Chapter I

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Oxygen in extreme environments

The importance of oxygen on the formation of Earth and life on it cannot be stressed enough.\(^1\) The majority of the organisms on our planet cannot function in the absence of adequate amounts of oxygen and will perish when oxygen levels drop below a critical level. The atmosphere consists of 78.1% nitrogen, 20.9% oxygen, and 1% other gases.\(^2\) This means that with every breath we take, we inhale approximately 21% oxygen. Although this is largely adequate in most circumstances, more oxygen is sometimes required for patients in emergency rooms and intensive care units and during anesthesia.\(^3\) When extra oxygen is supplied, for instance though a breathing mask, this increases the fraction of inspired oxygen (FiO\(_2\)). Based on Dalton’s law of partial pressures (\(p_{\text{total}} = p_1 + p_2 + \ldots + p_n\)), the partial pressure of oxygen (PO\(_2\)) at sea level (101.3 kPa, or 1 ATA) is equal to the FiO\(_2\). A higher PO\(_2\) leads to better oxygenation of cells and tissues and can be beneficial for many processes in the body, including wound healing.\(^6\)\(^,\)\(^7\)

The difference between FiO\(_2\) and PO\(_2\) becomes relevant when ambient air pressure changes. The ambient pressure slowly decreases with increased altitude. At an altitude of 5500 m, or 18,000 ft, the ambient air pressure is 50.7 kPa, or 0.5 ATA. Based on Boyle’s law (\(p \times V = c\)), each breath at 0.5 ATA has half the number of molecules at sea level. Thus, a FiO\(_2\) of 0.21 at 5500 m gives a PO\(_2\) of 0.10 (rounded down). To some degree, the human body can adapt and compensate for these changes in PO\(_2\), allowing short visits to these altitudes.\(^8\) Supplementary oxygen is required at higher altitudes.\(^9\) A continuous supply of additional oxygen is essential during flights at high altitudes (fighter pilots) or in space (astronauts) to prevent loss of consciousness.\(^10\)

In contrast to high altitude, divers are exposed to higher partial pressures of oxygen due to increased ambient water pressure. While it takes ascending to 5500 m to reduce the atmospheric pressure to 0.5 ATA, it only requires 10 m of sea water (msw) to double the pressure to 2 ATA. Environments with increased ambient pressure are called hyperbaric. When diving to 10 msw with compressed air, the FiO\(_2\) is still 0.21, but the PO\(_2\) is 0.42. Under hyperbaric conditions, the FiO\(_2\) remains constant, but the PO\(_2\) increases – even to partial pressures surpassing the partial pressure of 100% oxygen at sea level.
Oxygen toxicity

In 1878, the French physiologist Paul Bert published a paper on the toxic effects of oxygen. He found that animals, insects, plants and even fungi perished when exposed to high levels of oxygen for long periods of time. In humans, an increased partial pressure of oxygen can lead to ‘central nervous system oxygen toxicity’ (CNS-OT) with a wide range of symptoms varying from paranesthesia and nausea to visual and auditive disturbances and ultimately convulsions. Soon afterwards, in 1899, the Scottish pathologist James Lorrain Smith published an important paper concluding that oxygen can also be toxic for the pulmonary system of mice and larks. This ‘pulmonary oxygen toxicity’ (POT) can be divided in an early stage, with reversible changes such as inflammation and alveolar edema and a late stage, with irreversible changes such as fibrosis and emphysema. The late phase is reminiscent of extensive lung disease observed after many years of smoking; however, unlike lung disease, POT can appear within a relative short period of time. Both CNS-OT and POT are harmful effects of oxygen, which occur in experimental conditions under extreme PO2. It was unclear how long, or at which pressure, oxygen could safely be tolerated. Unlike today, at the beginning of the 20th century, preventive medicine was not as developed as it is today, thus, many occupational divers suffered from diving related illnesses at that time.

Diving with oxygen

Halfway the 19th century, compressed air exposure resulting in the accumulation of inert gasses, mainly nitrogen, in the body was recognized as the cause of decompression sickness. This resulted in the development of gas mixtures with less nitrogen. A cheap and widely available alternative was to replace nitrogen with oxygen. Mixtures with increased oxygen concentrations are called hyperoxic and are commonly known in diving as ‘enriched air nitrox’ (EAN) or simply nitrox. Depending on local regulations and training limitations sports divers can use nitrox mixtures containing up to 60% nitrogen and 40% oxygen. For instance, at 20 msw (3 ATA), the PO2 of a 40% nitrox mixture is 1.20 ATA. This is equal to 120% oxygen under normobaric conditions, which would be impossible to administer at sea level. This is an example of a hyperbaric environment with a hyperoxic mixture. These hyperbaric hyperoxic exposures are particularly toxic to the nervous system and the lungs. Nowadays, divers limit the depth of their dives to prevent CNS-OT and thus are relatively safe. In sports diving POT is usually not considered, since exposure is rarely frequent or long enough.
to cause symptoms. However, this is not the case for commercial, technical or military diving.

Certain scenarios (for instance clearance of sea mines) can require diving to great depths. These dives are often made with exotic mixtures, containing varying inspiratory fractions of helium, nitrogen, and oxygen (trimix) or helium and oxygen (heliox). It is beyond the scope of this thesis to expand on the additional dive medical problems these mixtures introduce in terms of decompression illnesses, but similar problems arise when considering $PO_2$ and oxygen toxicity.

A different type of diving is ‘tactical’ diving, of which an example is been given in the preface. In this type of diving military divers, or more specifically the combat divers of the Special Operations Forces (SOF), usually dive to relatively shallow depths but remain submerged for very long periods of time. Many military divers worldwide use closed-circuit rebreathers with 100% oxygen (O2-CCR) for diving under covert conditions. This is because O2-CCR produces almost no bubbles on exhalation (enhancing stealth), allows dives of several hours (expanding the operational area) and eliminates decompression sickness (which are difficult or impossible to treat in hostile or remote locations). However, diving with 100% oxygen ($FiO_2 = 1.0$) at a depth as shallow as 5 msw gives a $PO_2$ of 1.5 ATA. With dive times lasting several hours and divers diving several times a week over the course of their careers, CNS-OT and POT pose a plausible hazard to the health of these divers.

Hyperbaric Oxygen Therapy (HBOT)

Aside from diving, hyperbaric hyperoxia is also used as a treatment for wounds with damaged vascularization, such as those caused by diabetes mellitus or radiation. Patients are commonly treated daily in a recompression chamber for approximately 90 minutes at a $PO_2$ of 2.5 ATA, for a period of between 4 and 8 weeks. These treatments are also known as ‘dry dives’, as this form of hyperbaric hyperoxia does not involve submersion. HBOT is usually accepted very well by patients, and CNS-OT and POT very rarely occurs or are seldom reported. Although many explanations why CNS-OT and POT seldom occurs can be given, perhaps most relevant is that patients receiving HBOT have had severe complaints to begin with, therefore masking potential ‘subclinical’ changes. From an ethical point of view, it is paramount to assess the safety of this treatment when it is applied to frail patients, for instance those with delayed radiation
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Mathematical models

After the seminal work of Paul Bert and James Lorrain Smith several authors extended our understanding of oxygen toxicity and tested many parameters as potential markers of this illness. During World War 2, both Allied and Axis Forces deployed ‘frogmen’ using O2-CCR with devastating success. Although accurate reports of CNS-OT and POT during this period are lacking, it became clear that diving with O2-CCR was not without risk. This led to increased efforts to make diving with oxygen and O2-CCR safer. In 1970, James Clark, an American physician, published the first ‘safe limits’ for oxygen exposure aimed at preventing POT, based on a series of exposures in a recompression chamber (similar to HBOT). Based on a mathematical model including time exposed to PO2, oxygen damage defined as a decrease in vital capacity (VC) was quantified as Units of Pulmonary Toxicity Dose (UPTD). Although the UPTD has many methodological and practical limitations, which were recognized by the original authors, they are still used today. As the original authors put it: “The ability to detect the onset of pulmonary oxygen poisoning will be greatly enhanced if a toxicity index can be established which is more sensitive and more reliable than decrease in the VC.” In the 50 years following its introduction, other parameters than VC have been tested to replace the UPTD, but none have been successful so far.

Personalized medicine

Outside the realm of diving medicine, pulmonary medicine faced many similar challenges. Diagnosis of diseases such as asthma and chronic obstructive pulmonary disease (COPD) relied on clinical presentation and the results of pulmonary function tests (PFT). While many patients were comparable with respect to clinical presentation and the results of PFT, their responses to medication varied greatly. With the advent of gas chromatography and mass spectroscopy (GC-MS), it became clear that the asthma patients could be separated into groups based on the presence of specific volatile organic compounds (VOCs) in their exhaled breath. Where asthma was once considered a uniform entity, it can be divided into separated groups though specific VOCs, each with different responses to medication and different risks for exacerbation. Another application of exhaled breath analysis is its use in prediction whether immunotherapy will be effective in a particular patient, thereby sparing injuries or compromised grafts and flaps after surgery.
irresponsible patients long and costly treatments that can have a major impact on quality of life.\textsuperscript{38} Exhaled breath analysis and VOCs profiles assist pulmonary medicine in tailoring treatments according to specific patient characteristics.\textsuperscript{16,38,39}

GC-MS is both a time and resource dependent diagnostic modality.\textsuperscript{40} To enable clinicians to use breath markers in daily practice, a point-of-care instrument that rapidly analyses exhaled breath is required. A potential instrument is the electronic nose (eNose), which can generate exhaled breath profiles termed ‘breathprints’ (analogous to a fingerprint of exhaled air), allowing comparison to be made between the breathprints of patients and those in a database of ‘healthy’ and ‘affected’ individuals to identify disease.\textsuperscript{41,42} Considering the major impact VOCs have had in pulmonary medicine; it stands to reason that similar techniques could prove useful in diving medicine. However, before these instruments can be utilized in diving medicine to identify POT, the relevant VOCs and associated breathprints must be determined. Additionally, analyzing exhaled breath with an eNose has a significantly lower impact on resources than GC-MS, both with respect to cost and time. However, to enable large scale research on POT, the VOCs found by GC-MS analysis have to be linked to the sensor data generated by eNose.

**Thesis outline**

The aim of this thesis is to evaluate whether VOCs can be used to detect the onset and development of POT after hyperbaric hyperoxic exposure.

**Chapter 2** provides a narrative review of the available scientific literature on oxygen toxicity and its relevance to O2-CCR diving and HBOT. While POT is the main area of interest of this thesis, the history, clinical aspects and mathematical models of CNS-OT should be taken into account when designing studies investigating POT, as CNS-OT is an important limiting factor in hyperbaric hyperoxic exposure.

As submersion results in changes in ambient and hydrostatic pressure that alter pulmonary physiology, it would not be surprising if this reflected in changes in the chemical constituents of exhaled breath. To establish if exhaled breath can be analyzed to detect the ‘additional effect’ of oxygen diving, **chapter 3** presents a randomized double-blind cross-over trial in which Navy divers dove to 9 msw for 1 h with either oxygen (\(\text{FiO}_2\;1.0\), \(\text{PO}_2\;1.9\;\text{ATA}\)) or pres-
surized air (FiO$_2$ 0.21, PO$_2$ 0.40 ATA). Exhaled breath was collected both before and at several moments after the dive and analyzed using GC-MS. Additionally, PFT and diffusion capacity tests were also performed to allow comparison with the current gold standard (UPTD). The identified VOCs described within their pathophysiological framework.

The difference between wet (immersed) and dry (in a recompression chamber) hyperbaric hyperoxia has been addressed in chapter 2. To assess the effect of ‘dry’ hyperbaric hyperoxia, as well as to evaluate the effect of daily exposure to hyperbaric hyperoxia as used in HBOT, chapter 4 presents a longitudinal cohort study of Navy recompression-certified personnel who were subjected to six HBOT-sessions (80 min, FiO$_2$ 1.0, PO$_2$ 2.5 ATA). Again, like in the previous chapter, PFT and diffusion capacity tests are included to compare with the measured decrease in VC to the UPTD model and results are placed within context.

Because O2-CCR divers are perhaps most susceptible to the development of POT and to explore the possibilities of exhaled breath analysis under operational circumstances, chapter 5, describes a field study of divers from the Netherlands Maritime Special Operations Forces (NLMARSOF) in two scenarios simulating the actual working environment of the NLMARSOF. The first scenario consisted of a shallow (3 msw) endurance (4 h) O2-CCR dive with divers who were rested. In the second scenario the divers made a 3 h O2-CCR dive as part of a training scenario after five days of vigorous physical exertion, little sleep and very little food. The exhaled breath characteristics are described and compared with the results of chapter 3.

Previous chapters presented results obtained using GC-MS based exhaled breath analysis. Chapter 6 presents eNose data acquired after diving with oxygen for 1 h to 9 msw (FiO$_2$ 1.0, PO$_2$ 1.9 ATA). Additionally, this chapter utilizes two-way orthogonal partial least square (O2-PLS) regression to associate GC-MS and eNose data and evaluate the eNose sensors’ contribution to detection of targeted VOCs.

Lastly, chapter 7, is a summary of the main findings of in this thesis and elaborates on the future perspectives of using exhaled breath analysis for detecting POT.
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References


29. Clark JM, Lambertsen CJ. Pulmonary oxygen tolerance in man and derivation of pulmonary oxygen tolerance curves. Institute for Environmental Medicine, University of Pennsylvania Medical Center, USA; 1970.


32. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and...
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