

Differences Of Hyperbaric Oxygen Therapy With Compressed Air Before Decompression Diving Against Carbonyl Proteins, IL-6, Casp-3, Casp-9, Brain Histopathology Images, And Gait Analysis In Sprague Dawley (Rat)

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Abstract: Decompression dives are characterized by rapid ascent from significant depths, which can potentially lead to decompression sickness (DCS). Giving hyperbaric oxygen therapy (HBOT) before decompression diving can reduce the occurrence of DCS. This study used a model of 56 male Sprague Dawley rats, which were divided into two groups, namely the air group of 28 and the HBOT group of 28 heads. Each group was divided into 4-observation time for each group consisted of 7 heads. The 0,3,6 and 24-hour air group (KPU 0,1,2,3) and the hyperbaric oxygen therapy group 0,3,6 and 24 hours (KPT 0,1,2,3). The air group (KPU) was given compressed air at a pressure of 2.4 ATA and dived for 75 minutes. The treatment group (KPT) was given 100% oxygen HBOT at a pressure of 2.4 ATA and dived for 75 minutes. After the two groups rose to the surface at rest for 3 hours, they then did a decompression dive at a pressure of 6 ATA for 90 minutes, then rose to the surface at a rate of 3 ATA per minute. The results showed that the KPT group at each hour of observation showed a decrease in several biomarkers of DCS markers. Meanwhile, the KPU Group showed severe symptoms, including seizures, rapid breathing, paralysis, and death. Tissue analysis revealed a significant increase in the expression of carbonyl protein, IL-6, caspase 3, and caspase 9, in addition to a higher number of air bubbles in brain histopathology and poorer gait analysis results with p -values of <0.05 for all biomarkers. Meanwhile, the KPT group was lower in the occurrence of increased biomarkers of expression of carbonyl protein, IL-6, caspase 3, and caspase 9, fewer air bubbles in brain histopathology, and better gait analysis results with a p -value of <0.05 . The expression levels of the KPT group against the KPU group were most significantly observed at 6 hours post-diving, but the most severe symptoms appeared after 24 hours, suggesting a delayed but damaging impact. The study concluded that the administration of 100% HBOT 3 hours before decompression diving may decrease cell damage and bubble formation, and worsen gait analysis results.

Keywords: Animal model of decompression disease, Decompression diving, carbonyl protein, Interleukin 6, Caspase 3, Caspase 9, Histopathology of the brain, DCS histopathology, Gait Analysis.

1. INTRODUCTION

Decompression diving involves exposure to much higher atmospheric pressure compared to the Earth's surface, especially at very deep depths or during long durations of diving. This increase in pressure puts significant physiological pressure on the human body, especially on the central nervous system, lungs, heart, and brain. One of the main risks associated with this activity is decompression sickness (DCS), which occurs when divers rise to the surface too quickly, exceeding the safe Ascent rate of 9 meters per minute. DCS is mainly caused by the formation of nitrogen bubbles in the body, which fail to dissolve or exit properly during climbing. These bubbles can cause a variety of serious health problems, including brain tissue damage, neurological dysfunction, and even death.

One of the crucial factors involved in DCS is the formation of microparticles, which are vesicular structures derived from the walls of blood vessels. Research shows that microparticles can increase up to 3.4 times during decompression diving, which is driven by oxidative stress during diving. These microparticles interact with various components of the blood, including endothelial cells, platelets, and leukocytes, triggering neutrophil activation and coagulation processes. This condition not only interferes with blood flow but also increases free radicals, the body's inflammatory response, and cell death are characterized by increased expression of

carbonyl proteins, caspase-3 (casp-3), caspase-9 (casp-9), and interleukin-6 (IL-6), respectively. Carbonyl protein in this study is a marker of increased oxidants, and IL-6 is a pro-inflammatory cytokine that mediates inflammatory and immune responses in the body. Increased levels of IL-6 during decompression dives are triggered by skeletal muscle contractions and the release of stress hormones such as cortisol. Excessive levels of IL-6 can cause a variety of side effects, including decreased muscle strength, inflammation, and endothelial dysfunction of blood vessels. In addition, other biomolecular markers such as Casp-3 and Casp-9 play an important role in apoptosis or programmed cell death that occurs due to stress during decompression diving. In this study, DCS can cause an increase in nitrogen bubbles in brain histopathology examination and Basso, Beattie and Bresnahan (BBB) poor scoring as an assessment of gait analysis.

The problem that arises from these findings is the need for further understanding of the physiological mechanisms involved in DCS, in particular the role of IL-6 and Casp-3 and 9 in tissue damage within the brain, as well as the impact of oxidative stress on the formation of microparticles during diving characterized by increased expression of carbonyl proteins. Administration of HBOT 3 hours prior to the decompression dive simulation significantly lowered the incidence and mortality of DCS in Rat models and verified the involvement of carbonyl proteins, IL-6, Casp-3, Casp-9, the number of nitrogen bubbles in the brain, and the BBB scoring gait analysis. HBOT As moderate oxidative stress can have antioxidant, anti-inflammatory, and antiapoptotic consequences.

This study aimed to prove that the beneficial effects of HBOT administration before decompression diving simulation were related to decreased expression of Carbonyl Proteins, IL-6, Casp-3, and Casp-9, as well as the number of nitrogen bubbles in the brain and improvement in BBB Scoring gait analysis in a model animal of male Sprague Dawley rat. 56 male Spague Dawley rat were used to detect the expression of Carbonyl Protein, IL 6, Casp-3, Casp-9, Nitrogen bubbles in the brain, Scoring BBB on gait analysis, after a single administration of HBOT 3 hours before diving. Changes in the incidence rate of DCS, a decrease in the number of nitrogen bubbles, inflammation, apoptosis, and improvement in BBB scoring in gait analysis, may reflect the effect of giving HBOT 3 hours before decompression diving.

2. METHOD

This study is a pre-clinical study using interventional research methods with true experimental studies. The "Analytic Experimental" research design used Sprague Dawley experimental rats.

This study consisted of two treatments, the first treatment in the KPU group of 28 was given compressed air at a pressure of 2.4 ATA and dived for 75 minutes, and the KPT group of 28 was given 100% oxygen HBOT at a pressure of 2.4 ATA and dived for 75 minutes. This is a pretreatment before being given a decompression dive simulation.

The second treatment was administered after the Sprague Dawley experimental Rat had completed the first treatment and was already at surface pressure, then rested for 3 hours. After 3 hours of rest, they were given a simulated treatment of decompression diving at a pressure of 6 ATA for 90 minutes and then rose to the surface at ascent rate of 3 ATA per minute. The KPU and KPT groups, After being on the surface, 4 observation times were carried out, namely 0 hours, 3 hours, 6 hours and 24 hours. Each observation time was made in the form of gait analysis using Basso, Beattie, Bresnahan (BBB) Scoring, followed by the Sprague Dawley experimental Rat to undergo surgery to take brain tissue, brain tissue was divided into 2 parts, one part for biomarker examination of Carbonyl Protein, IL, 6, Casp-3 and Casp-9 and one part for histopathological examination of the number of air bubbles.

2.1 Termination and Retrieval Tissue

The Sprague Dawley experimental rats were terminated by administering a lethal dose of anesthesia with a dose of ketamine 50 mg/kgBB, xylazine 10 mg/kgBB injected intramuscular. The dead rats were confirmed with the pedal reflex. Then the tissue complex to be examined is taken, namely the brain (cerebellum). Brain tissue is divided into two parts. The first part is put into a labeled tube and glued to make it airtight, and then frozen and stored in a refrigerator minus 80°C for the examination of Carbonyl Protein Expression, IL-6, Casp-3, Casp-9, and some others are stored in a 10% formalin solution for histopathological examination. The Sprague Dawley experimental Rats are then buried properly.

3.2 Decompression diving simulation and Air or HBOT pre-treatment

The pre-treatment of giving air and HBOT was carried out using an chamber device at YARSI Hospital & University Jakarta Indonesia. The animals were put into small cages of 7 heads each. The hyperbaric chamber was given a pressure of 2.4 ATA with air for 75 minutes. The KPU group was given an air hose into the cage, while the KPT group was given a 100% oxygen hose into the cage. Then it rises to the surface at a slow speed \pm 10 minutes. To reduce discomfort in animals, try to reduce the pressure when descending or ascending as gently as possible. The noise due to the pressure process is muffled with sufficient sound suppression to reduce the noise. The temperature in the chamber is set to range from 18 - 26°C and the humidity ranges from 40 - 70 %.

2.3 Data collection techniques

Data collection consists of the following procedures:

1) Measurement of Biomarker Expression

Carbonyl proteins, analyzed using Western Blot (immunoblots). Study protein expression, protein modification, and protein interactions. Free radicals derived from oxygen have an important role in aging, apoptosis, and cancer. Meanwhile, the expressions of IL-6, Casp-3, and Casp-9 were analyzed using reverse transcriptional polymerase chain reaction (RT-PCR). Tissue samples from the brain were extracted after each recovery period for RNA isolation, followed by cDNA synthesis and RT-PCR to measure expression levels.

2) Histological Analysis of Air Bubbles

Brain tissue was processed for histology using Hematoxylin Eosin (HE) staining to visualize air bubbles. Pieces of tissue were examined under a light microscope with 40x magnification, and five fields of view were evaluated per sample to determine the rate of bubble formation.

2.4 Data Analysis

Prior to statistical analysis, the data were tested for homogeneity using the Levene test to check the consistency of weight between groups. The distribution of the data is then assessed using the Shapiro-Wilk test to determine normality:

- 1) If the data follows a normal distribution, an independent sample t-test is used to compare the groups.
- 2) For abnormally distributed data, the Mann-Whitney test is applied.

After the normality test, the main analysis involved a comparison of the expression levels of carbonyl proteins, IL-6, Casp-3, Casp-9, and air bubble formation, as well as the results of BBB Scoring for gait analysis between the KPU group and the KPT group for each sub-group of observation time. The SPSS software was used to perform statistical tests, with a significance level (α) set to 0.05. The analysis focused on whether there was a significant difference between the KPU group and the KPT group for each biomarker and histological output, as well as the results of BBB scoring for the gait analysis of experimental animals.

2.5 Ethical Considerations

All experimental protocols follow ethical guidelines for animal research, ensuring minimal suffering and proper care of Sprague-Dawley rat during the experimental process. By compiling this study, the researchers were able to effectively measure the physiological effects of decompression diving on the experimental group, thus forming a clear understanding of the relationship between dive duration, biomarkers, and brain tissue damage. The use of western blot, RT-PCR, histology, and gait analysis used the Basso, Beattie, and Bresnahan (BBB) scoring method. Rigorous statistical methods ensure that the results are reliable and meaningful to understand the benefits of HBOT in lowering the rate of decompression sickness in this animal model.

FINDINGS AND DISCUSSIONS

The physical animal is in a healthy condition with a body weight between 200 - 250 grams (238.6 ± 17 grams, average \pm sd). The physical characteristics of the body weight (gram) of male rats of the Sprague Dawley strain in the KPT group and the KPU group, with the Levene test ($p>0.05$), found that the weight data of the two groups were relatively homogeneous.

CARBONYL PROTEIN: Carbonyl protein expression check to measure the degree of oxidative damage to proteins. Carbonyl protein build up can occur as a result of oxidative stress. Oxidative damage to proteins can lead to decreased cell function, cell aggregation, and even cell death that occurs in DCS. The results of the measurement at the observation time of the 6th and 24th hours were analyzed with an unpaired t-test ($p < 0.05$), the results showed that the effect of HBOT on the KPT group was better in reducing the expression of Carbonyl Protein compared to the results of measuring the expression of Carbonyl Protein in the KPU group with an observation time of 6 hours and 24 hours (table 1). Meanwhile, at the beginning of observation, no significant difference was found in the expression of Carbonyl Protein in the two groups. The 6-hour measurement time showed very significant results. This means that the administration of HBOT to the KPT group during the 6th hour measurement was very useful in reducing the expression of Carbonyl Protein compared to the KPU group.

DCS can occur in the KPU group, while in the KPT group, it can reduce the incidence of DCS by decreasing the expression of Carbonyl Protein after diving decompression at 6 ATA. This can be seen in figures 1 and 2 in the KPU group, both at the 0,6 and 24th hour measurements. All KPU groups have high expression of carbonyl protein compared to the KPT group. However, in the use of HBOT, there will also be an increase in Carbonyl Protein levels at the 3rd hour measurement. This occurs due to the body's response to the high increase in oxygen in the body. HBOT is a moderate oxidant for the body that will increase considerably between 3 hours to 6 hours after the administration of HBOT.

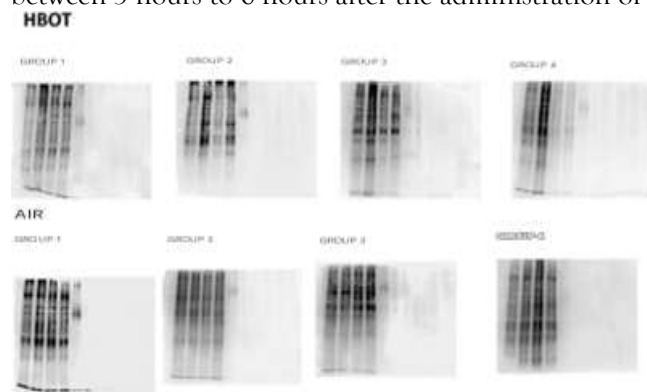


Figure 1 Western Blot Analysis of Carbonyl Protein Expression

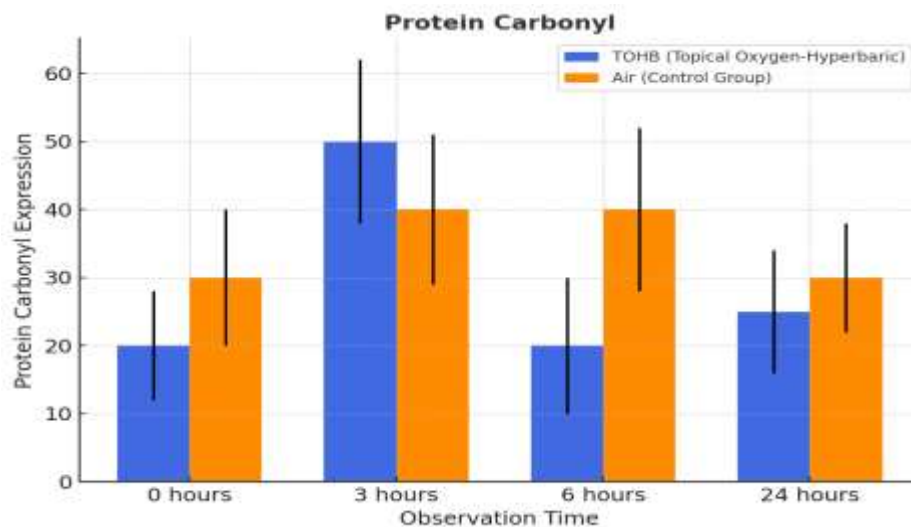


Figure 2: Difference in the Influence Between the KPT Group and the KPU Group on the Change in Carbonyl Protein Expression

Table 1 Results of Differential Test of Carbonyl Protein Expression at 0 Hour Observation, 3rd Hour Observation, 6 Hour Observation, 24th Hour Observation of KPT Group & KPU Group

Observation Time	KPT Group	KPU Group	p
0 hours	21,5490±7.	33.3515±9.	>0,05
3 hours	18286	2288	>0,05
6 hours	50,8355±7,	38,8368±1	<0.05
24 hours	94068 19,64±0 ,85 26,76±1 3,8	3,581 41,75± 12,08 34,17± 7,030	<0.05

The results of the 0, 6 and 24th hour measurement data were analyzed with unpaired t-tests in this group ($p < 0.05$). Meanwhile, in the 3-hour measurement, the data was analyzed with Mann-Whitney. ($p < 0.05$).

IL-6; Examination of IL-6 expression to measure the degree of tissue damage and activation of the immune system. This tissue damage triggers the release of pro-inflammatory cytokines, including the cytokine IL-6, as part of the body's response to injury. As a result of injury after decompression diving, immune cells such as macrophages, neutrophils, and endothelial cells will secrete IL-6 cytokines as part of the inflammatory response.

The process of activation and release of IL-6 from these cells takes several hours. The peak of IL-6 production usually occurs 4-6 hours after the inflammatory trigger. This is consistent with this study, when the Sprague Dawley experimental Rats arrive at the surface after decompression diving, and then 6 hours later, IL-6 expression is observed.

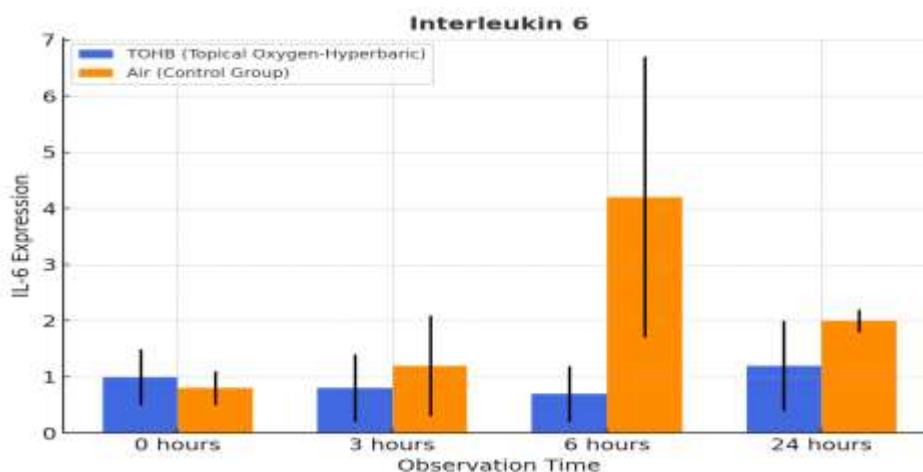


Figure 3 Difference in the effect between the KPT group and the KPU group on changes in Interleukin-6 expression 6

The results of the measurement data were analyzed with an unpaired t-test at the observation of the 0th hour, the 3rd hour, and the 24th hour. Observations at 0 and 3 hours showed no significant effect on the decrease in IL-6 expression in the KPT group compared to the KPU group. Next, an analysis was carried out with Mann-Whitney ($p < 0.05$) at the observation of the 6th and 24th hours. Observations at the 6th and 24th

hours showed significant results on the decrease in IL-6 expression in the KPT group compared to the KPU group (Table 2).

The results of the expression of IL-6 at the observation time of the 6th hour showed a very significant difference between the KPU group and the KPT group (Figure 3). This means that the administration of HBOT to the KPT group at the observation time of the 6th hour is very useful in reducing the expression of IL-6 compared to the KPU group.

Table 2 Results of Interleukin 6 Expression Difference Test 6 at 0 Hour Observation, 3rd Hour Observation, 6th Hour Observation, 24th Hour Observation KPT Group and KPU Group

Observation Time	KPU Group	KPT Group	p
0 jam	0,8480 ± 2,62	1,06 ± 0,52	>0,05
3 jam	1,83321 ±	0,848 ± 0,63	>0,05
6 jam	0,81	0,7679± 0,54	<0.05
24 jam	4,1924± 2,55	1,2496 ± 0,82	<0.05
	2,1107 ± 0,617		

Measurement data were analyzed by unpaired t-test in the 0th, 3rd and 24th hour groups ($p < 0.05$). Meanwhile, in the 6-hour measurement, the data were not normally distributed and were then analyzed with Mann-Whitney ($p < 0.05$).

CASP_3; Furthermore, the examination of Casp-3 expression measured the rate of cell death. The expression of casp-3 in this study was highest at the 6th hour measurement time, when on the surface after decompression diving. The results of the observation time measurement of the 0th, 3rd, and 24th hours were analyzed with an unpaired t-test ($p > 0.05$). When the 0, 3rd, and 24th hour measurements were taken, the results showed that the effect of HBOT on the KPT group did not show a real difference in reducing Casp-3 expression compared to the results of measuring Casp-3 expression in the KPU group. While the observation time of the 6th hour was analyzed with Mann withney ($p < 0.05$), the results showed that the effect of HBOT in the KPT group was better in reducing Casp-3 expression compared to the results of measuring Casp-3 expression in the KPU group with the observation time of 6 hours. (table 3)

The results of Casp-3 expression at the observation time of 6 o'clock showed a very significant difference between the KPU group and the KPT group (Figure 4). If there is an increase in Casp-3 expression, it is related to the process of apoptosis or programmed cell death due to tissue damage and stress that occurs during decompression diving activities. In decompression sickness, there is an increase in nitrogen bubbles in the blood and tissues. These bubbles will clog the blood capillaries and damage the endothelial cells of the blood vessels and surrounding tissues. These nitrogen bubbles will also press on the tissues of the body where these nitrogen bubbles are trapped, so that they will cause damage, which will then trigger cell death through the mechanism of apoptosis.

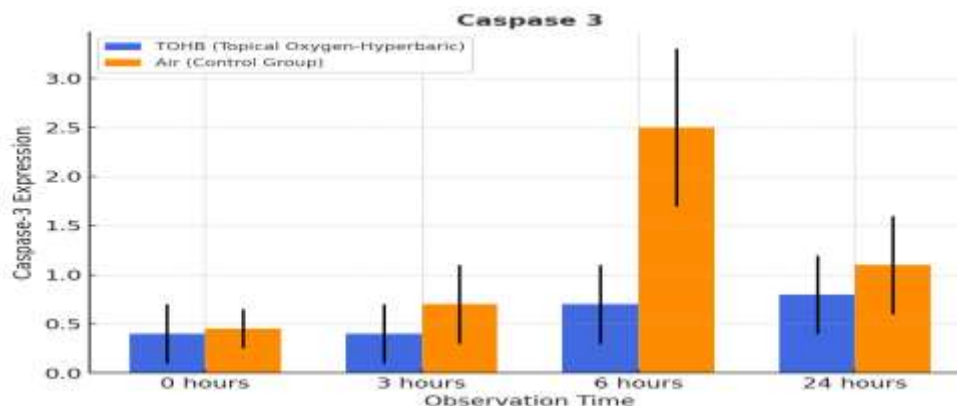


Figure 4: Difference in the influence between the KPT group and the KPU group on the change in Casp-3 expression

These pro-inflammatory cytokines can induce cell apoptosis through activation of extrinsic pathways involving Casp-8 and then activate Casp-3. In line with the magnitude of the release of IL-6 cytokines at the 6th hour after diving will trigger a similar increase in Casp-3.

Table 3. Results of Casp-3 Expression Differential Test on 0-hour observation, 3rd hour observation, 6th-hour observation, 24th-hour observation, KPT group, and KPU group.

Observation Time	KPU Group	KPT Group	p
0 jam	0,4365 ±	0,3947 ±	>0,05
3 jam	0,102	0,288	>0,05
6 jam	0,7116 ±	0,4001 ±	<0,05
24 jam	0,387	0,260	>0,05
	2,4510 ± 0,83	0,7115±	
	1,0679±	0,532	
	0,505	0,742 ±	
		0,253	

The results of the measurement data were analyzed with an unpaired t-test in the 0,3,24 hour group ($p > 0.05$). Meanwhile, in the 6-hour measurement, the data was not normally distributed and was then analyzed with Mann Withney. ($p < 0.05$).

CASP-9; Furthermore, the examination of Casp-9 expression is one of the indicators of cell death rate, along with Casp-3. Casp-9 is the initiator of cell death, and Casp-3 is the executor of cell death. The examination of Casp-9 expression in this study showed the highest difference in the 6th hour measurement time when on the surface after the decompression dive between the KPT group and the KPU group. Likewise, the measurement results at the observation time of the 3rd and 24th hours had a significant difference between the KPT group and the KPU group in the reduction of Casp-9 ($p < 0.05$). When the measurement of the clock was set to 0 did not show the effect of HBOT in the KPT group compared to the KPU group; there was no real difference in reducing the Casp-9 expression of the KPT group compared to the results of the measurement of Casp-9 expression in the KPU group (Table 4). The results of Casp-9 expression at the observation time of the 6th hour showed a very significant difference between the KPU group and the KPT group (Figure 5).

Casp-9 is a caspase that initiates a cascade of apoptosis through an intrinsic pathway (i.e., through mitochondria). Between 0 and 6 hours after decompression diving, oxidative stress will occur, so that mitochondrial damage occurs, and the apoptosis pathway is activated, so that Casp-9 levels reach peak levels. Meanwhile, at the 6th hour and 24th hour of measurement after the decompression dive, the apoptosis process takes place, the damaged cells are eliminated, and an antiapoptosis mechanism occurs so that Casp-9 activation begins to decrease. This process reflects the body's homeostasis mechanism in balancing cell damage, apoptosis, and healing after decompression diving. After 24 hours, many of the damaged cells have been repaired or eliminated, so that the apoptosis activation signal is reduced and Casp-9 levels decrease.

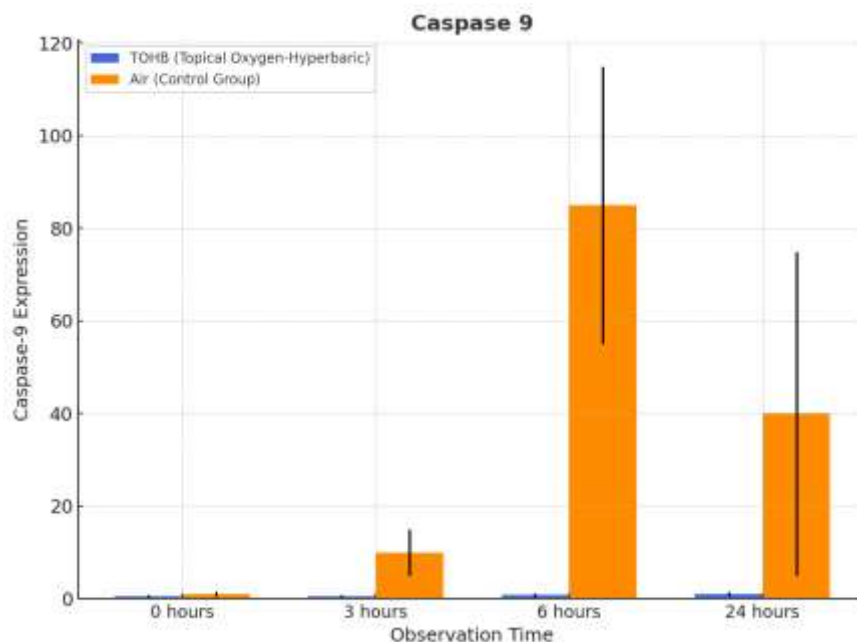


Figure 5 Differences in the influence between the KPT group and the KPU group on the change in Casp-9 expression

Casp-9 has a limited half-life; once activated and performs its job, this protein will be broken down by the cellular proteasome system to prevent excessive or prolonged activation of apoptosis.

Table 4. Results of the Casp-9 Expression Difference Test on the observation of the 0th hour, the observation of the 3rd hour, the observation of the 6th hour, the observation of the 24th hour of the KPT Group and the KPU group

Time Observation	KPU Group	KPT Group	p
0 jam	6,562 ±	1,156 ±	>0,0
3 jam	1,845;	0,985	5
6 jam	13,782 ±	2,837 ±	<0.0
24 jam	5,688	1,897	5
	89,4360	4,775±	<0.0
	34,229	1,534	5
	44,514 ± 37,848	5,618 ±	<0.0
		3,353	5

Histopathology of the Brain; Furthermore, the measurement of the number of nitrogen bubbles in the histopathological preparations of the brain is used to assess how large nitrogen bubbles form in the brain due to decompression and how much HBOT can reduce the number of nitrogen bubbles in the brain. The results of the 3rd, 6th, and 24th hour measurements showed that the effect of HBOT on the KPT group was better in reducing the number of nitrogen bubbles in the Brain Histopathology Picture compared to the results of measuring the number of nitrogen bubbles in the Picture.

Histopathology of the Brain in the KPU group ($p < 0.05$). Meanwhile, measurements at 6 hours showed a very significant difference in results. This means that the administration of HBOT to the KPT group during the 6th hour measurement is very useful in reducing the number of nitrogen bubbles in the Histopathology picture of the Brain compared to the KPU Group. However, in measurements with unpaired t-tests at the time of observation at 0 o'clock, the results showed that the effect of HBOT did not show a significant difference in decreasing the number of air bubbles in the Histopathological Brain picture in both groups ($p > 0.05$) (Table 5).

The formation of nitrogen bubbles in this study was seen as very high in the KPU group at the observation of the 3rd hour after the decompression dive. Has a high value. This condition occurs due to the process of releasing nitrogen from the tissue (desaturase). During the diving, nitrogen decompression from the breathing air used during the 3rd hour after the decompression dive, the capacity of the blood to carry nitrogen in dissolved form reaches its threshold, causing nitrogen bubbles to form in the blood and tissues.

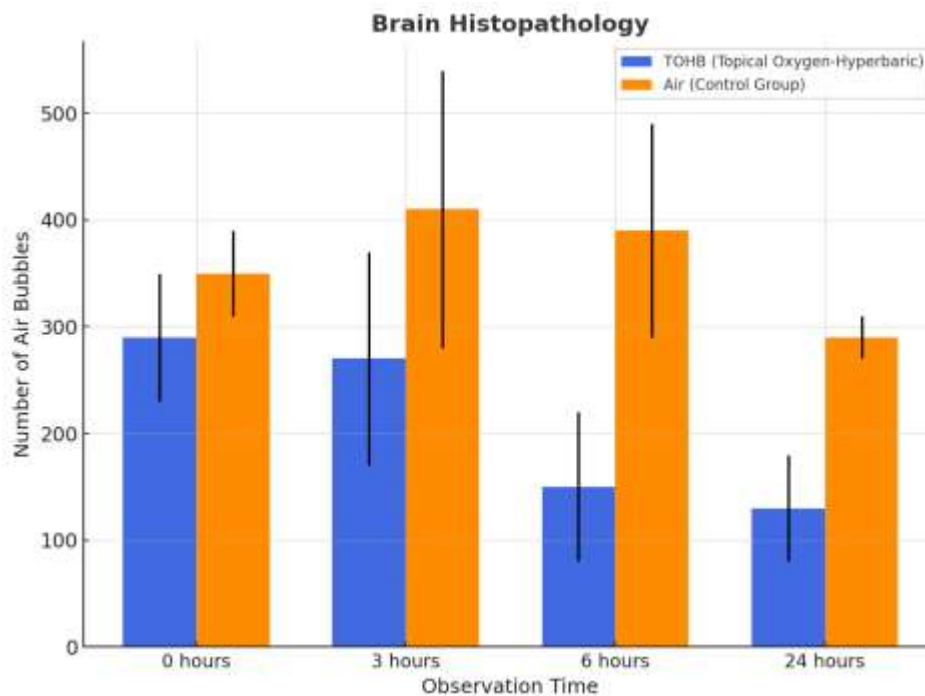


Figure 6: Difference in the effect between the KPT group and the KPU group on changes in the number of Air Bubbles in the histopathological picture of the brain

Figure 7 Histological view (hematoxylin and eosin staining) of brain tissue samples (observation time 0 hours) all images shown at 40 x magnification (control)

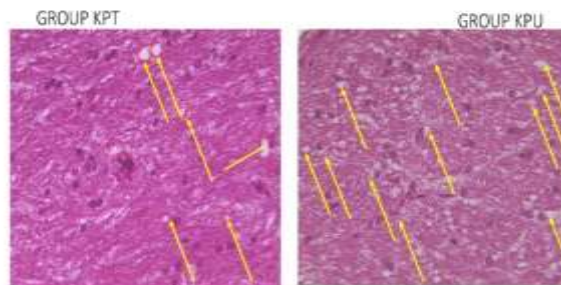


Figure 8 Histological view (hematoxylin and eosin staining) of brain tissue samples (observation time 3 hours) all images shown at 40 x magnification

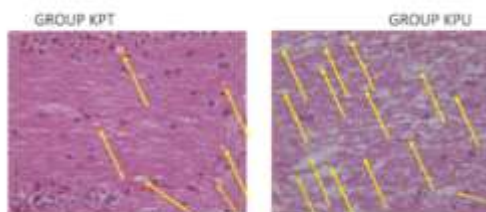


Figure 9 Histological view (hematoxylin and eosin staining) of brain tissue samples (observation time 6 hours) all images shown at 40 x magnification

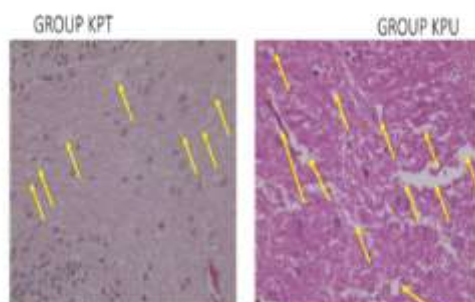
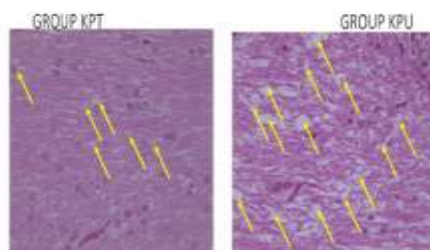


Figure 10 Histological view (hematoxylin and eosin staining) of brain tissue samples (observation time 24 hours) all images shown at 40 x magnification



During the dive, decompression and an increase in hydrostatic pressure will cause the partial pressure of nitrogen to also increase. Nitrogen can escape from blood vessels and enter the tissues to the deepest depths. When it rises to the surface, the external pressure decreases, and the nitrogen dissolved in the tissue begins to come out, which then re-enters the blood vessels and experiences a reduction in volume to normal (outgassing process). The 3rd hour after diving is the critical period at which the rate of nitrogen release from soft tissues and blood reaches its peak.

Body tissues have different desaturase speeds; tissues that have rapid desaturase include blood and brain. The blood and brain will release nitrogen faster than bones and fat. By the 3rd hour after the rapid tissue dive, it has begun to emit significant nitrogen.

Table 5. Results of the Difference Test of the Number of Air Bubbles in the histopathological picture of the brain at the observation of the 0th hour, the observation of the 3rd hour, the observation of the 6th hour, the observation of the 24th hour of the KPT group, and the KPU group

Observation Time	KPU Group	KPT Group	p
0 jam	347,14 ±	291,28 ±	>0,05
3 jam	36,615	59,523	<0.05
6 jam	412,43 ±	271 ±	<0.05
24 jam	140,576	108,354	<0.05
	378 ±	152 ± 61,537	
	112,918	138 ±	
	295 ± 15,556	45,595	

GAIT ANALYSIS; Gait Analysis disorders in DCS are often associated with neurological and vascular damage due to nitrogen gas embolism. This condition can worsen over time due to several interrelated mechanisms. The accumulation of nitrogen gas embolism at the 6th hour after the decompression dive begins to cause ischemia in the brain, spinal cord, and peripheral nerves. This initial breakdown triggers an inflammatory response that increases the release of pro-inflammatory cytokines such as IL-6. By the 24th hour after the dive, decompression, the inflammatory process intensifies, causing edema of the nerve tissue and wider damage, thereby interfering with the nerve signals that control gait movement and coordination.

This study shows that giving HBOT 3 hours before decompression diving can reduce the incidence of DCS. The concern of this study in the KPT group that had been given HBOT before diving was not found to have a single tail that experienced death, although there was also a lower neurological function change compared to the KPU group. The occurrence of an increase in the amount of antioxidants in the administration of HBOT is one of the positive factors for the improvement of the condition of experimental animals.

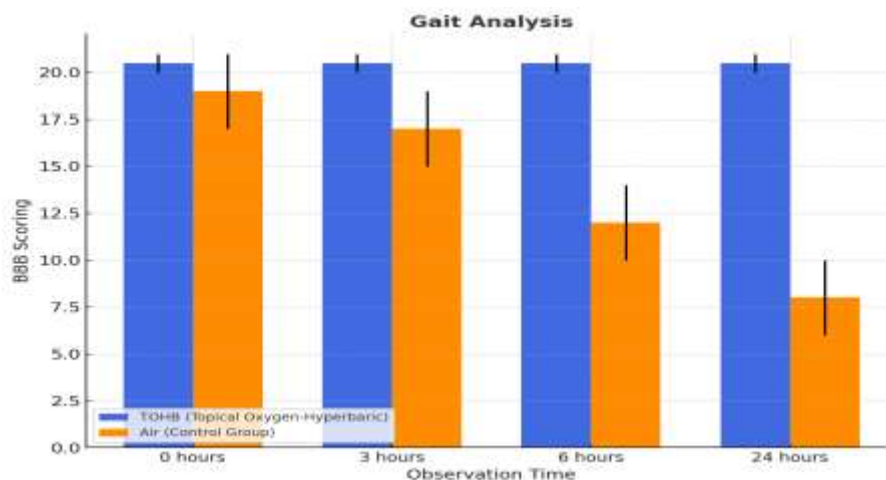


Figure 10: Difference in the influence between the KPT group and the KPU group on changes in BBB scoring in gait analysis

Measurement data on the observation of the 0th, 3rd hour, 6th hour and 24th hour were analyzed with Mann Whitney ($p < 0.05$), the results showed that the effect of HBOT with the measurement time on the observation of the 6th and 24th hours was better in improving the results of BBB scoring (Gait Analysis) compared to the results of BBB scoring measurements (Gait Analysis) in the Compressed Air Group.

Meanwhile, measurements at the observation time of the 6th hour showed a very significant difference in BBB scoring results. This means that the provision of HBOT to the KPT group during the observation at the 6th hour is very useful in improving the improvement of Gait Analysis compared to the KPU group. However, at the time of observation at 0 and 3 hours, the results showed that the effect of HBOT did not show a significant difference in improving the results of Gait Analysis in both groups.

DCS often affects *the spinal cord*, the thoracic and lumbar parts that control leg movement and gait coordination. At 6 hours after the decompression dive, the damage may be partial with some nerve function still working, but by 24 hours after the decompression dive, damage due to hypoxia, edema, and inflammation can cause more significant disturbance of motor nerve signals and even death.

Table 6. Results of the Difference Test, Basso, Beattie, and Bresnahan (BBB) score, Gait analysis on the observation of the 0th hour, the observation of the 3rd hour, the observation of the 6th hour, the observation of the 24th hour of the KPT group, and the KPU group

Time Observation	KPU Group	KPT Group	p
0 jam	21,00	21,00	>0,05
3 jam	±	± 0,52	05
6 jam	0,262	20,71	>0,05
24 jam	17 ±	± 0,50	05
	10,14	21,0 ±	<0,05
	00	0,00	05
	11,85	20,85	<0,05
	0 ±	±	05
	3,50	0,500	
	9,00 ±		
	10,500		

Nitrogen gas bubbles trapped in small blood vessels can disrupt blood flow to muscles and nerves, causing muscle fatigue and impaired motor control. At the 6th hour after the gas embolism dive is still in the active phase, and the apoptosis pathways of nerve and muscle cells have been activated (involving casp-3 and casp-9) due to oxidative stress and mitochondrial damage. While in the 24th hour after the decompression dive the impact of blockage and nitrogen bubble suppression will cause ischemia to worsen, damage due to nerve cell death (neuron apoptosis) increases leading to a more pronounced decrease in nerve function, edema of nerve and vascular tissues increases causing local pressure on the *spinal cord* or peripheral nerves thus inhibiting nerve signals necessary for walking and worsening gait disorders.

DISCUSSION

This study shows that giving HBOT 3 hours before decompression diving can reduce the incidence of DCS. The concern of this study in the KPT group that had been given HBOT before diving was not found to have a single tail that experienced death, although there was also a lower neurological function change compared to the KPU group. The occurrence of an increase in the amount of antioxidants in the administration of HBOT is one of the positive factors for the improvement of the condition of experimental animals.

Antonija Perovi, et al, in 2018, there was an increase in Carbonyl Protein levels after a 6-hour dive, during a 30-minute dive at a depth of 30 MSW. In this study, Carbonyl Protein measurements were carried out before and after 3 hours and 6 hours of diving. Immediately after rising to the surface, an increase in CAT and SOD2 was found and reached its maximum at 3 hours after the surface. Meanwhile, in the other study, subjects with measurements 6 hours after diving at a depth of 30 MSW, no increase in CAT, SOD2, and SOD was found, and no tissue damage was found.

The KPT group, after the administration of HBOT, from the results of the study showed that with the unpaired t-test ($p < 0.05$), it was found that the expression of Carbonyl Protein was lower after the administration of HBOT compared to the expression of Carbonyl Protein in the KPU group at the observation time of the 6th hour and observation at the 24th hour. The administration of the HBOT modification will increase the proportion of dissolved oxygen in the blood by five to twenty times. The effect of giving HBOT will increase antioxidants.

In previous studies, the administration of HBOT at a pressure of more than 3 ATA can cause oxygen poisoning and can cause immunosuppression. Therefore, in this study, the administration of HBOT is given with the aim of increasing optimal oxygen levels in the blood circulation. Therefore, in this study, HBOT was given with a measured composition, namely at a pressure of 2.4 ATA/14 MSW with 100% oxygen for 75 minutes.

HBOT can reduce oxidative stress by reducing lipid peroxidation by inhibiting the adhesion of neutrophils and by increasing the activity of antioxidant enzymes. Short-term administration of HBOT showed a significant increase in serum SOD levels. In Veronika Madzi's research in 2015, the application of HBOT can reduce oxidative damage, Plasma Carbonyl Protein, and 8-OHdG and increase Glutathione Peroxidase. The administration of HBOT will cause oxygenation in the mitochondria, so there is a decrease in free radicals. In this study, it was found that plasma Carbonyl Protein concentrations decreased significantly after administration of HBOT (77.1 vs 61.7 pmol/mg; $p < 0.001$).

In this study, changes in IL-6 were also observed after the administration of HBOT (KPT Group). The results showed that the KPT group with the Mann-Whitney Test ($p < 0.05$) expression of Interleukin 6 was lower after the administration of HBOT of the KPT group compared to the expression of IL-6 in the KPU group at the time of observation at the 6th hour. Similarly, the unpaired t-test ($p < 0.05$) at the 24th hour measurement found that IL-6 expression was lower in the KPT group compared to the IL-6 expression in the KPU group.

At the time of diving, most of the skeletal muscles, nitrogen bubbles are involved. The activity of skeletal muscle and nitrogen bubbles is closely related to an increase in IL-6 in muscle tissues and other body tissues. When IL-6 is released excessively during diving, there will also be an increase in the levels of cortisol, catecholamines, Interferon alpha, and IL-1 in the plasma. IL-6 can reduce Immunoglobulin-A A (IgA) levels, thereby increasing the acute inflammatory response and reducing immunity. The process of alteration of increased IL-6 levels plays an important role in the pathogenesis of DCS. The occurrence of DCS is closely related to endothelial dysfunction of blood vessels.

The administration of HBOT can increase the high concentration of oxygen in the blood circulation so that it can increase the body's ability to eliminate nitrogen bubbles in body tissues, including brain tissue. As a result of the reduction of nitrogen bubbles, the blockage and nitrogen suppression in the brain tissue will be lost. The loss of blockages and nitrogen suppression leads to decreased inflammation.^{16,19,22} DCS causes serious hypoxia-ischemic brain injury. The occurrence of hypoxia during decompression diving will result in an increase in HIF-1 protein. An increase in HIF-1 protein will stimulate the production of other proteins, such as Nuclear Factor Kappa Beta (NF-kB). NF-kB protein is responsible for stimulating pro-inflammatory cytokines. Giving HBOT Modification will negate the effect of HIF-1 protein so that NF-kB protein becomes inactive and IL-6 expression decreases.

In addition, the administration of HBOT can create a hyperoxic environment that increases protein I κ B α . Then the protein I κ B α mediates inhibition of NF- κ B proinflammatory genes by binding to NF- κ B and keeping it dormant in the cytoplasm. This condition ultimately inhibits the activation of the NF- κ B signaling pathway so that there is a decrease in the transcription of the pro-inflammatory cytokine IL-6.³⁵ In the administration of HBOT, there is an increase in antioxidant enzymes (CAT and SOD), which can decrease pro-oxidant enzymes, so that it can directly decrease inflammatory markers such as IL-6. It can be concluded that there is a strong relationship between oxidative stress and inflammation. In addition to HBOT can inhibit the production of pro-inflammatory cytokines and increase the release of anti-inflammatory cytokines, so that it can help restore cytokines to homeostatic levels by modulating IL-6 interactions.

Xavier Capo et al's research in 2023 can lower Oxidative Stress and inflammation. In this study, it was found that there was a progressive decrease in malondialdehyde (MDA) and carbonyl protein. In conclusion, HBOT reduces oxidative and pro-inflammatory mediators and may participate in activating healing, angiogenesis, and regulation of vascular tone by increasing the release of growth factors.

Another study from Thom, S.R. in 2019 showed that the administration of HBOT can reduce the inflammatory impact of diving. The administration of HBOT in this study provides the advantage of greater oxygen concentration, accompanied by an increase in partial pressure of oxygen in body tissues, which causes a decrease in inflammation. Increased blood oxygen pressure has a positive effect on the healing of inflammatory and microcirculatory disorders due to ischemic conditions.

In this study, changes in Casp-3 and Casp-9 were also observed after the administration of HBOT. The Mann-Whitney Test ($p < 0.05$) found that Casp-3 expression was lower after the administration of HBOT in the KPT group compared to Casp-3 expression in the KPU group at the 6th hour observation time (Table 3). It was also found that the Casp-9 expression of the KPT group, with the Mann-Whitney Test ($p < 0.05$) (Table 4), was lower than the expression of Casp-9 in the KPU group at the 3rd hour observation time and at the 6th hour observation. In addition, it was also found that with an unpaired t-test ($p < 0.05$) (Table 4) at the 24th hour measurement, Casp-9 expression was found to be lower in the KPT group compared to Casp-9 expression in the KPU group.

In Decompression Diving, there is an oxidative stress process. The occurrence of oxidative stress during decompression diving is due to muscle fatigue, endothelial dysfunction, and DNA damage, which results in cell death in the form of apoptosis or necrosis. Apoptosis or necrosis can be assessed with increased Casp-3 and Casp-9 levels. This cell damage is one of the predisposing factors for an increase in free radicals during decompression diving. The body tries to overcome oxidative stress through cytoprotective mechanisms, and this process is a form of adaptive response. During decompression diving, there is an increase in Casp-3 in the body. Casp-3 is a caspase protein that interacts with Casp-8 and Casp-9. Casp-9 plays a role in initiating Casp-3 increase, so this Casp-3 increase causes the initial process of apoptosis.

Damage from DCS is mainly caused directly by physical and secondary damage due to hypoxia, oxidative stress, as well as inflammation, which all lead to neuronal apoptosis. The administration of HBOT has a decreasing effect on oxidative stress. HBOT promotes the restoration of oxygen supply, antioxidant gene expression, reduction of inflammation, inhibition of apoptosis, and increased angiogenesis and neurogenesis. It has been proven that the number of apoptotic neurons and the levels of proapoptotic factors increase significantly in DCS. The activated Casp-3 protein (C-casp-3) is the most commonly used biomarker to assess apoptosis. The administration of HBOT will activate the phosphorylation of Akt (pAkt). Furthermore, pAkt will induce GSK3 β (pGSK3 β), while β -catenin levels due to increased phosphorylation of GSK3 β will decrease. Decreased levels of β -catenin will decrease Casp-3 expression, so that it can prevent apoptosis.⁴² TBT inhibits endoplasmic reticulum-induced apoptosis, which can alleviate secondary motor and sensory disorders, thereby improving neurological function.

Another previous study was conducted by Chin and Chen in 2021. The benefits of HBOT can markedly reduce the number of apoptotic cells and decrease the expression of BAX/BCL-2, Casp-3, and Casp-9 after burns. HBOT provides anti-inflammatory and neuroprotective effects. Furthermore, hypoxia-inducible factor (HIF)-1 α is reported to increase inflammation and apoptosis. HBOT has excellent potential in lowering HIF-1 α and its impact after burns.

The results of Chin and Chen's research showed that the administration of HBOT corrected the imbalance caused by burns. Post-burn, less atrophy and fibrosis of the gastrocnemius muscle after being given HBOT. HBOT weakens neuronal apoptosis and post-burn glia activation. Chin An Chen's research concludes that HBOT reduces neuronal apoptosis caused by burns in the ventral cornea through HIF-1 α signaling. From the previous description, it was shown that the administration of HBOT can reduce the expression of HIF-1 α and Casp-3 as well as Casp-9.

In this study, changes in the number of nitrogen bubbles in the Histopathological Brain picture were also observed after the administration of HBOT. With an unpaired t-test ($p < 0.05$), the measurement of the number of nitrogen bubbles in the Brain Histopathology Picture was lower in the KPT group compared to the KPU group at the 3rd hour observation time, at the 6th hour observation, and at the 24th hour observation.

The administration of HBOT has a protective effect against brain injury due to DCS. The mechanism of the protective effect is related to the reduction of oxidative damage by increasing antioxidant enzymes in the body, especially in the brain. The elimination of micronuclei in brain tissue is beneficial in preventing the formation of nitrogen bubbles. The growth of the nucleus into a bubble can be slowed down by the action of surface tension resulting from an increase in the partial pressure of oxygen (PPO₂). HBOT 3 hours before diving increased high-pressure oxygenation, increased PPO₂, significantly reduced bubble formation caused by decompression, which occurs due to the elimination of micronuclei in brain tissue. HBOT can remove most of the gas nucleus during decompression diving, thereby reducing the number and size of bubbles that develop.

When the administration of HBOT causes a higher arterial oxygen content which is achieved by increasing dissolved oxygen. Hyperoxic vasoconstriction during HBOT results in decreased perfusion of brain tissue and clearance of nitrogen bubbles. This increased oxygen content quickly diffuses into the micronuclei to be exchanged for nitrogen, which then quickly diffuses out. Oxygen is then absorbed by the surrounding tissues to cause rapid absorption of the micronucleus. A decrease in the number of micronuclei in brain tissue plays a role in the decrease in bubbles in brain tissue at the time of decompression diving. Vasodilation also occurs, which causes increased blood flow. This increase in blood flow will increase the elimination of micronuclei in brain tissue, so that the number of nitrogen bubbles in brain tissue decreases. Previous research by Ivana Šegrt Ribičić, et al. in 2019 found that administering high oxygen levels in diving can lower micronuclei in brain tissue so that the formation of nitrogen bubbles in the brain decreases.

The administration of HBOT will inhibit neuropathic pain after DCS injury, through the regulation of neuronal and inducible NOS expression in the spinal cord. The neuroprotective mechanism of HBOT in neuropathic pain caused by DCS is due to the involvement of microglia mythology. HBOT has neuroprotective properties after sciatic nerve degeneration through decreased lipid peroxidation, increased SOD activity, catalase, and attenuation of Casp-3 expression. The administration of HBOT after DCS injury lowered neuronal iNOS and NOS significantly. In addition, the administration of HBOT inhibits the production of intraneuronal TNF- α and reduces neuropathic pain after DCS injury.

HBOT in motor disorders due to DCS significantly improves mitochondrial dysfunction in the spinal cord and motor cortex. The occurrence of mitochondrial repair in the spinal cord will inhibit the occurrence of DCS. DCS involves a number of secondary damages caused by a series of biochemical, molecular, and cellular cascades, including apoptosis, inflammatory reactions, and lipid peroxidation. The administration of HBOT prevents oxidative damage to the spinal cord.

Administration of HBOT has another mechanism of neuroprotective effects on DCS by weakening interleukin (IL)-1 β and tumor necrosis factor- α (TNF- α) induced by severe DCS, and in turn significantly increases the number of glia cells derived from neurotrophic cells and vascular endothelial growth factor (VEGF) cells and spinal cord IL-10 production.⁴⁴

The administration of HBOT will increase the levels of spinal cord tissue enzymes, and other biochemical parameters such as nitric oxide, glutathione peroxidase, superoxide dismutase (SOD), and IL-10, as a result of spinal cord tissue injury caused by DCS. Inflammatory processes are one of the main causes of motor and sensory disorders due to DCS. The administration of HBOT will decrease spinal cord edema, improve neuronal function, and stabilize the blood-spinal cord barrier through decreased regulation of matrix

metalloproteinase (MMP)-2, IL-6, and MMP-9, thereby improving motor function scores and increasing myelin nerve fibers, as well as increasing VEGF regulation.

Other research results show that the effect of giving HBOT triggers an increase in fibroblasts and angiogenesis. Increased fibroblasts and angiogenesis lead to increased tissue neovascularization and collagen synthesis. In addition, the benefits of giving HBOT lead to increased and improved microvascular blood flow. It was also found that capillary density increased so that areas that experience ischemia will experience reperfusion. Another response with the administration of HBOT was found to increase the expression of Nitric Oxide (NO) by 4 to 5 times. Another effect of the administration of HBOT on endothelial cells causes an increase in the Vascular Endothelial Growth Factor (VEGF) protein. An increase in VEGF leads to increased angiogenesis or the formation of new vascularization in the tissues.

The increase in fibroblasts, as mentioned earlier, will encourage vasodilation, so that the condition of the body tissues becomes hypervascular, hypercellular, and hyperoxic.²¹⁻²³ Another positive impact of the administration of HBOT causes molecular changes in the central nervous system (SSP), increased mitochondrial function, and increased Cerebral Blood flow (CBF). Improved mitochondrial function plays an important role in the cell function of organelles responsible for supplying ATP energy to cells. Optimal ATP energy supply prevents cell apoptosis and improves the function of the motor and sensory nervous systems, as well as synaptic plasticity.

Previous research on the benefits of HBOT on neuroprotective effects was conducted by Fahimeh Ahmadi and Ali Reza Khalatbary, PhD, in 2021. Diseases of the nervous system are one of the leading causes of death and disability worldwide due to the limitations of effective treatment strategies. The use of HBOT as a method of improvement in nervous system diseases is due to the reason that during HBOT the amount of dissolved oxygen in plasma and hemoglobin saturated with oxygen increases, leading to greater availability of oxygen to all organs.

Regarding the neuroprotective effects of HBOT, evidence shows a link between beneficial effects with various biological properties, especially anti-oxidative, anti-inflammatory, and anti-apoptotic properties, in addition to increased oxygen supply and nerve metabolism. Another previous study on the benefits of HBOT in improving motor function due to injuries to the central nervous system was conducted by Ahmed Elshinnawy et al in 2021. Diabetic peripheral neuropathy develops with a decrease in both sensory and motor nerve function, which leads to high morbidity, mortality, and deterioration of the patient's quality of life.

CONCLUSION

Based on the results of the research analysis, it can be concluded that the administration of Hyperbaric Oxygen Therapy (HBOT) before decompression diving showed a better effect compared to the administration of compressed air. This can be seen from several indicators observed in Sprague Dawley rats in hyperbaric chambers. First, HBOT is more effective in lowering levels of carbonyl protein, which is one of the markers of oxidative stress. Second, this therapy also provides more significant results in lowering IL-6 levels, which is associated with the inflammatory response. In addition, the decrease in the expression of Casp-3 and Casp-9 as markers of the apoptosis process was also more prominent in the group that received HBOT than in the compressed air group.

Not only that, but brain histopathological examination shows that HBOT is able to provide better protection against brain tissue damage due to hyperbaric conditions. This finding was strengthened by the results of gait analysis, which showed an improvement in motor function in rats that received HBOT. Thus, overall, HBOT is superior to compressed air in preventing various negative impacts of decompression diving.

Suggestion

Based on the results of the study, there are several suggestions that can be considered. First, the administration of HBOT before decompression diving can be used as a prevention strategy against the risk of Decompression Sickness (DCS). Second, further research with variations in observation time is needed to more comprehensively evaluate the effectiveness of HBOT on the possibility of DCS. Third, clinical trial research in humans is very important to ensure the potential application of HBOT as an effective and safe DCS prevention method.

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