic flux.(22)

During conditions of cellular oxygen deprivation, energy supply becomes severely restricted, prompting autophagy to operate as a critical survival mechanism. (23) By systematically recycling damaged organelles and macromolecular complexes, autophagic processes supply essential metabolic substrates to preserve nutrient balance and energy homeostasis during acute oxidative stress. (8,24,25) Findings from the current study demonstrate that expression of the autophagic marker LC3 escalated in a frequency-dependent manner with successive hypobaric sessions, reaching maximal values after three exposures and remained elevated after four exposures without a significant difference from Group 3. Group 3 displayed the most pronounced autophagic response. This finding coincided with peak MDA levels and relatively preserved GSH levels. This pattern may reflect an autophagy-related response associated with cellular adaptation to oxidative stress. However, because the present study measured LC3B protein levels rather than autophagic flux, the observed increase in LC3B cannot by itself establish increased autophagic activity. This alignment suggests that LC3 response might initially prioritizes the selective removal of damaged intracellular structures to limit ROS-induced injury and prevent downstream neurodegeneration.(26) These observations followed the established molecular models establishing $H_2O_2$ and superoxide radicals ($O_2^-$) as key upstream triggers of autophagic induction.(27,28)

A significant divergence in LC3 expression was observed when comparing Group 2 to Group 3 and Group 4, reflecting a possible frequency-dependent escalation in autophagic induction as hypoxic exposures accumulated. This distinct shift between two and three sessions points to a critical physiological threshold required to achieve maximal autophagic engagement. Similar patterns have been documented in rat H9c2 cardiomyocytes and C57BL/6 mouse models subjected to intermittent hypoxic protocols (4–20% every 30 minutes across 1–4 days), where key autophagic markers, including LC3-II and Beclin-1, increased progressively from day-1 to -3 before reaching a peak on the third day.(29)

In Group 4, sustained autophagic turnover likely serves a vital compensatory function, clearing oxidized macromolecules to offset depleted GSH reserves and limit ongoing lipid damage. The lack of significant difference in LC3 levels between Group 3 and Group 4 indicates that autophagic capacity reaches a plateau beyond three exposure cycles. Together, these observations demonstrate that IHH triggers a robust autophagic response in cerebral tissue, with peak activation occurring after three exposures. These findings align with earlier work confirming that intermittent hypobaric exposure upregulates autophagic flux

in the central nervous system, thereby preserving vascular endothelial integrity and fostering neuronal survival.(30,31) Overall, autophagy-related processes and GSH antioxidant capacity may represent complementary cellular responses to oxidative stress during hypobaric hypoxia. Autophagy-related mechanisms can contribute to the removal of damaged cellular components, while GSH helps maintain intracellular redox balance and protect against oxidative damage. mTOR is a key regulator of cellular metabolism and autophagy that integrates nutrient availability and environmental stress signals.(32,33) While our results established statistically significant alterations in MDA, GSH, and LC3 markers, no statistically significant differences were observed in mTOR protein levels across any experimental groups relative to the control baseline. Under physiological, nutrient-replete conditions, active mTOR complex signaling exerts tonic repression on autophagic initiation; conversely, stress state transition disinhibits autophagic processing to facilitate organelle recycling and energy survival pathways.

In the present study, however, total mTOR protein levels did not differ significantly among the experimental groups, despite significant changes in MDA, GSH, and LC3. Although the IHH groups showed numerically lower mTOR values than the control group, these differences were not statistically significant and therefore should be interpreted with caution.

The increase in LC3 expression observed following repeated IHH exposure occurred without a corresponding change in total mTOR protein levels. This finding indicates that the increase in LC3 expression occurred without a detectable change in total mTOR protein abundance under the present experimental conditions; however, it does not establish whether mTOR signaling activity was altered. Because the present study measured total mTOR protein levels rather than phosphorylated mTOR or downstream signaling components, changes in mTOR pathway activity cannot be determined. Previous studies have reported that hypoxic stress can regulate autophagy through mTOR-dependent as well as mTOR-independent pathways.(34–37) Therefore, the increased LC3 expression observed in this study may reflect activation of autophagy-related processes through mechanisms that were not directly evaluated. Further studies examining mTOR phosphorylation, mTORC1 activity, and additional autophagy markers are needed to clarify the role of mTOR signaling during repeated intermittent hypobaric hypoxia.

Taken together, the combined evaluation of MDA, GSH, LC3, and mTOR provides an integrated view of the biological response to repeated intermittent hypobaric hypoxia. The increase in MDA indicates oxidative stress induced by repeated hypoxic exposure, whereas the preservation of GSH in Group 2 and Group 3 suggests the activation of antioxidant adaptation during intermediate exposure frequencies. Concurrently, elevated LC3 expression might indicate activation of autophagy-related processes during the same exposure period. In contrast, the absence of significant changes in mTOR protein levels suggests that alterations in oxidative stress and LC3 expression were not accompanied by detectable changes in total mTOR abundance under the present experimental conditions. Even though the small sample size limits the strength of the conclusions, but the result of current study provides a broader perspective and integrated assessment on the coordinated oxidative stress and autophagy-related responses to repeated IHH exposure. Further studies evaluating mTOR signaling activity and autophagic flux are warranted to clarify the mechanisms underlying these responses.

## Conclusion

In conclusion, repeated IHH exposure was associated with alterations in oxidative stress, antioxidant status, and LC3 expression in the rat brain. IHH exposure increased MDA concentrations across all treatment groups, while GSH levels showed a fluctuating response across increasing exposure frequencies. LC3 expression was also increased following repeated IHH exposure, with the highest level observed after three exposures. In contrast, total mTOR protein levels did not differ significantly among the experimental groups. Overall, these findings suggest that repeated IHH exposure may induce a dynamic oxidative stress and autophagy-related response in the rat brain, while no detectable alteration in total mTOR protein abundance was observed under the present experimental conditions.

## Acknowledgments

The authors would like to express their sincere gratitude to the Faculty of Medicine, Universitas Indonesia, and all individuals who contributed to the completion of this study. This study was funded by the Hibah Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) 2020, Grant No. NKB-2760/UN2.RST/HKP.05.00/2020, from the Directorate General of Higher Education, Research, and Technology, Indonesia.

## Authors Contribution

NM and ARP contributed to the conceptualization and planning of the research and assisted in the interpretation of the results and data analysis. R performed the data acquisition and collection, calculated the experimental data, and performed the data analysis. All authors contributed to the critical revision of the manuscript and approved the final version.

## Ethical Statement

Ethical clearance for all animal handling and experimental procedures was granted by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Approval No. KET-787/UN2.F1/ETIK/PPM.00.02/2022).

## Conflict of Interest

The authors declared that there were no conflicts of interests.

## References

1. Rahane D, Dhingra T, Chalavady G, Datta A, Ghosh B, Rana N, *et al.* Hypoxia and its effect on the cellular system. Cell Biochem Funct. 2024; 42(2): e3940. doi: 10.1002/cbf.3940.
2. Shaw DM, Cabre G, Gant N. Hypoxic hypoxia and brain function in military aviation: Basic physiology and applied perspectives. Front Physiol. 2021; 12: 665821. doi: 10.3389/fphys.2021.665821.
3. Dewi S, Yulhasri Y, Mulyawan W. The impact of intermittent hypobaric hypoxia exposures on triacylglycerol synthesis in rat liver. Rep Biochem Mol Biol. 2021; 10(3): 437–44.
4. Özugur S, Kunz L, Straka H. Relationship between oxygen consumption and neuronal activity in a defined neural circuit. BMC Biol. 2020; 18(1): 76. doi: 10.1186/s12915-020-00811-6.
5. Burtscher J, Citherlet T, Camacho-Cardenosa A, Camacho-Cardenosa M, Raberin A, Krumm B, *et al.* Mechanisms underlying the health benefits of intermittent hypoxia conditioning. J Physiol. 2024; 602(21): 5757–83.
6. Alam R, Ahsan H, Khan S. The role of malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP) in patients with hypertension. Mol Cell Biomed Sci. 2023; 7(2): 58–64.
7. Yun HR, Jo YH, Kim J, Shin Y, Kim SS, Choi TG. Roles of autophagy in oxidative stress. Int J Mol Sci. 2020; 21(9): 3289. doi: 10.3390/ijms21093289.
8. Mizushima N, Levine B. Autophagy in human diseases. N Engl J Med. 2020; 383(16): 1564–76.
9. Mohamed Ibrahim HA, Mohamed Hussein A, Gabr M, El-Saeed RA, Abd-Alhakem Ammar O, Hassan Mosa AA, *et al.* Effect of melatonin on alpha synuclein and autophagy in dopaminergic neuronal differentiation of adipose mesenchymal stem cells. Rep Biochem Mol Biol. 2023; 12(1): 13–26.
10. Lee YK, Lee JA. Role of the mammalian ATG8/LC3 family in autophagy: Differential and compensatory roles in the spatiotemporal regulation of autophagy. BMB Rep. 2016; 49(8): 424–30.
11. Oh S hee, Choi Y bok, Kim J hyun, Weihl CC, Ju J sun. Quantification of autophagy flux using LC3 ELISA. Anal Biochem. 2017; 530: 57–67.
12. Schmeisser K, Parker JA. Pleiotropic effects of mTOR and autophagy during development and aging. Front Cell Dev Biol. 2019; 7: 192. doi: 10.3389/fcell.2019.00192.
13. Hardiany NS, Sucitra S, Paramita R. Profile of malondialdehyde (MDA) and catalase specific activity in plasma of elderly woman. Health Science Journal of Indonesia. 2020;10(2): 132–6.
14. Hardiany NS, Karman AP, Calista ASP, Anindyanari BG, Rahardjo DE, Novira PR, *et al.* The effect of fasting on oxidative stress in the vital organs of New Zealand white rabbit. Rep Biochem Mol Biol. 2022; 11(2): 190–9.
15. Guan Y, Gu Y, Shao H, Ma W, Li G, Guo M, *et al.* Intermittent hypoxia protects against hypoxic-ischemic brain damage by inducing functional angiogenesis. Journal of Cerebral Blood Flow and Metabolism. 2023; 43(10): 1656–71. doi: 10.1177/0271678X231185507.
16. Shichiri M. The role of lipid peroxidation in neurological disorders. Vol. 54, Journal of Clinical Biochemistry and Nutrition. 2014; 54(3): 151–60.
17. Soetjipto, Murbani ID, Harnanik T. Hyperbaric oxygen ameliorates the expression of tumor growth factor-β and malondialdehyde in pristane-induced lupus nephritis mice model. Indones Biomed J. 2023; 15(1). 54–60.
18. Hou Y, Wang X, Chen X, Zhang J, Ai X, Liang Y, *et al.* Establishment and evaluation of a simulated high altitude hypoxic brain injury model in SD rats. Mol Med Rep. 2019; 19(4). 2758–66.
19. Wardaya W, Sukmawati D, Ibrahim N, Ferdinal F, Mudjihartini N, Sadikin M, *et al.* Intermittent exposure to hypobaric hypoxia increases VEGF, HIF-1α, and Nrf-2 expressions in brain tissue. Indones Biomed J. 2025; 17(2): 197–206.
20. Coimbra-Costa D, Alva N, Duran M, Carbonell T, Rama R. Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain. Redox Biol. 2017; 12: 216–25.
21. Coimbra-Costa D, Garzón F, Alva N, Pinto TCC, Aguado F, Torrella JR, *et al.* Intermittent hypobaric hypoxic preconditioning provides neuroprotection by increasing antioxidant activity, erythropoietin expression and preventing apoptosis and astrogliosis in the brain of adult rats exposed to acute severe hypoxia. Int J Mol Sci. 2021; 22(10): 5272. doi: 10.3390/ijms22105272.
22. Fan X, Wang H, Zhang L, Tang J, Qu Y, Mu D. Neuroprotection of hypoxic/ischemic preconditioning in neonatal brain with hypoxic-ischemic injury. Rev Neurosci. 2021; 32(1): 23–34.
23. Fitriana N, Iswanti FC, Sadikin M. The role of hypoxia-inducible factor in Mycobacterium tuberculosis-infected macrophages. Mol Cell Biomed Sci. 2024; 8(1): 23–30.
24. Farnung J, Muhar M, Liang JR, Tolmachova KA, Benoit RM, Corn JE, *et al.* Semisynthetic LC3 probes for autophagy pathways reveal a noncanonical LC3 interacting region motif crucial for the enzymatic activity of human ATG3. ACS Cent Sci. 2023; 9(5): 1025–34.
25. Gómez-Virgilio L, Silva-Lucero MDC, Flores-Morelos DS, Gallardo-Nieto J, Lopez-Toledo G, Abarca-Fernandez AM, *et al.* Autophagy: A key regulator of homeostasis and disease: An overview of molecular mechanisms and modulators. Cells. 2022; 11(15): 2262. doi: 10.3390/cells11152262.
26. Antonio José Cabrera-Serrano, José Manuel Sánchez-Maldonado,

Carmen González-Olmedo, Carretero-Fernández M, Díaz-Beltrán L, Gutiérrez-Bautista JF, *et al.* Crosstalk between autophagy and oxidative stress in hematological malignancies: Mechanisms, implications, and therapeutic potential. Antioxidant. 2025; 14(3): 264. doi: 10.3390/antiox14030264.

27. Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: The clash between damage and metabolic needs. Cell Death Differ. 2015; 22(3): 377–88.
28. Chen Y, Azad MB, Gibson SB. Superoxide is the major reactive oxygen species regulating autophagy. Cell Death Differ. 2009; 16(7): 1040–52.
29. Chang JC, Hu WF, Lee W Sen, Lin JH, Ting PC, Chang HR, *et al.* Intermittent hypoxia induces autophagy to protect cardiomyocytes from endoplasmic reticulum stress and apoptosis. Front Physiol. 2019; 10: 995. doi: 10.3389/fphys.2019.00995.
30. Song S, Tan J, Miao Y, Zhang Q. Effect of different levels of intermittent hypoxia on autophagy of hippocampal neurons. Sleep Breath. 2017; 21(3): 791–8.
31. Cui F, Guan Y, Guo J, Tian YM, Hu HF, Zhang XJ, *et al.* Chronic intermittent hypobaric hypoxia protects vascular endothelium by ameliorating autophagy in metabolic syndrome rats. Life Sci. 2018; 205: 145–54.
32. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell. 2017; 168(6): 960–76.
33. Szwed A, Kim E, Jacinto E. Regulation and metabolic functions of mTORC1 and mTORC2. Physiol Rev. 2021; 101(3): 1371–426.
34. Kimball R, Wayment M, Merrill D, Wahlquist T, Reynolds PR, Arroyo JA. Hypoxia reduces placental mTOR activation in a hypoxia-induced model of intrauterine growth restriction (IUGR). Physiol Rep. 2015; 3(12): e12651. doi: 10.14814/phy2.12651.
35. Koren I, Reem E, Kimchi A. DAP1, a novel substrate of mTOR, negatively regulates autophagy. Current Biology. 2010; 20(12): 1093–8.
36. Xie Y, Lei X, Zhao G, Guo R, Cui N. mTOR in programmed cell death and its therapeutic implications. Cytokine Growth Factor Rev. 2023: 71–72: 66–81.
37. Yu L, McPhee CK, Zheng L, Mardones GA, Rong Y, Peng J, et al. Termination of autophagy and reformation of lysosomes regulated by mTOR. Nature. 2010; 465(7300): 942–6.