RESEARCH PLAN

Effect of Hydrogen Gas on Hyperbaric Oxygen Toxicity: A Randomized Controlled Cross-Over Trial

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1.1. PROJECT TITLE: Effect of Hydrogen Gas on Hyperbaric Oxygen Toxicity:
- A Randomized Controlled Cross-Over Trial.

1.2. SWEDISH TITLE: Effekt av vätgas på hyperbar syrgastoxicitet:
- En randomiserad kontrollerad studie.

2.2 INVESTIGATING RESEARCH FACILITIES

1. Blekinge Institute of Technology (BTH) - Marine System Engineering
2. Swedish Armed Forces (FM DNC) - Diving and Naval Medicine Centre in Karlskrona.
3. Blekinge Hospital (BLS) Karlskrona, Department of Pulmonary Medicine
4. Lund University, Department of Respiratory Medicine, Allergology, and Palliative Medicine
5. Lund University, Dept. of Translational Medicine
6. University of Gothenburg - Sahlgrenska Academy, Occupational and Environmental Medicine, Department of Public Health and Community Medicine
7. Karolinska Institutet (KI), Department of Physiology and Pharmacology
8. Karolinska University Hospital, Perioperative Medicine and Intensive Care
9. Blekinge Institute of Technology (BTH) – Institute of Health/THIA
10. Sahlgrenska University Hospital, Gothenburg, Department of Anaesthesia and Intensive care

3. ESTIMATED DURATION OF THE STUDY
Approximate study period of 5 years *(2025 – 2030).* The duration of the study for each participant is four days in total.

4. ABSTRACT (SYNOPSIS)
Introduction: Professional divers within the Swedish Armed Forces are frequently exposed to extreme environments where the human body is not fully adapted. To mitigate diving-related risks such as decompression sickness (DCS), caused by the accumulation of inert nitrogen bubbles, oxygen-enriched breathing gases are commonly used by both military and technical divers. Despite its benefits, the use of oxygen-enriched breathing gases is limited by the risk of adverse effects from increased partial pressures of oxygen (PO_2) and the increased formation of reactive oxygen species (ROS). The maximum allowable PO_2 is defined by the risk of CNS-toxicity, while exposure duration is primarily limited by pulmonary oxygen toxicity (POT)(1). Current limits for exposure are primarily based on reductions in lung vital capacity (VC)(2), although other markers may better reflect oxidative stress. Despite strict regulations, there is currently no established method to counteract the oxidative damage during prolonged oxygen exposure. Recent studies suggest that the already known diving gas, hydrogen, has antioxidative properties, neutralizing cytotoxic oxygen radicals and providing potential cellular protection.

Aim: We aim to investigate if a diving gas with a small fraction of hydrogen could reduce Pulmonary Oxygen Toxicity. 1-2% fraction of hydrogen to an oxygen-enriched breathing gas (FO_2 = 0.98-0.99, FH_2 = 0.02-0.01) reduce Pulmonary Oxygen Toxicity (POT) in divers exposed to a partial pressure of 1.75 ATA for 240 minutes. In parallel, the study aims to elucidate the underlying pathophysiological mechanisms associated with POT.

Materials and methods*:* The study will follow a double-blind, randomized, crossover design with two exposure sessions, 1-2% of hydrogen (intervention) and 1-2% of nitrogen (control) to oxygen breathing gas (FO_2 = 0.98-0.99) in divers exposed to a partial pressure of 1.75 ATA for 240 minutes. These exposures will be separated by a minimum of 2 weeks. Each session will be followed by a post-exposure assessment day. Pulmonary Functions Tests (PFTs) and biochemical markers of oxidative stress and of neuronal injury will be measured before and after each intervention. With each participant undergoing both the intervention (H_2-group) and control (N_2-group) they will serve as their own control for comparative analysis.

Participants: Recruitment will be conducted via email inquiries, targeting active professional divers (N=32) from the Swedish Armed Forces.

Statistical Analysis: Baseline characteristics will be summarized using descriptive statistics. Within-participant differences in FVC, will be analyzed using paired t-tests, while categorical variables will be assessed with Wilcoxon matched-pairs signed-rank tests, as well as using regression models. Statistical significance is set at a two-tailed p-value < 0.05.

Significance: If successful, the findings of our study could contribute to the development of safer diving protocols and optimize operational efficiency while minimizing oxygen toxicity risks.

Keywords:
Diving; Hyperoxia; Ventilation; Oxygen Toxicity; Pulmonary Oxygen Toxicity; Oxidative Stress;

Abbreviation list:
ATA = Atmosphere absolute
ATS = American Thoracic Society
BIBS = Built-In Breathing System
BLS = Blekinge Hospital Karlskrona
BTH = Blekinge Institute of Technology
CCRs = Closed-Circuit rebreathers
DAN = Divers Alert Network
DCS = Decompression Sickness
D_LCO = Diffusion Lung Capacity for Carbon Monoxide
EANx = Enriched Air Nitrox
ELF= Epithelial Lining Fluid
FeNO =
FM DNC = Swedish Armed Forced Diving and Naval Medicine Center
FVC = Forced Vital Capacity
Heliox = Gas mixture with oxygen and helium
HBOT = Hyperbaric Oxygen Therapy
HPNS = High-pressure Nervous Syndrome
iOS= Index of [Oxygen Stress]
Nitrox = Oxygen-enriched gas with nitrogen
NOAA = National Oceanic and Atmospheric Administration
PExA = Particle in Exhaled Air
PEFR = Peak Expiratory Flow Rate
PFTs = Pulmonary Function Tests
POT = Pulmonary Oxygen Toxicity
ROS = Reactive Oxygen Species
SOB = Shortness of Breath
SVC = Slow Vital Capacity
TLC = Total Lung Capacity
TLV = Total Lung Volume
VC = Vital Capacity
RV = Residual Volume
FRC = Functional Residual Capacity

5. AIM, OBJECTIVES AND OUTCOMES

Aim:
To investigate whether a high-oxygen diving breathing gas enriched with a small fraction of hydrogen

(1–2%) can reduce pulmonary oxygen toxicity (POT) in divers. In parallel, the study aims to elucidate the underlying pathophysiological mechanisms associated with POT.

Objectives:
1. To characterize the oxidative stress response in the lungs during hyperoxic exposure to diving breathing gases by means of pulmonary function tests and molecular analysis of exhaled breath.

2. To characterize systemic oxidative stress during hyperoxic exposure to diving breathing gases through the measurement of circulating and urinary biomarkers.

3. To determine whether the addition of hydrogen to a high-oxygen diving breathing gas confers measurable protective effects against POT, as assessed by lung function, exhaled breath particels, and systemic biomarkers.

4. To identify predictive markers associated with individual susceptibility or resistance to oxygen-induced damage and the potential efficacy of hydrogen-enriched diving breathing gases.

5. To evaluate the effect of breathing hyperbaric oxygen, with and without addition of hydrogen, on the central nervous system.

Exposure:
Participants will be exposed to two different high-oxygen diving breathing gas mixtures at a partial oxygen pressure of 1.75 ATA for 240 minutes:

- Intervention gas: Fraction of oxygen (FO_2) = 0.98–0.99; fraction of hydrogen (FH_2) = 0.01–0.02
- Control gas: FO_2 = 0.98–0.99; fraction of nitrogen (FN_2) = 0.01–0.02

This study design allows for a direct comparison between the physiological effects of hydrogen- and nitrogen-enriched diving breathing gases under hyperbaric conditions.

Primary Outcome

- The primary outcome will be the difference in the absolute change in forced vital capacity (ΔFVC) between the two diving breathing gas exposures.
- ΔFVC is defined as the individual change from pre-exposure to post-exposure values within each condition. Main comparison will be the mean ΔFVC following hydrogen-enriched gas exposure versus control gas exposure, reflecting the physiological impact of hydrogen on pulmonary oxygen toxicity.

Secondary Outcomes:

- Changes in diffusing capacity for carbon monoxide (DLCO)
- Changes in exhaled particle concentration and composition measured using Particle Exhaled Air (PExA) analysis
- Changes in fractional exhaled nitric oxide (FeNO), as a marker of airway inflammation
- Changes in the Index of Oxygen Stress (iOS)
- Biomarkes in blood and urine.
- Changes in concentrations of biomarkers of neuronal injury in blood

6. BACKGROUND AND RATIONALE

The choice of breathing gas mixture is a crucial factor in optimizing diver safety and reducing physiological risks associated with high pressure exposure in underwater environments. Among the most well-documented risks in diving medicine are the physiological challenges arising from accumulation of inert gases, leading to conditions such as nitrogen narcosis and decompression sickness (DCS) (3) While nitrogen narcosis intensifies with increasing partial pressure, the risk of DCS is determined by a combination of factors. These include the duration of hyperbaric exposure, ascent rate, physical exertion and breathing gas composition. One strategy to reduce the accumulation of inert gas is to increase the fraction of oxygen (O_2). This principle forms the basis for the use of alternative gas mixtures such as pure oxygen, Nitrox (Enriched Air Nitrox, EANx) and Heliox (helium and oxygen) (4), each optimized for specific depth ranges and physiological demands (5).

However, the use of oxygen-enriched breathing gas mixtures is limited by the risk of oxygen toxicity. Under high pressure conditions where the partial pressure of oxygen (pO_2) exceeds established safety thresholds (6,7). In such environments, the elevated pO_2 significantly increase the formation of reactive oxygen species (ROS), contributing to oxidative stress. Despite technological advancements in gas composition, oxygen toxicity remains a critical limiting factor, restricting both depth and duration in diving operations.

At sea level, where the partial pressure of oxygen is approximately 0.21 atmospheres absolute (ATA), natural defense mechanisms within the body, are generally sufficient to mitigate oxidative stress by neutralizing ROS, thereby effectively preventing cellular injury that leads to tissue damage. However, when oxygen is administrated at partial pressures exceeding 0.6 ATA, prolonged exposure may overwhelm these endogenous defense mechanisms (8,9). Organs particularly susceptible to oxygen-induced damage include the lungs and the central nervous system, leading to conditions collectively known as pulmonary oxygen toxicity (POT) and central nervous system (CNS) toxicity.

To mitigate the risks of acute toxicity, professional divers strictly adhere to oxygen exposure limits regulated by various safety guidelines over the past few decades Traditionally, the most widely used parameter for assessing POT has been a reduction in vital capacity (VC) (10) Although researchers have tried to find better biomarkers for POT, but no useful correlations have yet been found (11). However, some physiological measurements have been found that may reflect pulmonary oxidative damage better than VC (12). Tailoring safety measures based on these measurements may represent a significant advancement in the optimization of exposure guidelines for professional divers.

There is currently no established method to directly mitigate the formation of ROS and associated oxidative toxicity encountered during diving. However, emerging research suggests that hydrogen possesses antioxidative properties through its ability to selectively neutralize cytotoxic oxygen radicals, thereby offering potential cell-protective effects (13–16).

Although the antioxidative role of hydrogen in counteracting POT remains a subject for investigation, its application as an inert gas in diving has already been investigation with high pressures of hydrogen without any toxically effect besides narcosis at very high pressures (17) A diver who had previously experienced HPNS symptoms under similar conditions while using trimix blend, reported no such effects when using a *helihydrox* gas mixture of 3% O_2, 59% He, 38% H_2. This observation does not only support the hypothesis that H_2 may alleviate HPNS, but also contributes to our understanding of its narcotic potential, which has only been observed at absolute pressures exceeding 24 ATA, corresponding to depths equivalent to 230 meters, way beyond the range of most conventional operational dives. (18)

Further historical and experimental data reinforce hydrogen's physiological promise, the practice of using hydrogen as a diluent gas is well-documented in several high-profile research programs, most notably those conducted by *Compagnie Maritime d'Expertises* (COMEX). Being a pioneer in

demonstrating the feasibility of hydrogen-oxygen breathing under extreme hyperbaric conditions. Around 100 hydrogen dives were carried out in controlled hyperbaric chamber environments and offshore operations. Among these, the Hydra VIII experiment in 1988 reached a simulated depth of 701 meters, representing one of the deepest hydrogen-based dives ever recorded (19). Parallel efforts by the Institute of Marine Research in Norway involved dives with similar breathing gas mixtures to depths of 300 meters, while the U.S Navy conducted numerous experimental H_2-dives under chamber conditions. Although the exact depth profiles of these U.S. Navy dives remain classified or unpublished, they add further weight to the international interest in H_2 hyperbaric applications. (20) Collectively, these efforts provide a robust empirical foundation for renewed exploration of hydrogen as a therapeutic and operational tool in hyperbaric physiology. (21)

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7. CLINICAL SIGNIFICANCE

Even though oxygen-enriched breathing gases have been extensively studied, the potential benefits of incorporating small fractions of hydrogen remain largely unexplored. *As outlined above,* hydrogen-enriched breathing gas emerges as a promising strategy for mitigating oxidative stress, offering a potential means of extending the operational limits of deep diving. Investigating its physiological and neurological effects could provide valuable insights into the management of ROS and oxygen toxicity under hyperbaric conditions. By emphasizing the need for further research into strategies for neutralizing oxidative stress and enhancing diving safety, our intervention aims to explore the potential benefits of hydrogen-enriched breathing gas in detail. A deeper understanding of potential protective effects of hydrogen could significantly enhance diver safety, expanding the boundaries of modern diving medicine.

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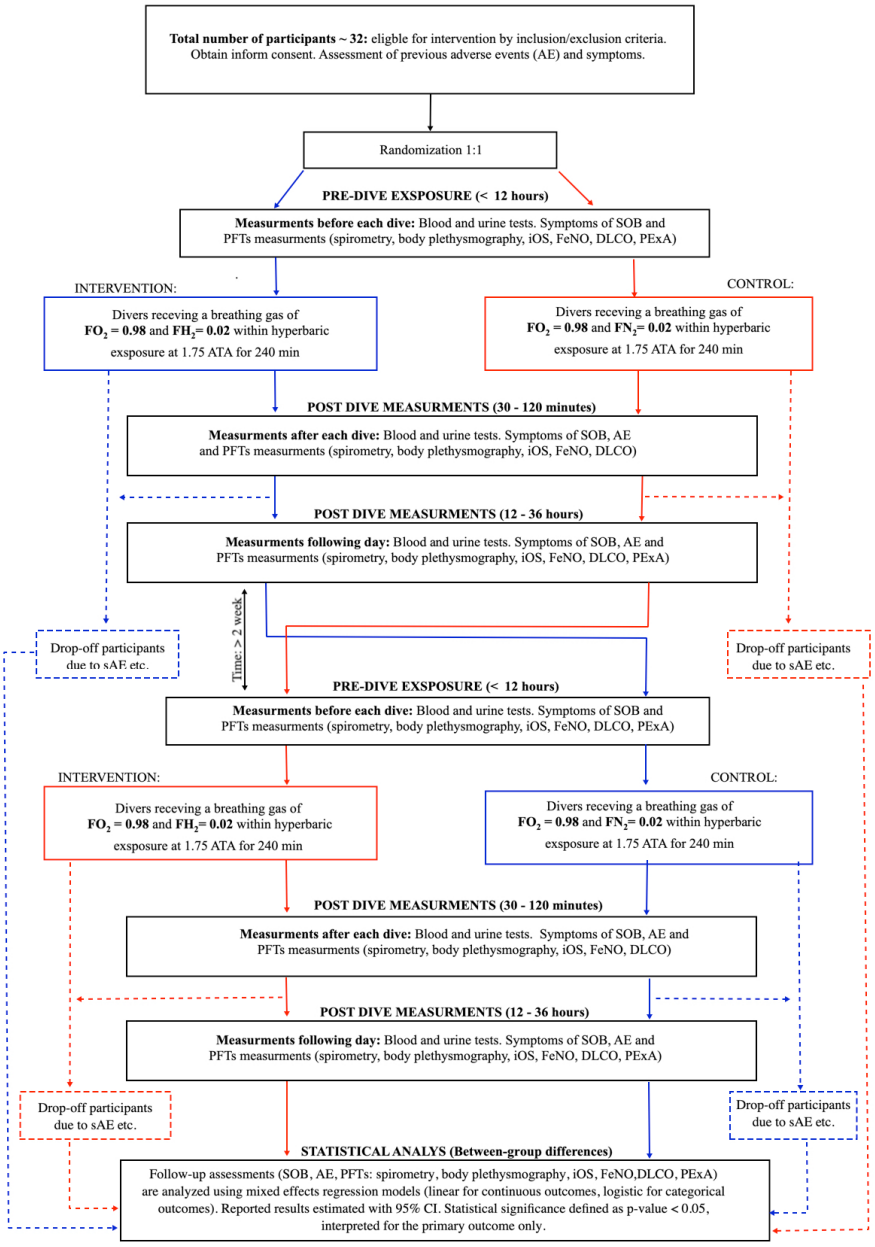
8. MATERIAL AND METHODS:

Study Design:

This is a double-blind, randomized, crossover trial (Figure 1). Both participants and investigators conducting the assessments and analyses will be unaware (blinded) of which treatment is assigned to each group during the intervention, to mitigate placebo and confounding factors.

Figure 1. Outline of the trial



8.2 INTERVENTION OF THE TRIAL

The study is conducted over four days, with each exposure followed by a post-exposure assessment the next day. This design minimizes variability and ensure an accurate evaluation of the results in a controlled environment. Exposures are divided into two separate sessions, with at least two weeks between each crossover visit. However, participants will not be continuously monitored between sessions, no additional visits are planned beyond scheduled pre- and post-intervention assessments.

Screening Visit (week 0): Randomization

1. Consenting Process (obtained inform of consent)
2. Screening for inclusion/exclusion criteria
3. Medical History + previous sAE correlated to diving
4. Randomization 1:1 to either intervention or control group

Study Visit #1 (week 1) pre-exposure

→ Assessments are carried out prior to each individual intervention, on the same day and shortly before the first scheduled intervention in both groups.

1. Self-reported symptoms (breathlessness, cough and other respiratory symptom[s])
2. Tremoflo - Ocillometry (iOS)
3. Fractional Exhaled Nitric Oxide (FeNO)
4. Spirometry (FVC, FEV_1, IC, FEF25, FEF50 and FEF75, PEF, SVC) and body plethysmography (TLC, RV, FRC)
5. Diffusing lung capacity for carbon monoxide (D_{LCO}).
6. PExA, Blood and Urine collection (for later analysis of biomarkers of oxidative stress and neuronal injury)

Study Visit #1 (week 1) post-exposure

→Assessments are carried out 30-120 min after (post exposure) of each individual intervention

1. Self-reported symptoms
2. Tremoflo - Ocillometry (iOS)
3. Fractional Exhaled Nitric Oxide (FeNO)
4. Spirometry: FVC, FEV_1, IC, FEF25, FEF50 and FEF75, PEF, SVC. Body plethysmography (TLC, RV, FRC)
3. FeNO
5. D_LCO.
6. Blood and urine collection (for later analysis of biomarkers of oxidative stress and neuronal injury)

Study Visit #2 (week 1) post-exposure

→ Assessments are carried out the following day, approximately 12-36 h post exposure of each individual intervention.

1. Self-reported symptoms
2. Tremoflo - Ocillometry (iOS)
3. Fractional Exhaled Nitric Oxide (FeNO)
4. Spirometry: FVC, FEV_1, IC, FEF25, FEF50 and FEF75, PEF, SVC. Body plethysmography (TLC, RV, FRC)
5. D_LCO
6. PExA, Blood and Urine collection (for later analysis of biomarkers of oxidative stress and neuronal injury)

→ Intervention Crossover 1:1

By implementing a 1:1 crossover design with fixed randomization, equal number of participants are allocated to either the intervention or the control group. This ensures that each group receives both interventions and effectively allow each participant to serve as their own control. By balancing the

sequence in which participants receive the interventions, potential carryover effects are evenly distributed across both groups minimizing the risk of wash-over effects between the phases. (22)

Study Visit #3 (week 2) pre-exposure
→ Assessments are carried out prior to each individual intervention, on the same day and shortly before the second scheduled intervention in both groups.
1. Self-reported symptom[s]
2. Tremoflo - Ocillometry (iOS)
3. Fractional Exhaled Nitric Oxide (FeNO)
4. Spirometry: FVC, FEV_1, IC, FEF25, FEF50 and FEF75, PEF, SVC. Body plethysmography (TLC, RV, FRC)
5. D_LCO
6. PExA, Blood and Urine collection (for later analysis of biomarkers of oxidative stress and neuronal injury)

Study Visit #3 (week 2) post-exposure
Assessments are carried out 30-120 min after (post exposure) the second individual intervention
1. Self-reported symptom[s]
2. Tremoflo - Ocillometry (iOS)
3. Fractional Exhaled Nitric Oxide (FeNO)
4. Spirometry: FVC, FEV_1, IC, FEF25, FEF50 and FEF75, PEF, SVC. Body plethysmography (TLC, RV, FRC)
5. D_LCO
6. Blood and urine collection (for later analysis of biomarkers of oxidative stress and neuronal injury)

Study Visit #4 (week 2) post-exposure
→ Assessments are carried out the following day, approximately 12-36 h after (post exposure) of the second scheduled individual intervention.
1. Self-reported symptoms (breathlessness, cough and other respiratory symptom[s])
2. Tremoflo - Ocillometry (iOS)
3. Fractional Exhaled Nitric Oxide (FeNO)
4. Spirometry: FVC, FEV_1, IC, FEF25, FEF50 and FEF75, PEF, SVC. Body plethysmography (TLC, RV, FRC)
5. D_LCO
6. PExA, Blood and Urine collection (for later analysis of biomarkers of oxidative stress and neuronal injury)

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9. 1 PARTICIPANTS AND RECRUITMENT

Study population:
Recruitment will be conducted via mail inquiry, targeting (N=32) individuals that are active military divers, with no history of serious diving-related injuries or long-term complications.

Inclusion criteria:

- Military divers actively serving, aged 20 years or older
- Meeting the Swedish Armed Forces physical standards for diving

Exclusion criteria:

- Ongoing infection or illness that may impact pulmonary function or immune response.
- Use of alcohol or smoking cigarettes within 48 hours, as this may influence oxidative stress
- Diving within 48 hours or oxygen diving within 2 weeks, to minimize potential residual effects of the intervention.
- Use of medications that could affect oxidative stress, lung function, or neurological status
- Medical history of serious diving-related injuries or long-term complications

Informed consent:
Following orally and written information with a detailed explanation of study procedures, potential risks and anticipated benefits. Participants will have the opportunity to ask questions. Each participant will sign an informed consent before any study related procedures takes place. Subjects may at any given time withdraw their consent and leave the study without having to motivate the reason for withdrawal.

9.2 RANDOMIZATION

Following screening and inclusion, participants will be randomly assigned to begin with either the *intervention* or the *control*. A fixed 1:1 allocation scheme will be used to ensure that an equal number of participants (N=16) start in each side of the trial. This design minimizes potential washover-effects where the first exposure might influence the subsequent phase. After the initial phase, a crossover will be implemented, allowing each participant to serve as their own control. Comprehensive pulmonary function tests (PFTs), including spirometry, body plethysmography, diffusing capacity for carbon monoxide (D_{LCO}), and regional ventilation assessments, will be performed before and after each exposure.

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10. BLINDING PROCEDURE:

To minimize the risk of bias and prevent confounding factors that could lead to placebo effects, a double-blinded design is implemented, meaning that both participants and investigators involved in outcome assessments remain blinded to group allocation. This approach is essential to ensure that any observed physiological effects or symptoms are directly attributed to the intervention rather than expectations of the investigator or preconceived beliefs related to the participants.

Participants: The breathing gas mixtures, consisting of oxygen with or without a low fraction of hydrogen, will be administered either via demand regulators during submerged (in-water exposure) or via the Built-In Breathing System (BIBS) used in the hyperbaric chamber (HAUX 2300). All delivery systems will be identical in external appearance with standardized components to ensure that no visual differences could reveal group allocation. Preparation and handling of the gas mixtures will be carried out by unblinded technical personnel. These are designated staff members are exclusively assigned to gas logistics and are not involved in data collection or outcome evaluation, thereby maintaining participant blinding throughout the study.

Investigators: Those conducting pulmonary function assessments and analysing data will remain unaware of which exposure each participant is receiving to ensure objectivity in measurements and interpretations. Only personnel who is responsible for the mixing of gas are aware of in which order each participant is undergoing the intervention or the control, respectively. By blinding investigators, we reduce the risk of "observer bias," preventing their beliefs or expectations from affecting data collection or analysis. This ensures that both investigators and participants remain blinded throughout the study, maintaining an unbiased data collection process.

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11. Hyperbaric Oxygen Dive Protocol:
Participants will perform wet dives or "dry dives" inside a hyperbaric chamber at an elevated atmospheric pressure of 1.75 ATA for 240 minutes, corresponding to the highest approved oxygen exposure according to Swedish dive regulations (RMS Dyk 2013). Within the intervention the participants are administrated one of the exposure breathing gases.

Risk management:
Participation in this study entails potential risks related to both medical safety and personal integrity. However, the overall risk of harm is assessed to be low. The planned medical assessments are predominantly non-invasive and include standard pulmonary function testing, supplemented by urine

collection and venous blood sampling. The latter procedure may, in some cases, cause mild discomfort, such as pain or localized hematoma at the site of needle insertion.

The study involves controlled exposure to hyperbaric oxygen using diving breathing gases at 1.75 ATA for 240 minutes. During this time, participants will inhale a gas mixture containing 98–99% oxygen. Although this exposure is compliant with the Swedish Armed Forces' current diving regulations for oxygen diving (RMS Dyk 2013) and adheres to established safety protocols, it is well known that oxygen doses within this range are relatively high and can affect pulmonary function. Similar exposures have previously been associated with a transient group-level reduction in vital capacity (VC) of approximately 2%. This effect is reversible and has not been linked to long-term functional impairment in earlier studies. One of the key scientific objectives of this study is to systematically investigate the pathophysiological processes underlying pulmonary oxygen toxicity (POT), in order to contribute to more evidence-based and relevant safety limits for future oxygen use in hyperbaric environments.

General diving-related risks include barotrauma to the middle ear, sinuses, or lungs due to inadequate pressure equalization. This risk is considered low, as all participants are professional divers trained in pressure exposure and hyperbaric safety. Decompression sickness (DCS), typically caused by inert gas accumulation, is not relevant in this study. The diving breathing gases contain 98–99% oxygen and only 1–2% nitrogen (in the control condition), a level far below what would pose any significant risk of DCS.

The use of hydrogen gas (1–2%) in one of the diving breathing gas mixtures is considered physiologically and technically safe. The gas is delivered directly via a breathing regulator (or chamber mask), preventing accumulation in free volumes and eliminating ignition risk. Hydrogen has previously been used safely in diving breathing gases at substantially higher concentrations, without any reported toxic effects. Notably, the Compagnie Maritime d'Expertises (COMEX) conducted hydrogen-based dives, both in chambers and during offshore operations. The most extreme project, Hydra VIII in 1988, simulated a depth of 701 meters with no reported adverse events.

The study includes the collection and handling of sensitive personal data, which always carries a potential risk to personal privacy. Data handling will be conducted in full compliance with the General Data Protection Regulation (GDPR). All data will be pseudonymized, and the associated code key stored separately in a secure, access-restricted location. The risk of unauthorized access or data misuse is considered low.

There is also a possibility that participants may be informed of previously unknown health conditions, such as unexpected pulmonary findings or reduced oxygen saturation. Any pathological findings will be communicated to the participant—regardless of the presence of symptoms—and appropriate information about associated risks will be provided. No health-related information will be shared with the Swedish Armed Forces or any third party without the participant's explicit consent.

Based on operational data from the Swedish Navy, no incidents of central nervous system oxygen toxicity (oxygen-induced seizures) have been reported in the past 10 years under diving conditions regulated by RMS Dyk 2013. Therefore, the risk of such events is assessed as extremely low. Furthermore, the fact that exposure in this study occurs at rest—rather than during physical activity—further reduces the risk.

The hydrogen concentration in the **diving breathing gas** is well below the lower flammability limit in oxygen (4%). Thus, the participant is not exposed to any risk of fire or explosion. Although theoretical accumulation of hydrogen in a closed space due to leakage from tanks or chamber masks could occur, continuous hydrogen leak detection will be implemented to mitigate these already minimal risks. Medical preparedness on site will follow the safety protocols in RMS Dyk 2013.

12. OUTCOME MEASUREMENTS:

12.1 Technical devices and medical equipment

Our study includes a wide range of measurements to evaluate outcomes, many of which require the use of specialized technical devices. While certain assessments can be performed using standardized instruments commonly used in clinical settings, others specifically demand advanced techniques for gas composition analysis, imaging technologies, or laboratory-based biochemical evaluation. By integrating multimodal endpoints into the study design, we aim to strengthen our hypothesis and capturing both functional and molecular changes associated with POT, as well as the potential protective effects of hydrogen supplementation. Following equipment will be used to conduct the respective outcome assessments:

Vitalograph™ Devices

Vitalograph™ (23) is a provider of advanced pulmonary function testing devices designed for clinical use and unlike standalone spirometry units, these systems are PC-based and require connection to a computer to operate. This setup enables real-time visualization of results and allows analysis to be performed via dedicated software. By integrating advanced flow-sensing technology with user-friendly interfaces, these systems facilitate accurate and efficient lung function assessment in clinical environments. Airflow is measured using a *Fleisch-type pneumotachograph*, which captures laminar flow through micro-capillary tubes. Lung volumes are derived through flow integration, with data collected at high sampling rates and automatic temperature compensation. This construction ensures high precision and long-term stability in accordance with international standards ATS/ERS 2019 and ISO 26782:2009 (24) with measurements of following parameters.

Spirometry with body plethysmography:: FVC, FEV_1, FEV_1/FVC ratio, FEF25–75%, IC and PEF. TLC (Total Lung Capacity), RV (Residual Volume), and FRC (Functional Residual Capacity).
→ *Device model: Vitalograph™ Pneumotrac or equivalent.*

Airway inflammation: Fractional exhaled nitric oxide (FeNO)
→ *Device models: Vitalograph™ FeNO, NIOX VERO™* [1]

Oscillometry Testing

Oscillometry is a non-invasive technique for assessing airway resistance and reactance during tidal breathing. Serving as a valuable tool for detecting minor deviations in peripheral airway mechanics. Key parameters such as R5, R20 and X5 offer detailed insights into both central and small airway function, without the need for forced respiratory maneuvers. Beyond its clinical applications, oscillometry is particularly relevant for detecting early-stage toxicological responses and respiratory changes associated with inhalation exposures and pharmacological interventions.
→ *Tremoflo™ C-100 (Thorasys)* [2]

Devices for Diffusing Capacity (D_{LCO}) Testing

Assessment of pulmonary gas exchange efficiency is essential in both clinical diagnostics and respiratory trials, providing a direct measure of alveolar-capillary function. Typically performed using single-breath D_{LCO} method, which requires administration of a standardized gas mixture containing carbon monoxide (CO) and helium (He**)**, as well as advanced gas analysis to quantify the amount of gas diffused across the alveolar-capillary membrane. D_{LCO} values are adjusted for hemoglobin levels to improve measurement accuracy. Device solutions employed in this project include:
→ *MGC Diagnostics™ DLCO, Vyaire™ Vmax*[3]

[1] https://www.niox.com/en/niox-vero/about-niox-vero/
[2] https://www.thorasys.com/solutions/tremoflo-c-100
[3] https://www.vyaire.com/products/vyntus-one-pulmonary-function-system

→ *ndd® EasyOne Pro*[4]
→ *Vitalograph™ COMPACT™ Medical Workstation (DLCO integration)*[5]

PExA (Particle in Exhaled Air)
The method to sample lining fluid from small airways with the PExA method (Particles in Exhaled Air) is a unique, non-invasive technique allowing sampling endogenous aerosol particles from the small airways during a special breathing maneuver. It represents a pioneering technology developed in Sweden, originating from research at the Department of Occupational and Environmental Medicine at the Sahlgrenska Academy, University of Gothenburg. The method enables the collection of particles that are formed when the epithelial lining fluid (ELF) burst when small airways open during inhalation. The samples can be processed for advanced molecular analysis, including mass spectrometry for both proteins and lipids. This provides a novel window into lung biology and is particularly valuable for identifying non-invasive biomarkers associated with airway inflammation, epithelial integrity, and early toxicological responses.

Since the PExA technique is relatively new, there are currently no established guidelines by European or Swedish authorities. By this, the procedure is based on incorporated protocols from peer-reviewed research and will strictly follow the manufacturing instructions to ensure consistent and reliable data collection.

The subjects are instructed to perform a standardized breathing maneuver starting with an exhalation at normal flow rate to residual volume, breath holding for 5 seconds, followed by a maximal inhalation to total lung capacity, immediately followed by a normal exhalation to functional residual capacity. Exhalation flow is measured by an ultrasonic flow meter (OEM flow sensor; Spiroson-AS, Medical Technologies, Zürich, Switzerland), enabling visualization of the expiratory flow and volume. Between breathing manoeuvres, the subject breaths particle-free air tidally for 10 to 30 seconds. Each sampling session continued until 200 ng of exhaled particles is collected.

12.2 Pulmonary Function Tests (PFTs)

Following assessments are integrated into the study protocol to provide a comprehensive evaluation of lung function and furthermore to determine efficacy of hydrogen supplementation in mitigating POT.

Spirometry and Body plethysmography:
All subjects will perform forced spirometry from total lung capacity (TLC) to residual volume (RV) in triplicate, meaning that three acceptable measurements are obtained. The highest values recorded from these acceptable tests will be used for analysis. Following parameters will be measured:

- Forced Vital Capacity (FVC)
- Slow Vital Capacity (SVC)
- Forced Expiratory Volume in one second (FEV_1)
- FEV_1/FVC ratio
- Peak Expiratory Flow Rate (PEFR)
- Total Lung Capacity TLC
- RV, FRC)

Briefly the maneuver is as follows: Spirometry is performed according to the guidelines of the European Respiratory Society (ERS) (25). These guidelines dictate requirements for equipment calibration, test execution, and data analysis. In Sweden, national lung function testing guidelines are largely aligned with these international standards, ensuring that measurements of FVC, FEV_1, FEV_1/FVC ratio, and PEFR are accurate and comparable.

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[4] https://nddmed.com/products/complete-pulmonary-function-machine/easyone-pro/
[5] https://vitalograph.com/products/compact-medical-workstation/

Fractional Exhaled Nitric Oxide (FeNO)
FeNO will be used as a non-invasive biomarker of airway inflammation and oxidative stress. Nitric oxide (NO) is produced endogenously in the respiratory epithelium, primarily via the enzyme inducible nitric oxide synthase (iNOS), which is upregulated in response to inflammatory stimuli. Hyperoxic conditions, such as those encountered during oxygen-rich diving exposures, are known to induce both oxidative stress and secondary inflammatory responses in the pulmonary system. Measuring exhaled NO-levels provides a real-time indicator of airway inflammation that contribute to evaluation of the antioxidative effects of hydrogen supplementation.

Oscillometry
Oscillometry serves as a composite measurement designed to quantify the overall impact of oxygen-induced oxidative stress on lung tissue. This method will be performed in accordance with established standards (26) to ensure accurate and reliable measurement of respiratory impedance and resistance.

Briefly the maneuver is as follows:
Measurements of pulmonary function are taken before and after each dive to assess changes in pulmonary function and oxidative stress. This allows us to evaluate the potential mitigating effects of hydrogen supplementation on POT. All calculations and interpretations of the oscillometry data will strictly follow published methodologies to ensure consistency and reliability in data collection. In accordance with these methodologies, the oscillometry technique will be implemented in our study using the Tremoflo system, which is known for its ability to provide detailed assessments of small airways function—particularly relevant for detecting subtle changes in pulmonary oxidative stress levels in this context.

Diffusion Capacity for Carbon Monoxide (D_{LCO})
The D_{LCO} test is a standardized test to assess a pulmonary disease, by measuring the ability of the lungs to transfer carbon monoxide to the red blood cells in pulmonary capillaries. Carbon monoxide (CO) is used due to its 200-fold higher affinity for hemoglobin than oxygen (O_2)

Briefly the maneuver is as follows: Participants will be instructed to stay in a sitting position while breathing from a mouthpiece connected to a CO-gas mixture. Participants will first exhale smoothly to residual volume (RV) and then take a maximal inhalation to total lung capacity (TLC) of a gas mixture (0.3 % CH_4, 21% O_2, 0.3% CO, balanced with N_2). After a 10-second breath-hold, they will perform a final exhalation to RV, during which the concentration of CH_4 and CO will be measured. Alveolar volume (V_A), inspired volume (V_I), will be calculated. Since hemoglobin concentration affects D_LCO variability, Hb will be analyzed to minimize errors.

13.2 Biological sample collection and biochemical analysis

Blood sampling: 5ml EDTA (Proteinomics, Lipidomics, Metabolomics), 2,5ml PAX gene RNA (Transcriptomics/Metabolomics), full blood on FTA dry-blood cards (Epigenomics)
5 ml of EDTA blood is kept on ice and centrifuged within 60 min,10 min at 5000G and aliquoted. Snap-frozen and stored on dry ICE and then transfered to –80 °Celscius (°C)

2,5 ml PAX gene RNA blood is stored in refrigerator for up to 5 days and then transferred to –80 °C
FTA dry-blood cards are stored in individual plastic bags in room temperature or fridge +4-8°C.
PExA Are kept on ICE, snap freezed and aliquoted, stored on dry ICE and then transfered to –80°C .
Urine sampling: 5ml, kept on ICE, centrifuged 10min at 1000G and aliquoted. Snap-frozen and stored on dry ICE and then transfered to –80 °C.

Venous blood samples that will be used to analyze fluid biomarkers of neuronal injury are collected in plasma EDTA (ethylenediaminetetraacetic acid) tubes and centrifuged for 15 minutes at 2400G and 4 degrees centigrade. Directly after centrifugation, aliquots of 500µL serum will be frozen on dry ice and then stored at -78 degrees centigrade until analysed.

Description of main analyses:
Blood and Urine samples: Transcriptomics to study changes in immune activation, stress responses, inflammation, and other biological processes at the mRNA level. Metabolomics to capture changes in energy metabolism, oxidative stress, inflammation, and nutrient processing. Proteinomics to quantify and characterize circulating proteins, including enzymes, cytokines, and signalling molecules, providing insights into systemic inflammation, stress responses, and regulatory pathways affected by the intervention. Epigenomics to assess methylation patterns, which may reflect immunological stress in response to the exposure. Lipidomics including phospholipids, sphingolipids, and fatty acids to uncover shifts in lipid metabolism, oxidative damage, and systemic stress responses. All –omics will used in conjunction with targeted, orthogonal, and quantitative methods to confirm/validate high-throughput findings and explore novel biomarkers for inflammation and oxidative stress.
PexA samples will be analyzed for lipid-profile using a a targeted LC-MSMS method, developed specifically for PexA. Proteins in PExA samples will be analysed with Olink (proteomics) but also a targeted protein-profile using mass-spectrometry. Fluid biomarkers of neuronal injury will be analyzed using either a NULISA^{TM} proteomics assay technology or a Neurology 4-Plex A assay on an HD-1 Single molecule array (Simoa) instrument.

13.3 Self-reported symptoms

Subjective assessment of shortness of breath (SOB) along with other respiratory symptoms will be assessed using a structured self-assessment form, specifically developed to detect early signs of pulmonary oxygen toxicity (POT). Participants will be asked to report whether they currently experience any of a predefined set of symptoms, including uncomfortable or labored breathing, cough, shortness of breath, increased respiratory effort, chest tightness or airway irritation. If a specific symptom is present, the severity of the symptom is graded on a numerical rating scale ranging from 0 (no discomfort) to 10 (worst imaginable discomfort). An open-ended category of "other symptom" is included to capture unexpected or atypical reactions.

These assessments will be conducted among with PFTs and sampling biomarkers for oxidative stress, before and after each hyperbaric intervention to capture both immediate (acute) effects or any delayed onset of symptom as a response to the intervention. This custom designed scale provides a more symptom-specific assessment of objective pulmonary endpoints in our study protocol, replacing previously considered instruments such as the modified BORG-scale and VAS.

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13. STATISTICAL ANALYSES

Descriptive Statistics
Baseline characteristics and outcome variables are summarized using descriptive statistics. Continuous variables will first be assessed for distribution, where normally distributed variables are presented as means with standard deviations (SD) and categorical variables will be summarized as frequencies and percentages. Rating scales, such as the BORG-scale and (VAS) for dyspnea, are considered ordinal variables and will therefore be reported as medians with interquartile ranges (IQR).

Primary Outcome Analysis
Difference in the absolute change in forced vital capacity (ΔFVC) between the two diving breathing gas exposures. ΔFVC is defined as the individual change from pre-exposure to post-exposure values within each condition. The main comparison will be the ΔFVC following hydrogen-enriched gas exposure versus control gas exposure within subjects. This will be analyzed using mixed effects regression models, Wilcoxon sign rank test or paired t-test.

Secondary Outcome Analysis
Additional lung function variables will be analyzed using mixed effects regression models, linear for continuous outcomes and logistic for categorical outcomes. The reversibility of FVC and FEV_1 will be evaluated according to standard spirometric guidelines, assessing the following parameters:

- FVC (Forced Vital Capacity)
- Slow Vital Capacity (SVC)
- FEV_1 (Forced Expiratory Volume in One Second)
- FVC% (FVC as percentage of predicted value)
- FEV_1% (FEV_1 as percentage of predicted value)
- FEV_1/FVC-ratio

Results for fluid biomarkers of neuronal injury will be reported using mean (with standard deviation) and median (with both interquartile range and minimal/maximal) values. Both relative and absolute changes will be presented. Non-parametric statistical techniques will be used in analyses.

Explorative analyses
For the explorative analyses of lipid- and protein-profiles multivariate statistical methods will also be used including SIMCA and MUVR2 including Random Forest and Elastic Net.

Statistical Significance
A two-tailed p-value < 0.05 will be considered statistically significant.

Sample Size Calculation
To ensure a sufficient sample size, 32 participants are recruited for the study to account for potential dropouts or missing data. A minimum of 20 participants is required to achieve 80% statistical power with valid primary outcome data.

Sample Size requirements
The sample size estimation is based on a healthy 30-year-old male (180 cm, 75 kg) with an expected FVC of 7.2 L. Spirometry data, including (FVC) are calculated with a percentage of predicted values using Global Lung Initiative (GLI) reference standards. (27)

- Relevant difference (Δ)**:** 144 mL (2% change in FVC)
- Expected standard deviation (SD) (σ): 216 mL
- Significance level (α): 0.05
- Power (1-β): 80%

Sample size calculation – Dykstudie

Consider Δ as the absolute difference between control and intervention regarding change in FVC (post-pre dive). Significance level is fixed at α=0.05.

Table 1: Sample sizes for different levels of Δ, standard deviation, and power. Sample size is calculated based on a paired t-test

Δ	Standard deviation of Δ	N (power 80%)	N (power 85%)	N (power 90%)
144	180	15	17	19
144	216	20	23	26
144	252	27	30	35
180	180	10	12	13
180	216	14	15	18
180	252	18	20	23
216	180	8	9	10
216	216	10	12	13
216	252	13	15	17

Based on a man 30 years old, 180 cm tall with weight 75 kg and expected FVC 7.2 L:
2% = 144 mL 2.5% = 180 mL 3% = 216 mL 3.5% = 252 mL

15. ASSESMENT AND OUTCOME:
To evaluate the effect of the intervention, a series of standardized measurements will be conducted at predefined time points in relation to each exposure **(seen in Table 2).** These assessments include both subjective and objective evaluations of pulmonary function, inflammatory markers, and potential adverse effects. Assessment of primary and secondary outcomes is designed to evaluate changes in oxidative stress, with within-participant comparisons made before and after the intervention.

Assessment	**Description**	Pre 1st visit	Post 1st dive	Post 1st dive	Pre 2nd dive	Post 2nd dive	Post 2nd dive
Date and time	Tracking record of exposure timeline	Before 1st dive	30-120 min	12-36 h	Before 2nd dive	30-120 min	12-36 h
Anthropometric measurements	Weight, height, sex, age, BMI	X					
Self-reported symptoms	SOB: Dyspnea etc.	X	X	X	X	X	X
Oscillometry (Tremoflo)	Airway resistance	X	X	X	X	X	X
FeNO (Levels of NO)	Airway inflammation	X	X	X	X	X	X
Spirometry and body plethysmography	FVC, FEV1, IC, FEF 25-75%, PEF, TLC, RV, FRC	X	X	X	X	X	X
D_{LCO}	Diffusion capacity	X	X	X	X	X	X
PExA	Airway lining fluid particle analysis	X		X	X		X
Blood sample + urin sample	Analysis of oxidative stress, inflammatory biomarkers, biomarkers of neuronal injury	X	X	X	X	X	X
Alveolar integrity	Epithelial permeability (via DTPA uptake)			X			X
Adverse events	Systematic monitoring for safety	X	X	X	X	X	X

Table 2. Time relationship for each measurement, in chronological order relative to each exposure.

Spirometry and Body plethysmography (Δ FVC, SVC, FEV_1, IC, FEF_{25}-FEF_{50}, FEF_{75}, PEF, TLC, RV, FRC)
As the spirometry results include assessment of forced vital capacity (FVC) as our primary outcome, these measurements play a central role in evaluating the intervention's impact on pulmonary function. To provide a more comprehensive evaluation, additional spirometry parameters are included in our assessment; forced expiratory volume in one second (FEV_1), forced expiratory flow, inspiratory capacity (IC) and peak expiratory flow (PEF). Pre- and post-exposure values are compared to determine whether hydrogen supplementation offers a protective effect on lung function. To further investigate the effects of hydrogen supplementation, we will examine its impact on:

Diffusing Capacity for Carbon Monoxide (Δ D_{LCO})
Measuring D_{LCO} to assess the alveolar-capillary membrane and the lung's gas exchange efficiency. A stable or improved D_{LCO} post-dive in the H_2-supplemented group suggest a protective effect against the oxidative stress induced by high oxygen partial pressures.

Exhaled Breath Particle Analysis (Δ PExA)
PExA measurements will provide direct insight into biochemical changes within the epithelial lining fluid (ELF) of the lungs**,** potentially revealing early signs of pulmonary oxygen toxicity and the mitigating effects of hydrogen supplementation.

Oscillometry (Tremoflo)
Oscillometry will be performed to assess airway resistance and reactance**,** providing insight into potential changes in airway mechanics following exposure.

Fractional Exhaled Nitric Oxide (FeNO)
FeNO will be measured as a marker of airway inflammation, with pre- and post-exposure values analyzed to determine whether the intervention mitigates inflammatory responses associated with oxygen exposure.

Self-Reported Symptoms (Dyspnea and SOB)
Self-reported symptoms of dyspnea and shortness of breath (SOB) will be collected to **c**apture subjective respiratory changes**,** allowing for correlation with objective pulmonary function measures.

Biomarkers and blood samples:
Blood samples will be analyzed to assess biomarkers of oxidative stress and systemic inflammation**,** enabling a comparative evaluation of metabolic responses before and after exposure to hyperbaric oxygen with or without hydrogen supplementation. Fluid biomarkers of neuronal injury will be analyzed to evaluate the effect of breathing hyperbaric oxygen, with and without addition of hydrogen, on the central nervous system.

Adverse Events (AE)
Adverse events (AE) will be systematically recorded throughout the study to ensure participant safety and assess any potential intervention-related effects, contributing to the overall risk-benefit evaluation of hydrogen supplementation.

Serious Adverse Events (SAE)
Given that the study follows a well-established safety protocol and remains within recognized exposure limits, the risk of serious adverse events (SAE) is considered low. However, any serious injuries or indications of an SAE will be systematically documented to ensure participant safety and evaluate potential risks associated with the intervention. In the event of an SAE, the participant will be advised to terminate their participation to mitigate further risk.

15. ETHICAL CONSIDERATIONS

This study will be conducted in line with the International guidelines for good clinical practice (ICH-GCP E6 (R2)(28) and the latest version of the Declaration of Helsinki.(29) All dive candidates will be clearly informed of their right to withdraw from the study at any time without any penalty. To ensure support and address any concerns, a designated researcher will be present during all study investigations.

Ethical Review Authority

The study has been reviewed and approved by the Swedish Ethical Review Authority (Etikprövningsmyndigheten) on September 10th, 2025 with the diary number: 2025-05124-01. Responsible applicant is the Swedish Armed Forces through the Diving and Naval Medicine Centre

Registration and Reporting

In addition to obtaining ethical approval, our study will prospectively be registered in a recognized clinical trial database (ClinicalTrials.gov) before recruitment of the first participant. Initially designed in accordance with Consolidated Standards of Reporting Trials (CONSORT) guidelines from 2010 (30) and later on updated to the most recent CONSORT extensions and best practice standards as of 2025 (31) to ensure transparency and publication of reliable results.

Biological Materials for Future Research

To ensure that all collected biological materials are handled in compliance with ethical and legal standard concerning participant privacy and data integrity, a biobank agreement will be established in accordance with the procedures of the Swedish Ethical Review Authority. The application will follow the Biobanks Act (2023:38)(32) and could only be submitted after EPM approval has been granted.

Confidentiality and Data Protection

All participant data will be handled with strict confidentiality. Data will be securely stored and accessible only to authorized study personnel. Personal identifiers will be removed, and data will be coded to ensure participant anonymity in compliance with applicable data protection regulations.

16.1 Potential Risks:

- *Hyperbaric Exposure:*

Exposure to elevated pressures environments carries risks such as barotrauma to the ear or lungs. However, there is no risk of DCS due to the very low partial pressures of inert gases used.

- *Oxygen Toxicity:*

Neurological effects are unlikely at the partial pressures of oxygen planned for this experiment. Oxidative pulmonary stress effects are expected, but prior studies and experience have not demonstrated any long-term consequences following single exposures. (33) See also Section 12: Risk management.

Hydrogen mixed with oxygen poses a risk of explosion. See also Section 12: Risk management.

- *Handling of Personal Data:*

Ensuring secure and ethical management of sensitive participant data.

16.2 Risk Mitigation Measures:

- *Hyperbaric Exposures:*

The experiments will adhere to established military safety protocols. Additionally, the participants are experienced military divers, which minimizes the risk of barotrauma. We will remain within national oxygen exposure limits for military diving. Furthermore, participants are military divers Assessed by a physician to have healthy lungs.

- *Hydrogen and Oxygen Mixtures:*

It's well known that hydrogen can be explosive when mixed with oxygen, thus, this reaction doesn't occur if the hydrogen fraction is kept sufficiently low. Gas cylinders will be pre-filled according to current standards to ensure an adequately low hydrogen fraction. See also Section 12: Risk

management.
Handling of Personal Data:
Data will be pseudo-anonymized, and individual keys will be securely stored in a safe at Blekinge Institute of Technology.

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17. Potential Benefits:
- *Potential Intervention Development:*
The findings could contribute to reducing the toxic effects of oxygen on tissues, enabling dives that are impossible today because of high inert gas load and/or to high pressure of oxygen. Additionally, such interventions could improve treatments for critically ill patients (e.g., intensive care unit)
- *Improved Understanding:*
The experiment may provide a better understanding of the toxic effects and pathophysiological effects of oxygen on lung tissues. We assess that the potential benefits outweigh the risks in this study setup. The planned exposures are no greater than those already approved, and our intervention and outcomes are likely to provide valuable knowledge for both divers and patients.
- *Enabling Longer and Safer Dives:*
By mitigating oxygen toxicity, hydrogen-enriched gas mixtures may enable longer and deeper dives than currently possible. Reducing the oxygen toxicity risk could allow divers to safely increase the exposure time at high pressures, optimizing operational efficiency and reducing decompression-related constraints. A higher possible oxygen fraction also means a lower inert gas fraction, further decreasing nitrogen absorption and reducing the risk of decompression sickness
- *Improved Clinical Applications*
The ability to reduce oxygen toxicity could have profound medical implications, particularly in neonatal care, intensive care and post-organ transplantation. Basically, all areas where oxygen therapy is crucial but carries risks of oxidative damage. Understanding how to modulate oxidative stress in a controlled setting could enhance critical care treatments, potentially improving patient outcomes in high-oxygen environments.

Risk-Benefit Assessment
We assess that the potential benefits outweigh the risks in this study setup. The planned oxygen exposure strictly adheres to diving regulations set by the Swedish Armed Forces' and does not exceed levels approved in existing hyperbaric research and clinical practice. These standards are specifically designed to ensure participant safety during hyperbaric regulations. Furthermore, hydrogen has been repeatedly incorporated into breathing mixtures in professional deep-sea diving to safely reduce the concentration of oxygen or other active components.

The practice of using hydrogen as a diluent gas is well-documented in several experimental programs who demonstrated its feasibility under hyperbaric conditions simulated at depths of up to 700 meters (19). As outlined above, the planned oxygen exposure of 1.7 ATA in our study is well below the limits investigated in previous hyperbaric research. It can therefore be concluded that the exposure falls within established safety margins in accordance with existing scientific evidence and diving regulations. Given the high potential for both scientific and clinical impact, the results of our study are expected to provide valuable insights for diving professionals as well as for the care of critically ill patients requiring high oxygen concentrations.

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18. Summary
The antioxidative potential of hydrogen gas presents a promising strategy for reducing pulmonary and neurological oxygen toxicity, reaching implications for diving safety. This study aims to explore the feasibility of the antioxidative potential of hydrogen, proposing a novel approach to safer diving protocols and improved clinical interventions. Specifically, it aims to assess whether incorporation of a small fraction of hydrogen into a breathing gas could effectively reduce symptoms of POT within oxygen-enriched diving exposed to elevated partial pressure of 1.75 ATA over 240 minutes. If

successful, this approach could provide several key benefits**,** including extended dive durations by mitigating the limitations of oxygen toxicity.

WORK PLAN:
1) Study preparation: 2025
2) Recruitment and data collection: 2026-2027.
3) Analysis and reporting: 2027-2030

19. GUIDELINES
The study will be conducted under the oversight of the Swedish Armed Forces Diving and Naval Medicine Centre (DNC), ensuring full compliance with internationally recognized hyperbaric safety standards. The Diving Medicine Section is responsible for all medical aspects of diving within the Swedish Armed Forces**,** including research and operational support. By adhering to guidelines set by the European Diving Technology Committee (EDTC) and the European Committee for Hyperbaric Medicine (ECHM) upholding highest standards of protection and operational integrity. (34) To ensure both safety and reliability within our study while meeting the highest medical and scientific standards.

20. DISSEMINATION
Findings will be published in peer-reviewed journals and presented at international diving and medical conferences. Planned publications include of: 1) primary outcome and main secondary outcomes; 2) PeXA outcomes; 3) blood inflammatory profile outcomes; 4) exploratory analyses of mechanisms underlying oxygen toxicity. Authorship will be determined in accordance with guidelines from the International Committee of Medical Journal Editors (www.icmje.org/). Further, relevant professionals and organizations in diving medicine will be informed through targeted communications, to ensure the results contribute to both scientific advancements and practical applications in the field.

22. BUDGET AND RESOURCES
Current Funding: The project is supported by grant 5005113/22FMV2951.

Key Resources and Collaborations:
Hyperbaric Facilities:
Access to hyperbaric chambers and associated medical support is secured through a collaboration with DNC, Karlskrona. Including the availability of a certified physician during procedures.

Measurement Equipment:
Required instrumentation and technical equipment will be provided through collaboration with the Blekinge Institute of Technology, Department of Translational Medicine, *Lund* University and Occupational and Environmental Medicine, *Sahlgrenska Academy*

Personnel: The research team includes physicians with expertise in diving medicine, pulmonary physiology, nuclear medicine and environmental medicine, combined with specialists in advanced marine systems engineering. The team further comprises research assistants and biostatisticians with additional technical and administrative personnel as needed throughout the project.

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