POTENTIAL APPLICATIONS FOR HUMAN HYPOXIA MODELS

TITIAAN POST

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CHAPTER I | INTRODUCTION

Understanding human hypoxia models and their applications

Hypoxia, the condition in which the body or a region of the body is deprived of adequate oxygen supply, is a critical factor influencing human physiology in various environments. On Earth, hypoxia plays a critical role in the study and treatment of conditions such as stroke and lung diseases. Beyond clinical settings, hypoxia is also significant in high-altitude environments, affecting those involved in mountaineering and aerospace missions. Understanding how the human body responds to low oxygen levels is crucial for advancing medical science, enhancing human performance, and ensuring safety in extreme environments. This dissertation explores the multifaceted role of hypoxia through a series of studies that leverage human hypoxia models, initially developed for aerospace medicine, to better understand its effects on the body and its potential applications in both clinical and non-clinical settings.

The foundation of human hypoxia models

Human hypoxia models are essential tools for simulating low-oxygen conditions in physiological and clinical investigations. Originally developed for aerospace medicine, these models allow researchers to replicate hypoxic conditions in a controlled environment, providing invaluable insights into the physiological changes that occur during oxygen deprivation. Despite their potential, these models are currently underutilized. **Chapter 2** introduces the concept of hypoxia and its relevance across various fields, from clinical settings where hypoxia is a common complication in diseases such as chronic obstructive pulmonary disease (COPD) and heart failure, to high-altitude environments and space, where ambient oxygen levels are altered. The chapter also highlights the need for standardized and validated hypoxia models to ensure the reliability and accuracy of research findings, which is critical for advancing our understanding of human physiology and developing new therapeutic interventions.

Enhancing oxygenation in aviation through elevated CO_2 levels

Commercial air travel exposes passengers to hypoxic conditions due to the high cruising altitude. Cabin pressurization partly mitigates effects of reduced oxygen levels, but it is not always sufficient to prevent hypoxemia, particularly in passengers with underlying health conditions. **Chapter 3** explores an innovative approach to counteracting hypoxemia risks by investigating the potential benefits of elevated carbon dioxide (CO_2) levels in airplane cabins. The study tests whether maintaining a 1% CO_2 concentration can improve blood oxygen saturation (SpO_2) levels without negatively impacting cognitive functions, offering a potential strategy for enhancing passenger safety and well-being during flights. This chapter also discusses the environmental and operational implications of this approach, emphasizing the need for further research to optimize cabin conditions while balancing efficiency and sustainability in aviation practices.

Cognitive impairment under hypoxic conditions

One of the earliest symptoms of hypoxia exposure is impaired cognitive functioning, which poses significant safety risks for occupational and recreational settings at high altitudes. During high-altitude activities, cognitive performance plays a critical role in tasks that require attention, decision-making and memorization of safety-relevant protocols. Impaired cognitive function predisposes to mistakes, which can have catastrophic consequences. Over the years, numerous tests have been developed to evaluate cognitive performance. However, due to the wide range of tests used in studies examining cognitive performance during acute hypoxia exposure, it is challenging to compare results across studies. **Chapter 4** provides an overview on the wide range of tests used to assess cognitive performance during acute hypoxia in healthy volunteers, to evaluate the differences in test sensitivity to different levels of hypoxia, to explore the role of barometric pressure, and to ultimately identify the tests that best detect functional hypoxia effects. The chapter also highlights the need for standardized cognitive testing to better understand the effects of hypoxia on executive functions and to develop strategies for mitigating these risks, particularly in environments where cognitive performance is critical for safety and mission success.

Insights from the naked mole-rat's hypoxia tolerance

The naked mole-rat, a species with exceptional hypoxia tolerance, offers fascinating insights into potential adaptations that could benefit humans. This small rodent can survive in nearly anoxic conditions by metabolizing fructose instead of glucose, a unique adaptation that allows naked mole rats to thrive in extreme hypoxia. **Chapter 5** explores the possibility of leveraging fructose metabolism to enhance human endurance and cognitive function under hypoxic conditions. Given the limited evidence regarding human fructose metabolism, systemic availability and metabolic

response following oral fructose loading was assessed. In a second part, it was determined whether fructose ingestion acutely improves endurance capacity and visual and cognitive performance in humans exposed to acute hypoxia in a randomized, double-blind, placebo-controlled crossover study.

Hypoxia and circadian rhythm regulation

Circadian clocks regulate daily cycles in physiology and behavior, with a master clock in the brain aligning these rhythms to the day-night cycle and coordinating clocks in peripheral tissues. Recent discoveries in rodents and cell cultures suggest that tissue oxygenation cycles might help reset and synchronize circadian clocks. **Chapter 6** explores whether exposure to ambient hypoxia in humans can reset the circadian melatonin rhythm, potentially providing the first direct evidence of an interaction between the body's hypoxia-sensing pathway and circadian clocks. The chapter explores how behaviors that alter tissue oxygenation, such as exercise and fasting/eating, might influence circadian timing. The chapter also examines the implications for hypoxia-related diseases, like chronic obstructive pulmonary disease and sleep apnea, which may lead to circadian misalignment and associated health issues. Given the crucial roles of hypoxia-sensing and circadian pathways in health and disease, this study offers insights with broad, cross-disciplinary relevance.

General discussion and future directions

Chapter 7 summarizes the findings from previous chapters, highlighting the potential of hypoxia research to advance medical science, particularly in understanding and treating hypoxia-related conditions. The chapter emphasizes the need for more advanced and standardized human hypoxia models to enhance our knowledge and translate it into clinical practice. It also underscores the importance of cross-disciplinary collaboration, especially with aerospace medicine, to fully utilize hypoxia models and gain new insights into human physiology. Looking ahead, research into hypoxia and its effects on the body remains promising for breakthroughs in both clinical and non-clinical settings.

Potential applications of experimental human hypoxia models

Experimental human hypoxia models serve as valuable tools across multiple disciplines. In pathophysiological research, they provide insights into how low oxygen levels impact the body, aiding the study of conditions such as stroke, chronic lung diseases, and heart failure. These models also contribute to clinical drug development, enabling the controlled testing of new treatments and improving understanding of their potential in addressing oxygen-deprivation-related conditions. Moreover, hypoxia studies can serve as benchmarks of functional relevance, for (novel) CNS-active drug effects in healthy participants.

Beyond healthcare, hypoxia models are instrumental in occupational medicine, supporting the safety and performance of individuals working in low-oxygen environments, such as high-altitude workers, miners or military personnel. Similarly, in aerospace safety, they simulate conditions encountered by pilots and astronauts, informing strategies to enhance safety and performance in flight and space missions. These applications highlight the practical value of hypoxia models in bridging research and real-world challenges across diverse fields.

CHAPTER II | HUMAN HYPOXIA MODELS IN AEROSPACE MEDICINE: POTENTIAL APPLICATIONS FOR HUMAN PHARMACOLOGICAL RESEARCH

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ABSTRACT

Aerospace medicine required controlled terrestrial models to investigate influences of altered atmosphere conditions, such as hypoxia, on human health and performance. These models could potentially be expanded to encompass disease conditions or treatment targets regulated through hypoxia or hypercapnia. Hypoxia, a condition in which the body is deprived of adequate oxygen supply, profoundly affects human physiology at multiple levels and contributes to the pathogenesis of various diseases. Experimental exposure to hypoxic conditions has gained recognition as a model for studying diseases such as pulmonary hypertension, chronic obstructive pulmonary disease, obstructive sleep apnoea, migraine and kidney disease. This approach may be particularly useful in mechanism-oriented early-stage clinical studies. This review discusses the ability of hypoxia models from space medicine research to mimic or induce these conditions in a controlled laboratory setting as a tool for testing the efficacy and safety of new pharmaceutical interventions.

INTRODUCTION

Challenges in space and aeronautics environments

In space and in aeronautics, human beings are exposed to harsh environmental conditions that pose risks for health and performance. An important example is altered atmospheric pressure and composition. Even in pressurized aeroplane cabins, atmospheric pressure and oxygen partial pressure are significantly reduced. In spacecraft, atmospheric pressure and relative oxygen content vary profoundly between vehicles. Moreover, during extravehicular activities in free space or on another celestial body, pressure in the spacesuit is substantially reduced to reduce its stiffness and astronauts breathe pure oxygen.[1] Carbon dioxide concentrations in space- and aircraft cabins can increase significantly due to the closed environment.[2,3] These conditions pose risks for human health and performance in space but could also be tweaked to achieve desirable health effects.

Terrestrial models in space medicine

Testing influences of atmospheric conditions in space is difficult given the relatively low number of astronauts and limited availability of medical and psychological testing capabilities. Therefore, space medicine developed highly controlled terrestrial models exposing human beings to environmental conditions that are relevant to space or aeronautics. Influences of these conditions on human health and performance are then investigated using high-fidelity phenotyping. Our study testing the interaction between simulated weightlessness through head-down tilt bedrest and elevated ambient carbon dioxide is prime example for this approach.[4] This review will discuss how this approach could be used in modelling disease conditions in clinical drug development with a particular focus on hypoxia.[5] We will focus on commonly used human preclinical hypoxia models and their relevance to human pathophysiology, with the aim of providing a comprehensive analysis of the translational gap filled by these models.

Understanding hypoxia: causes and effects

Hypoxia is a state in which the body or parts of the body are inadequately supplied with oxygen. The condition can occur for various reasons, including high altitude exposure, heart failure, intoxications, anaemia,

chronic vascular diseases, obstructive sleep apnoea (OSA), as well as lung diseases such as cystic fibrosis, asthma and chronic obstructive pulmonary disease (COPD).[6–9] Oxygen plays is crucial for tissues to generate energy and to maintain cellular functions.[10,11] Furthermore, hypoxia on a tissue level develops when capillaries are rarefied, poorly perfused or when the distance between capillaries and cells is increased, for example, by oedema formation. As the final electron acceptor in the electron transport chain, oxygen is necessary for aerobic respiration, which typically generates the majority of the cell's chemical energy.[12] Thus, hypoxia arises when oxygen levels drop and fall below energetic demands, where causing inadequate oxygenation of tissues and a poorly regulated response can contribute to chronic diseases.[13]

Using hypoxia as a controlled human disease model

Experimentally induced hypoxia has the potential to serve as a research tool. Hypoxia could be used as a human disease model mimicking or inducing the pathological responses observed in certain diseases. A potential advantage of the approach is that hypoxia exposure can elicit physiological responses in isolation from common confounding variables, in controlled and measurable amounts, with graded doses, and safely in otherwise healthy individuals. Furthermore, hypoxia could be utilized to validate potential treatment targets and pathways that are regulated through oxygen.

SPACE MEDICINE DISCOVERIES: LESSONS FROM TERRESTRIAL MODELS IN WEIGHTLESSNESS ENVIRONMENTS

Weightlessness is a defining challenge in the space-related environmental conditions that astronauts must navigate. As they effortlessly float through their spacecraft, the physical demands on their bodies are significantly reduced, resulting in a state of immobility. Comparable to bedrest on Earth, prolonged periods of weightlessness lead to a decline in bone mass and muscle weakening. Consequently, spaceflight serves as a unique testing ground for drugs combating bone loss.

A pharmacological study involving 7 astronauts, with an average stay of 5.5 months on the International Space Station, revealed the superior effects of resistive exercise on bone health when combined with alendronate therapy compared to exercise alone.[14] Remarkably, classical anti-osteoporotic drugs also demonstrated positive effects on bones in ground-based bedrest immobilization studies.[15] These findings align with current guidelines for osteoporosis treatment, emphasizing the importance of a combination of pharmacological and nonpharmacological interventions.[16]

Beyond musculoskeletal challenges, spaceflight induces deconditioning of the circulatory system and cardiac atrophy, leading to orthostatic intolerance and fainting upon return to Earth. To address this, pharmacological countermeasures have been explored both in space and terrestrial models. For instance, a study administering 5 mg of the α-agonist drug midodrine orally to subjects following up to 16 days of head-down tilt bedrest reduced the risk of presyncope during tilt table testing from 75 to 28%.[17] Another study involving astronauts returning to Earth found that 10 mg of midodrine orally could reduce tachycardia while maintaining safety.[18] Clinical guidelines for syncope management now recommend midodrine for orthostatic hypotension.[19]

While immobility is a classic risk factor for thromboembolic events, space has seen only 1 symptomatic venous thrombosis, successfully treated with enoxaparin and subsequent apixaban, without progressing to pulmonary embolism.[20,21] Speculation surrounds the activation of natural thromboprotection mechanisms in space, although these have not been thoroughly investigated during in-flight conditions. Notably, a recent study uncovered a novel mechanism that shields bears in hibernation, individuals undergoing head-down bedrest and patients with spinal cord injuries—all enduring chronic immobilization—from thrombosis.[22] Both bear and human thrombocytes exhibit a joint antithrombotic phenotype marked by decreased heat shock protein (HSP) 47 expression. These promising findings may pave the way for innovative classes of antithrombotic drugs, warranting further testing and refinement on spaceflight-related research platforms.

All these examples demonstrate that knowledge and methodologies derived from studies on how the space or aeronautics environment affects human health can be exploited for terrestrial applications. We suggest that influences of atmosphere conditions on human beings deserve more attention in that regard.

PHYSIOLOGICAL RESPONSE TO HYPOXIA

The physiological response to hypoxia involves a complex cascade of adaptive mechanisms aimed at restoring oxygen delivery to vital organs

and maintaining cellular homeostasis. The response to acute, intermittent and chronic hypoxia, is sensed by chemoreceptors. Peripheral chemoreceptors are chemical sensory cells in the aortic and carotid bodies that are activated by changes in oxygen, carbon dioxide and pH blood levels, which are conveyed to the central nervous system. The response restores homeostasis by increasing ventilation, optimizing pulmonary ventilation–perfusion ratio, adjusting cardiac function and raising the oxygen carrying capacity of the blood.[23,24] In addition to optimizing gas exchange, inadequate supply triggers hyperacute responses within seconds to minutes of vascular beds, which are initiated by mitochondria, acting as oxygen sensor.[25] A hypoxic vasomotor response leads to vasodilation and increases tissue blood flow in most organs, with the exception of the lungs, where hypoxia induces vasoconstriction. This hyperacute response is followed by a subacute response over minutes to hours during which the master switch of cellular hypoxia defence, known as hypoxia-inducible factors (HIFs), are activated. HIFs regulate the expression of various hypoxia-sensitive genes such as erythropoietin (EPO), endothelin-1 (ET-1) and vascular endothelial growth factor (VEGF).[6,23,26] Nitrate oxide (NO) contributes to oxygen sensing by modulating the activity of proteins such as prolyl hydroxylase 2 (a key oxygen sensor in the HIF-1 pathway). Hypoxia upregulates NO synthases which increases NO production. NO goes on to inhibit prolyl hydroxylase 2, which prevents HIF-1α hydroxylation and degradation. NO is also crucial to the hypoxic response by regulating vasodilation and blood flow.[27] Nitrite acts as NO reservoir and is converted back to NO in oxygen-deficient conditions. Dietary nitrate, especially from beets, can affect nitrite levels, which provides an opportunity for therapeutic interventions in hypoxic related diseases.[28–30] The European Space Agency (ESA) suggests growing beets, spinach, lettuce and rocket salad (nitrate-rich vegetables) as a food source for long-term space missions.[29]

HIFs are rapidly broken down by prolyl-hydroxylase under normoxic conditions, but accumulate and alter gene transcription under hypoxia due to the oxygen dependent activity of their degrading enzymes. Increased EPO and VEGF expression promote erythropoiesis and angiogenesis respectively, which augments oxygen delivery to cells and tissues.[31] HIF prolyl hydroxylase inhibitors such as roxadustat and molidustat stimulate EPO production in renal anaemia[32,33] HIF-1α can also induce glucose transporter genes to augment glucose transport and metabolism.[35] Furthermore, inflammatory stimuli trigger a metabolic shift in immune cells from

oxidative phosphorylation towards glycolysis.[35] HIF may also be activated hypoxia-independent under normoxic conditions for instance during severe systemic bacterial infection.[36,37] Similarly, HIF activation can occur in situations mimicking hypoxia, such as severe iron deficiency.[38] Whereas HIF-1α is the dominating HIF molecule during the first 24 h of hypoxia exposure, HIF-2α gains dominance thereafter.[39] HIF-2α upregulation contributes to serious systemic diseases such as pulmonary hypertension, pulmonary and cardiac fibrosis, and polycythemia.[40] Furthermore, HIF activates the transcription of genes which are pivotal for cancer genesis, progression and metastasis.[41] Studying high-altitude populations, such as Andeans, Ethiopians and Tibetans, reveals genetic adaptations to chronic hypoxia, including variations in erythrocyte homeostasis, angiogenesis, vasoregulation and immune response.[42,43] These adaptations, particularly the lower expression of the endothelin receptor type B gene, provide insights into hypoxia tolerance and offer valuable models for understanding and potentially treating cardiac diseases.[44,45] Additionally, HSP70, known for its protective effects under hypoxic conditions, may serve as a potential biomarker for hypoxia tolerance, with genetic variations in HSP70 genes influencing susceptibility to high-altitude illnesses.[46] Currently, HIF-1, HSP70 and NO are identified as potential biomarkers to gauge hypoxia tolerance in experimental animals and in humans.[47]

LEVEL-RESPONSE RELATIONSHIP AND LIMITS OF HYPOXIA TOLERANCE

The time-dependent patterns of physiological responses to hypoxia observed during the acclimatization process at high altitude are shown in Figure 1. Hypoxia severity determines intensity and extent of the physiological response.[48,49]

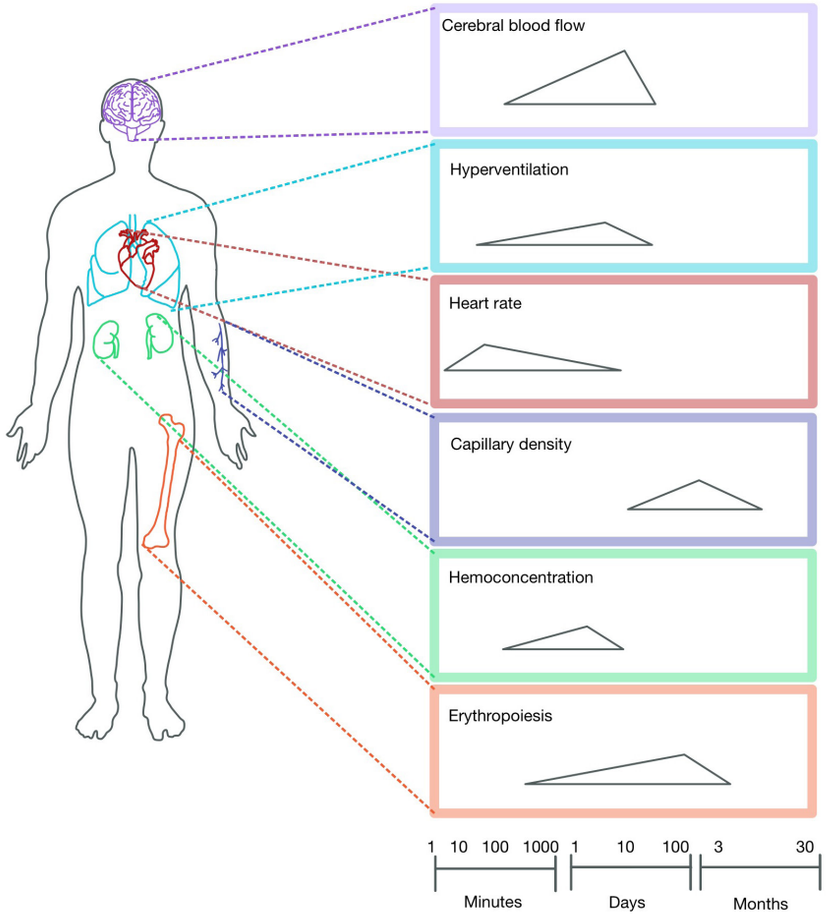
In mild hypoxia, the body initiates compensatory responses to mitigate the impact of oxygen deficiency. These responses include sympathetic nervous system activation, increased ventilation, peripheral vasodilation, increased cardiac output and enhanced oxygen extraction by tissues.

As hypoxia worsens to moderate levels, HIF activation affects genes involved in oxygen transport, angiogenesis and metabolism. Red blood cell production is increased to enhance oxygen-carrying capacity and cell metabolism increasingly shifts towards anaerobic energy generation with increased lactate production and subsequent metabolic acidosis.

In severe hypoxia with critical oxygen deficiency, breathing becomes

more laboured with increased respiratory effort. Anaerobic metabolism and lactate production further increase resulting in severe metabolic acidosis. Cognitive function becomes impaired, resulting in confusion, impaired judgement and potential loss of consciousness. Cardiovascular disturbances may arise, including arrhythmias, decreased cardiac output and increased pulmonary artery pressure, which can lead to organ failure.

FIGURE 1 **The time-dependent patterns of physiological responses to acute hypoxia observed during the acclimatization process at high altitude (modified from Burtscher *et al.*, 2022[50] and Mallet *et al.*, 2023[51]).**



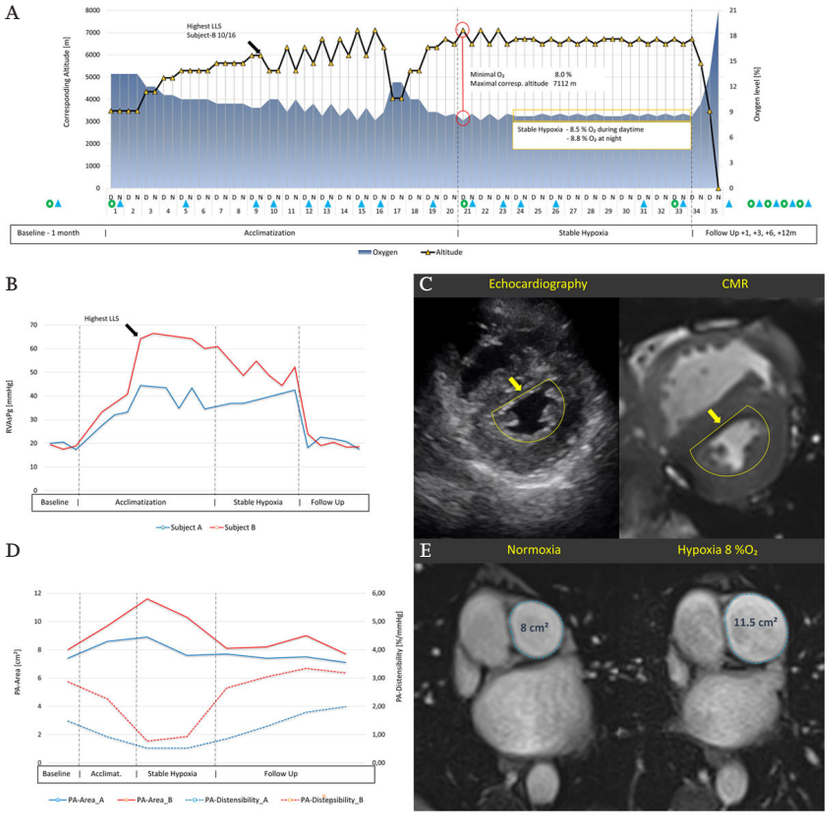
The limits of hypoxia tolerance at which the body's compensatory mechanisms are overwhelmed and critical physiological dysfunction and potentially irreversible damage ensues is referred to as defence zone. The defence zone lies around 35 mmHg of arterial oxygen partial pressure.[52] Furthermore, each organ exhibits a distinctive normoxic tissue oxygen partial pressure threshold, below which physiological functions become compromised: 72 mmHg for kidneys, 58 mmHg for intestinal tissue, 41 mmHg for liver, 34 mmHg for brain and 29 mmHg for skeletal muscle.[11,53] Interindividual variability in hypoxia tolerance results from genetic background, age, health status, physical fitness and acclimatization.[47]

Understanding the limits of hypoxia tolerance is crucial for assessing risks associated with high-altitude activities, occupational settings and medical conditions involving hypoxia. Careful monitoring and assessment are necessary to ensure safety and mitigate potential health risks. However, the Operation Everest studies I–III, all performed at 8848 m in hypobaric chambers, have demonstrated safety and feasibility of exposing highly selected, healthy, young individuals, under well controlled conditions to extreme hypoxia over several weeks.[54,55] In these studies, subjects experienced increased ventilation–perfusion mismatch and higher pulmonary artery pressure with altitude and exercise. Despite substantial weight loss in a 40-day ascent and difficulties in achieving maximal altitude acclimatization, the subjects reached the summit in improved physiological conditions, thanks to controlled acclimatization and environmental factors. In our recent series of pilot trials, we have demonstrated the safety and feasibility of subjecting not only healthy (Figure 2), middle-aged individuals but also those with prior myocardial infarction to normobaric hypoxia approaching the human hypoxic limit (Figure 2).[56–59] Both kinds of study series pave the way for pharmacological studies using human hypoxia models.

HYPOBARIC AND NORMOBARIC HYPOXIA

The two approaches to elicit ambient hypoxia are hypobaric hypoxia and normobaric hypoxia. These techniques have different characteristics that are important for practical use. In hypobaric hypoxia, as described by the Dalton's law of partial pressures, oxygen concentration of air remains constant at approximately 21% but hypoxia results from reduced ambient air pressure. A large meta-analysis of high-altitude studies showed a linear decrease in arterial oxygen partial pressure by 1.6 kPa for every 1000 m of altitude ascended to 6000 m.[60]

FIGURE 2 Experimental setup at the German Aerospace Center's :envihab facility, illustrating a 35-day exposure of two healthy professional mountaineers to severe sustained hypoxia. The study assessed pulmonary artery hypertension, echocardiographic images and cardiac magnetic resonance imaging (CMR): providing valuable insights into the impact of prolonged hypoxia on cardiovascular parameters (obtained from Hoffmann *et al.* 2020[56] and conducted at the German Aerospace Center).



In normobaric hypoxia, oxygen concentration is lowered by adding an inert gas, typically nitrogen, while ambient air pressure remains unchanged. Differences in the physiological response to hypobaric and normobaric hypoxia exist but have not yet been fully characterized.[61,62] Effects of reduced pressure on the middle ear and other closed air-containing organs are evident. Important disadvantages of hypobaric hypoxia compared with normobaric hypoxia are that sophisticated and costly hypobaric chambers are required and that study participants and staff cannot easily move in and out of the hypoxia environment. Decompression to severe hypoxia may cause decompression illness. Despite these challenges, hypobaric chambers offer distinct advantages over alternative methods for simulating hypoxia. These chambers can be used to precisely control the level of hypoxia, which is important for studies that require consistent and repeatable conditions. Through air pumps, pressure regulators and control systems, chamber pressure can be adjusted rapidly. Furthermore, hypobaric chambers are often used for research or training programmes that require large groups to be exposed to hypoxic conditions simultaneously. By contrast, normobaric hypoxia is easier to implement, but requires more time to adjust the oxygen concentrations. Nitrogen can be supplied onsite through concentrators, which operate with molecular sieves, or from a nitrogen tank. Room-in-room solutions for normobaric hypoxia are commercially available. For short term applications, hypoxic gas mixtures can also be supplied through a face mask, which excludes experimenters from hypoxia and is inexpensive.

RESPONSE TO ACUTE HYPOXIA: A CHALLENGE TO MIMIC DISEASE

Because hypoxia affects human physiology at multiple levels ranging from reflex mechanisms, such as the peripheral chemoreflex, to specific cellular pathways regulated through oxygen, experimental hypoxia could have utility in various clinical research settings. Tonic chemoreceptor hyperactivity with subsequent sympathetic nervous system activation has been implicated in the pathogenesis of arterial hypertension.[63] Thus, peripheral chemoreceptor modulation could have therapeutic utility in this condition, particularly in patients not responding sufficiently to established therapies.[64] Our recent studies demonstrated that acute hypoxia during high-resolution functional magnetic resonance imaging can be used to trace peripheral chemoreceptor responses in human beings.[65] Because changes in CO_2 confound the response to hypoxic peripheral chemoreceptor stimulation, isocapnic hypoxia protocols have been proven useful in clinical research.[66]

Acute hypoxia can also be used to test the tolerance in patients or those in occupational settings such as in fighter pilots. Moreover, hypoxia may produce a phenotype resembling a clinical condition, which could then be utilized to probe new therapies. However, hypoxia may also regulate a disease-relevant signalling pathway. Indeed, hypoxia plays a major role in a multitude of human diseases, either as a result of the disease, such

as in the case of pulmonary dysfunction, or by modifying the disease process as seen in some forms of cancer, where local hypoxia may affect differentiation of the tumour to more aggressive phenotypes.[67] These numerous implications create ample opportunity to utilize hypoxia in clinical testing.

PULMONARY ARTERIAL HYPERTENSION

Pathophysiological background of the disease

Pulmonary arterial hypertension is a serious, progressive vasculopathy of the lungs of different aetiologies. Increased arterial pulmonary pressure results from increased vascular resistance of pulmonary resistance vessels.

There are five main classifications of both acute and chronic mechanisms that can provoke pulmonary arterial hypertension. One group contains idiopathic and hereditary forms of primary vascular pathologies with normal lung function and basically no cardiopulmonary comorbidities. These patients show only mild to no hypoxia.[68] Pulmonary arterial hypertension can also result from left heart failure, chronic thromboembolic disease or mixed or unknown origin. Because the latter diseases do not originate from the pulmonary system, they may be less suitable to be modelled by human hypoxia. This model is better suited for the types of pulmonary arterial hypertension caused primarily by impaired lung functions or hypoxia, such as COPD, interstitial lung disease, sleep-disordered breathing and chronic high-altitude exposure.

Disease mechanisms modelled with experimental hypoxia

Systemic hypoxia at high altitude or in hypoxia chambers leads to hypoxic pulmonary vasoconstriction and increases pulmonary arterial pressure. Pulmonary arterial hypertension up to 66 mmHg systolic arterial pressure has been safely induced over weeks in healthy individuals by normobaric hypoxia.[56] Pulmonary arterial hypertension at altitude is not a disease per se, but it can progress to life-threatening high-altitude pulmonary oedema. Studies in mountaineers have revealed that the vasoactive factors that are involved in the development of the hypoxic pulmonary vasoconstriction at altitude are also responsible for the pulmonary arterial hypertension in patients.[56] Chronic sojourn at high altitude may eventually result in pulmonary arterial hypertension and, remodelling of the pulmonary vasculature and right heart failure. These similarities are what makes induced hypoxia an excellent tool to model this disease in a controlled manner.

Pulmonary arterial hypertension is caused by disbalance between vasodilatory and vasoconstrictive factors. Patients with pulmonary hypertension exhibit reduced levels of the key vasodilators NO, its second messenger cGMP and prostacyclin while vasoconstrictors endothelin-1 and thromboxane-A2 are increased. Additionally, reactive oxygen species production increased through induction of oxidase systems.[69]

Open questions which can be addressed with human hypoxia models

Human hypoxia models could be particularly suitable when testing pharmacological interventions for pulmonary arterial hypertension associated with arterial hypoxia such as in high altitude or pulmonary disease. In this setting, hypoxia exposure recapitulates a fundamental pathogenic mechanism increasing pulmonary vascular resistance. Considering that home oxygen therapy is often effective in reducing dyspnoea and improving physical capacity in these conditions,[70] clinical trials under controlled ambient hypoxia can serve as a meaningful research tool. Episodes of desaturation during sleep is another trait of pulmonary arterial hypertension, which could be easily modelled by periodic reduction of ambient oxygen. However, hypoxia-exposure in healthy probands could also add useful information in early-stage clinical trial of drugs developed for pulmonary hypertension treatment not primarily caused by hypoxia.

The 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension call on to perform more studies on the responses of patients with pulmonary arterial hypertension at altitude.[71] There are 120 million people worldwide who live above 2500 m. Because research at geographic altitude can be a logistical challenge, these conditions could be more safely simulated under highly controlled laboratory conditions.

Limitations of pulmonary arterial hypertension hypoxia models

While arterial hypoxia and increased pulmonary vascular resistance can be effectively modelled, other disease characteristics are less reproducible. The duration of the experimental hypoxic exposition will not be long enough to induce relevant pulmonary remodelling in healthy participants. Trials should therefore aim to investigate acute responses in the vasculature such as oxygen metabolism and the hypoxia-induced inflammation and fibrosis. Furthermore, pulmonary arterial hypertension-related comorbidities should be carefully considered. Patients with chronic pulmonary arterial hypertension may develop right heart failure, which can affect the whole cardio-circulatory system. Healthy

participants will maintain right heart function with higher cardiac outputs than pulmonary arterial hypertension patients.

COPD

Pathophysiological background of the disease

COPD encompasses inflammatory diseases that cause structural abnormalities to the airways and or pulmonary parenchyma,[72] usually caused by smoking or inhaled particulates.[73] Pronounced ventilation–perfusion inequalities within poorly ventilated, yet well-perfused alveoli, result in hypoxaemia and, in certain patients, hypercapnia.[74] As the disease progresses, inflammation-induced hyperplasia of respiratory glands significantly increases the production of viscoid mucus, leading to the obstruction of both smaller and larger airways. Consequently, this obstruction culminates in hypoxia and respiratory epithelial cell failure. Cough and dyspnoea represent the primary symptoms of this condition. Hypoxic respiratory epithelial cells in COPD lungs exhibit an increased sodium absorption, attributed to the upregulated expression of epithelial Na^+ channels, consequently leading to mucus thickening.[75] Paradoxically, the opposite effect has been observed in healthy individuals who developed high altitude pulmonary oedema. In these individuals, hypoxia reduced transepithelial sodium transport mediated by epithelial Na^+ channels, resulting in the accumulation of fluid in the alveoli.[76]

COPD is associated with systemic inflammation and numerous comorbidities, most notably cardiovascular disease.[77,78] Hypoxia plays a vital role in this process because the increase in the HIF cascade stimulates angiogenesis within atherosclerotic plaques.[77] There is a vicious cycle between the COPD-induced inflammation and hypoxia where inflammation increases metabolic demand and hypoxia increases the levels of reactive oxidative species, an inflammatory agent. In mice, the combination of a high/fat diet and chronic intermittent hypoxia has been shown to have a significant negative impact on atherosclerosis.[7] However, the direct effect of hypoxia in connecting COPD and atherosclerosis in the absence of associated inflammation requires further investigation, which could be addressed through laboratory-induced hypoxia studies.

Disease mechanisms modelled with experimental hypoxia

By isolating hypoxia effects in the absence of inflammation, laboratory-induced hypoxia studies can provide insights into the direct relationship between hypoxia and atherosclerosis. Additionally, human hypoxia models can be utilized to study respiratory failure in COPD.[79] Type I respiratory failure, characterized by a ventilation–perfusion mismatch with normal or low arterial partial pressure of carbon dioxide levels and a reduced arterial partial pressure of oxygen (PaO_2), can be modelled using hypoxia in combination with hypocapnia or isocapnia. Type II respiratory failure, characterized by elevated arterial partial pressure of carbon dioxide levels and reduced PaO_2 levels, can be modelled using hypoxia and hypercapnia. Human hypoxia models facilitate refining ventilation techniques, evaluating the effectiveness of pharmaceutical interventions and tailoring personalized therapeutic approaches to the distinct stages and characteristics of respiratory failure.

Open questions which can be addressed with human hypoxia models

Human hypoxia models could be applied to selectively assess hypoxia influences on atherosclerosis progression. Effects of different hypoxia levels and durations on COPD progression, exacerbations and the underlying mechanisms can also be explored.[80] Furthermore, treatment optimization for respiratory failure in COPD during both type I and type II phases can be studied using human hypoxia models.

Limitations of the COPD hypoxia model

The model does not fully replicate the complex pathophysiology of COPD: as the disease involves multiple factors beyond hypoxia, such as chronic inflammation, respiratory endothelial failure and airway remodelling. Additionally, individual variations and comorbidities associated with COPD, including cardiovascular diseases, may influence the response to hypoxia and limit generalizability. While the ability of the model to isolate a single factor within this disease can be used as an advantage, as previously described, cautious interpretation and consideration of these limitations are still necessary when using the COPD hypoxia model.

CENTRAL SLEEP APNOEA AND OSA

Pathophysiological background of the diseases

In central sleep apnoea (CSA), a dysfunctional respiratory drive results in apnea events during sleep. These events appear periodically together with phases of hyperventilation. CSA is common in patients with heart failure.[81] OSA is a sleep disorder characterized by recurrent episodes of

cessation of airflow, with or without partial or complete upper airway obstruction during sleep, leading to disruptions in normal breathing patterns. The obstruction results in intermittent hypoxia and hypercapnia, as well as sleep fragmentation. OSA is primarily caused by anatomical and physiological factors that contribute to airway collapse, such as obesity, anatomical abnormalities and decreased upper airway muscle tone. Newer data also suggest a pathomechanism in OSA that is dependent on respiratory drive.[82] The repetitive episodes of hypoxia and hypercapnia trigger physiological responses, including sympathetic activation, systemic inflammation, oxidative stress and endothelial dysfunction.[83] These responses contribute to the development of neurocognitive, cardiovascular and metabolic comorbidities commonly associated with OSA, such as daytime sleepiness, hypertension, coronary artery disease and insulin resistance.[84]

Disease mechanisms modelled with experimental hypoxia

Above 2000 m, sleep in hypobaric and normobaric hypoxia produces a characteristic periodic breathing pattern, similar to CSA, which is called Cheyne–Stokes breathing. The apnoea hypopnoea index is directly proportionally associated with increasing sleeping altitude whereas mean oxygen saturation during sleep is inversely associated.[85]

Intermittent hypoxia, which mirrors repetitive hypoxia and reoxygenation cycles experienced by individuals with OSA during sleep can be used to model OSA. This model allows researchers to study effects of intermittent hypoxia on various physiological processes. Oxidative stress, another important mechanism in OSA, can be replicated through hypoxia-induced imbalance between reactive oxygen species production and neutralization.[86] Furthermore, hypoxia-induced inflammation and endothelial dysfunction, key contributors to OSA-related complications, can be investigated by simulating the inflammatory responses and impaired vascular function associated with hypoxia exposure.[87,88] Importantly, hypoxia models should replicate the intermittent nature of hypoxia during sleep and consider specific OSA characteristics, including upper airway obstruction and sleep architecture. Controlling duration and severity of hypoxia exposure is crucial to mimic the varying degrees of intermittent hypoxia observed in OSA patients. By employing hypoxia as a modelling tool, researchers can gain insights into the underlying disease mechanisms of OSA and its associated complications, paving the way for the development of targeted therapeutic strategies.

Open questions which can be addressed with human hypoxia models

Animal and human models used to study OSA through intermittent hypoxia induction do not fully replicate all disease aspects.[89] Therefore, healthy human OSA models are continually improved through technological adjustments. To maximize construct validity, experiments should be conducted overnight on sleeping participants rather than during the waking hours.[90] Secondly, intermittent hypoxia models should include hypercapnia, which is present during obstruction in OSA. Thirdly, the therapeutic potential of these models depends on factors such as dose, duration and frequency. Acute mild hypoxia (9–16% inspired O_2) exposure with a lower number of cycles (3–15 episodes/day) leads to positive effects without inducing pathology. Conversely, chronic severe hypoxia (2–8% inspired O_2) combined with a higher number of cycles (48–2400 episodes/day) leads to increasing progression of pathological conditions.[91] Fourthly, intermittent hypoxia should mimic characteristic slow oxygen desaturation and rapid re-saturation during obstructive events, as opposed to the current model with a square-wave design of rapid desaturation and re-saturation. Lastly, full polysomnography is necessary to characterize sleep architecture, including frequent brain arousals. Dial-down CPAP during sleep can induce upper airway obstruction and negative intrathoracic pressure swings, thus creating an experimental model that closely simulates OSA.[92] However, this technique is labour-intensive, invasive and not without risks. The experimental model for OSA should be tailored to the research question at hand and may or may not require the complete simulation of OSA.

Limitations of the OSA hypoxia model

There are obstacles in the model, as it mainly emphasizes the lack of oxygen in OSA and may not fully understand the complexities of other factors like airway collapse and disrupted sleep patterns. Additionally, duration and frequency of hypoxic exposure in experimental settings may not perfectly replicate the intermittent hypoxia experienced during sleep apnea episodes. Individual variations and comorbidities in OSA patients, as well as the influence of sleep architecture, may affect the response to hypoxia and limit generalizability. These limitations should be considered when using OSA hypoxia models.

MIGRAINE

Pathophysiological background of the disease

Recurrent migraine is a common, debilitating and highly elusive disorder that is difficult to treat. The condition is characterized by recurrent, enduring, unilateral and pulsating headaches often accompanied by nausea, light and sound sensitivity, and sometimes preceded by a period of altered sensory experience (often visual hallucination) called auras. The origin of migraine is argued to be vascular and/or neurogenic but this is still under investigation,[93] however, the aura symptoms are known to result from a wave of neuron depolarization and subsequent depression propagating across the cortex, whereas the pain results from the activation of the trigeminovascular system and meningeal blood vessels, both through unconfirmed mechanisms.[94]

Disease mechanisms modelled with experimental hypoxia

Symptoms of acute mountain sickness, a disease of the brain which is developed by individuals who ascent to high altitudes too fast, are often migraine-like and include headache, nausea and vomiting.[95] An association between migraine and hypoxia has been suggested for patients with patent foramen ovale. In these patients, who also suffer from migraine, PaO_2 was lower than in healthy controls and normobaric oxygen treatment attenuated the frequency and severity of their migraines.[96] Furthermore, hypoxia can trigger migraine.[97–99] Indeed, 6 h normobaric hypoxia at 12.6% oxygen triggered migraines in 80% of participants with > 16% presenting with aura.[97] Hypoxia was a more reliable migraine trigger than nitroglycerine, which is the current experimental standard.[99] Hypoxia offers safety advantages over the pharmacological models as it can be easily reversed, whereas nitroglycerine cannot be withdrawn such that rescue medications like triptans may be required.[100] However, only few studies with relatively small populations applied the hypoxia model. Possibly, migraine research could benefit from hypoxia, both as a dependable and physiologically accurate trigger for mechanistic and interventional studies.

Hypoxia models have been utilized to study various disease mechanisms associated with migraine. One such mechanism is cortical spreading depression (CSD), a wave of neuronal depolarization and subsequent depression that spreads across the cerebral cortex.[100] CSD has been implicated in the generation of migraine aura and is hypothesized to contribute to the initiation and propagation of migraine attacks. Hypoxia-induced CSD models have provided insights into the underlying mechanisms and potential therapeutic targets for migraine.[102] Furthermore, experimental hypoxia can be used to investigate the role of oxygen levels in modulating neurovascular function and neurotransmitter release, such as serotonin and calcitonin gene-related peptide, which are involved in migraine pathophysiology.[103] Hypoxia models can provide information regarding the interplay between hypoxia and migraine mechanisms including cerebral blood flow, vascular reactivity and neuroinflammatory processes.

Open questions which can be addressed with human hypoxia models

Human hypoxia models offer a unique opportunity to address several open questions in migraine research. For instance, the impact of hypoxia on the trigeminovascular system and its contribution to migraine attacks can be studied in controlled settings. Understanding how hypoxia affects the release of vasoactive substances, neuronal excitability and the propagation of CSD can provide insights into the triggers and mechanisms of migraine. Additionally, human hypoxia models can shed light on the interplay between hypoxia and other migraine triggers, such as stress, exercise or sleep disturbances.[104] Investigating how hypoxia interacts with these triggers and influences migraine susceptibility can help uncover the complex interactions between multiple factors involved in migraine pathogenesis. Importantly, improving our ability to reliably trigger migraines safely would facilitate the clinical testing of any future migraine medications. Furthermore, studying the effects of hypoxia on sensory processing and perception may provide insights into the mechanisms underlying migraine-associated sensory hypersensitivity.[105]

Limitations of the migraine hypoxia model

Migraine is a heterogeneous disorder with various triggers and individual variations, which may not be fully captured in experimental settings. Furthermore, translating findings from hypoxia models to clinical practice may be challenging. Severity, duration and frequency of hypoxia-induced migraine-like symptoms may differ from those experienced during spontaneous migraine attacks. Moreover, hypoxia models may not capture contributions of genetic factors, cortical excitability or neuroinflammatory processes on migraine. Genetic studies and advanced neuroimaging techniques could conceivably improve the model.

KIDNEY FUNCTION

Pathophysiological background of the disease

Renal oxygen sensors can translate a measure of plasma volume into a signal for tissue oxygen pressure, through the effects of sodium reabsorption on renal energy use and oxygen consumption. These processes are required for the regulation of EPO production.[106] However, in severe hypoxia below pO_2 40 mmHg, glomerular filtration rate declines, leading to sodium retention and water retention.[107,108] Hence, renal function is often impaired in conditions associated with hypoxia such as OSA or COPD and predisposes to fluid and sodium retention.[109] A similar phenomenon has been described at high altitude, especially in altitude maladapted individuals.[110] Hypoxia effects on patients with impaired renal function can be studied in hypoxia models with implications risks of long air travel or dwelling at high altitude.

Disease mechanisms modelled with experimental hypoxia

Experimental hypoxia models have been used to study the mechanisms underlying kidney dysfunction. Hypoxia, or reduced oxygen availability, can occur in various renal diseases due to impaired blood flow, ischaemia or inadequate oxygenation.[111] Hypoxia can trigger cellular responses, including the activation of HIFs, which play a crucial role in adaptive mechanisms to maintain cellular homeostasis under low-oxygen conditions.[112] Experimental hypoxia models can simulate and study the impact of reduced oxygen levels on kidney cells and tissues, providing insights into the molecular and cellular responses involved in renal hypoxia-related diseases.

Furthermore, experimental hypoxia models allow researchers to investigate the effects of hypoxia on renal blood flow, glomerular filtration rate, tubular function and electrolyte handling.[113] These models can simulate renal ischaemia–reperfusion injury, a common cause of acute kidney injury, and elucidate the mechanisms underlying renal tissue damage, inflammation and impaired renal function in hypoxic conditions.[114,115]

Open questions which can be addressed with human hypoxia models

Human hypoxia models may help in elucidating cellular and molecular responses of the kidneys to hypoxic stress in real-time, providing insights into the adaptive mechanisms and potential therapeutic targets. Furthermore, human hypoxia models can aid in studying the interplay between hypoxia and other factors contributing to kidney disease progression, such as oxidative stress, inflammation and metabolic disturbances. Additionally, human hypoxia models can help explore the potential benefits of oxygen-based therapies and interventions in kidney diseases.[116]

Limitations of the kidney function hypoxia model

The kidney function hypoxia model may oversimplify complex pathophysiological processes involved in renal hypoxia. The kidney comprises multiple cell types, intricate blood supply and complex regulatory mechanisms. Second, experimental hypoxia may not fully replicate the conditions seen in human kidney diseases. Experimental models often involve controlled and acute hypoxia, whereas human kidney diseases are characterized by chronic and multifactorial hypoxia. Therefore, extrapolating findings from experimental models to human conditions should be done cautiously. Third, the model primarily focuses on the role of hypoxia in kidney dysfunction, overlooking other contributing factors such as inflammation, oxidative stress, immune responses and genetic factors. Neglecting these factors in the model may limit its ability to provide a comprehensive understanding of kidney dysfunction. Lastly, the model's generalizability may be limited. Human kidney diseases exhibit considerable heterogeneity, and the response to hypoxia can vary among individuals and diseases. The model may not fully capture this heterogeneity and may not be applicable to all kidney disease scenarios.

CONCLUSIONS

Human hypoxia models that originated from aerospace medicine offer a tool for studying various physiological processes and diseases. These models have gained popularity, particularly for replicating hypoxic conditions in healthy humans and studying the effects of hypoxia on the body in a controlled manner. Similarly, hypoxia models could aid in the study of cardiovascular and neurological diseases, which are also characterized by a decrease in oxygen supply to tissues. The models have not yet been used widely (Table 1) but we recommend their application as facilities for inducing hypoxia are available in aerospace medicine departments and often open for collaborative projects. Furthermore, by understanding how the body responds to hypoxia in extreme environments such as high altitudes and space travel, researchers could develop

new ways to improve human health in the future. There is a need for more standardization and validation of the different published models.[117] Overall, healthy human hypoxia models offer significant potential for advancing our understanding of various diseases and physiological processes. However, other human models developed for space medicine may also have applications for human drug development. A good example are our head-down tilt bedrest studies conducted in collaboration with DLR, ESA and NASA, which produces musculoskeletal and cardiovascular deconditioning as well as cephalad fluid shifts resembling those produced by real weightlessness.[118]

TABLE 1 Human hypoxia models used as platform for pharmacological interventions.

Reference	Drug	Class	Biomarker	Design	Hypoxia inducer	Outcome
PULMONARY HYPERTENSION						
Hall *et al.* (2021)[5]	GSK258688	Recombinant angiotensin-converting enzyme-2	PASP	n = 10 HV, 4000 m ± 10% (NH), 70 min, rest and exercise (phase 1 study)	Normobaric chamber	Dose was well tolerated but did not impact the acute HPV response in healthy volunteers.
Watt *et al.* (2000)[119]	Amlodipine	Calcium channel blocker	Pulmonary haemodynamics	n = 14 mountaineers, 12.5% O_2 (HH), 10 min, rest and exercise	NA	Increased heart rate, influenced HPV dose-dependently, increased breathlessness perception during exercise.
Pham *et al.* (2010)[120]	Bosentan	Endothelin (ET) A and ETB receptor blocker	PASP	n = 10 HV, 12% O_2 (NH), 90 min, rest and exercise	Normobaric chamber	Reduced hypoxia-induced PASP elevation during rest, but not during exercise.
Faoro *et al.* (2009)[121]	Bosentan	ETA and ETB receptor blocker	PVR	n = 11 HV, 12% O_2 (NH), 90 min, rest and exercise	Facial masks	Reduced hypoxia-induced PASP elevation during exercise during rest.
Ghofrani *et al.* (2004)[122]	Sildenafil	5-phosphodiesterase (5-PDE) inhibitor	PASP	n = 14 mountaineers, 5400 m/10% O_2 (HH), 14 days, rest and exercise	Mount Everest/ facial mask	Reduced pulmonary hypertension both in rest and during exercise.
Ricart *et al.* (2005)[123]	Sildenafil	5-PDE inhibitor	PASP	n = 14 HV, 5000m (HH), 90 min, rest and exercise	Hypobaric chamber	Reduced the hypoxia-induced increase in PASP, and after exercise.
Damy *et al.* (2015)[124]	Sildenafil	5-PDE inhibitor	RVTG	n = 18 patients with CHF, 15% (NH), 2 h, rest and exercise	Facial masks	Reduces RVTG at rest and prevented hypoxia-induced increases, but not by exercise.
CHRONIC OBSTRUCTIVE PULMONARY DISEASE						
Burghuber (1987)[125]	Nifedipine	Calcium channel blocker	PAP, PVRI	n = 11 COPD patients with normal PAP, 13% O_2, 5% CO_2 and 72% N_2 (HH + HC), 10 min, rest	Facial masks	Acutely dilates the constricted vascular bed associated with hypoxia in these patients with COPD.

Reference	Drug	Class	Biomarker	Design	Hypoxia inducer	Outcome
DISORDERED SLEEP						
Straus *et al.* (2021)[126]	Baclofen	GABA$_B$ agonist	Coefficient of variation of respiratory cycle total time	n = 14 HV, 12-14% O_2+ 6.2% CO_2, (NH + HC), NA, sleep	Tent	Baclofen destabilises breathing during sleep.
Beaudin *et al.* (2014)[90]	Celebrex	Selective COX-2 inhibitor	BP, CBF, and urinary prostanoids	n = 12 HV, IH, 6 h (1 min cycles), rest	Mask and normobaric chamber	COX-2 and COX-1 play distinct roles in regulating vascular responses to both acute and chronic intermittent hypoxia (IH). In addition, inhibiting COX-1 may alleviate cardiovascular and cerebrovascular issues in OSA.
	Indomethacin	Nonselective COX inhibitor				
Foster *et al.* (2010)[127]	Losartan	Type I angiotensin II receptor	BP, CBF, and ventilation	n = 10 HV, IH, 6 h (1 min cycles), rest	NA	IH raises blood pressure through type I angiotensin II receptor activation, without affecting the cerebrovascular or ventilatory response to acute hypoxia.
HEART FAILURE						
Pavelescu & Naeije (2012)[127,128]	Epoprostenol	Prostacyclin analog	PAP	n = 10 HV, 12% (NH), 1 h, rest	Tightly collar fitted helmet	At maximum tolerated doses in healthy volunteers, neither substance influenced cardiac function or demonstrated intrinsic positive inotropic effects.
	Sildenafil	5-phosphodiesterase (5-PDE) inhibitor				
MIGRAINE						
Didier *et al.* (2022)[129]	Ketorolac	nonselective COX inhibitor	ASL-MRI	n = 6 HV, 9-13% O_2 (Isocapnic H), NA, rest	NA	CBF did not change globally or regionally with hypoxia stimulation and Ketorolac did not change CBF during hypoxia in any region.

Abbreviations: ASL-MRI, arterial spin labelling–magnetic resonance imaging; BP, blood pressure; CBF, cerebral blood flow; CHF, chronic heart failure; COX, cyclooxygenase; HC, hypercapnia; HH, hypobaric hypoxia; HPV, hypoxic pulmonary vasoconstriction; HV, healthy volunteers; IH, intermittent hypoxia; NH, normobaric hypoxia; PAP, pulmonary artery pressure; PASP, pulmonary artery systolic pressure; PVR, pulmonary vascular resistance; PVRI, pulmonary vascular resistance index; RVTG, transtricuspid systolic pressure gradient

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CHAPTER III | JUDICIOUS ELEVATION OF AMBIENT CARBON DIOXIDE DURING HYPOBARIC HYPOXIA TO IMPROVE OXYGENATION IN AIRLINE PASSENGERS – A RANDOMIZED FEASIBILITY STUDY

Submitted

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ABSTRACT

Despite pressurization of airliner cabins, some passengers experience in-flight hypobaric hypoxia with blood oxygen saturation dropping below 90%, potentially causing discomfort and increasing the risk of medical events. Enrichment of the cabin air with CO_2 may augment passengers' blood and tissue oxygenation by stimulating respiratory drive, thereby increasing health and safety during air travel. In a randomized double-blind crossover study we exposed 17 healthy adults (8 women; mean age ± SD: 27.8 ± 4.2 years) on separate days to two ambient CO_2 levels (partial pressure: 1 vs.10 hPa) during 6 hours of hypobaric hypoxia (~8,000 ft or 2,438 m; 753 hPa) in an altitude chamber. We measured blood oxygen saturation (SpO_2), brain and muscle tissue oxygen saturation, respiration and cognitive function (sustained attention, working memory, hand-eye coordination) at hourly intervals. In addition, we conducted capillary blood gas analyses at baseline, 15 minutes and 6 hours after hypoxia onset. High ambient CO_2 increased levels of pCO_2, pO_2, and SpO_2, while reducing the time fraction of $SpO_2 < 90\%$ from 18.8 ± 5.3% to 2.5 ± 0.9% ($p = .005$). Tissue oxygenation increased in the brain but not in muscle. Minute ventilation and respiratory rate increased. Increase of CO_2 had no effect on cognitive performance. Elevating ambient CO_2 can improve blood oxygenation in healthy individuals during moderate hypoxia as experienced in an airliner cabin. These findings have implications for the health and safety of passengers as well as for the regulation and design of aircraft environmental control systems.

INTRODUCTION

Commercial jet aircraft often fly at altitudes around 30,000 - 43,000 ft. At such altitudes, oxygen partial pressure outside the airliner cabin is profoundly reduced and incompatible with human life. Therefore, aircraft cabins are pressurized to a level that varies by flight with the maximum allowed cabin altitude during normal operations set at 8,000 ft (~2,438 m).[1,2] Cabin pressurization ensures adequate oxygenation for most passengers. However, in some individuals, oxygen saturation (SpO_2) decreases below 90%,[3–6] increasing the risk of hypoxemia-related adverse events.[3,7,8] One potential solution is to increase cabin pressure. However, this measure would increase aircraft weight and fuel consumption due to the need of reinforced cabin structure, subsequently worsening its environmental impact.[9] Given the continuously increasing air travel volume, alternative strategies to improve oxygenation in passengers are currently explored.

We assessed the potential of enriching the cabin atmosphere with carbon dioxide (CO_2) as a countermeasure to in-flight hypoxia. Our objective was to assess whether ambient CO_2 increases blood SpO_2 in healthy individuals during hypobaric hypoxia as typically experienced during air travel.[1] Exposure to reduced oxygen partial pressure (pO_2) elicits an increase in ventilation, the so-called "hypoxic ventilatory response". The ventilatory response increases CO_2 elimination leading to hypocapnia which in turn opposes respiratory drive. Consequently, in most people exposed to modest hypoxia, ventilation stabilizes slightly above baseline levels.[10]

Although these counteracting regulatory circuits maintain overall homeostasis, they can limit the ability to cope with hypoxia. Conversely, increased inspired CO_2 prevents hypocapnia and augments ventilation under hypoxia for prolonged periods of time.[11] Moreover, because CO_2 is a potent cerebral vasodilator, maintaining adequate levels of arterial CO_2 can help ensure proper brain perfusion and prevent light-headedness, thereby supporting overall brain function and alertness.[12]

Our primary hypothesis was that during a realistic simulation of a 6-hour flight a sea-level equivalent of 1% CO_2 inside the airliner cabin increases our primary outcome, i.e., blood SpO_2, in healthy individuals compared to a flight condition without CO_2 enrichment (control). To gain insight into the underlying mechanisms, we measured secondary endpoints including capillary blood gases and respiration. Additionally, we

estimated brain and skeletal muscle oxygen saturation using near-infrared spectroscopy (NIRS). Finally, we evaluated cognitive performance as reflected in sustained attention, working memory, and hand-eye coordination.

STUDY DESIGN AND METHODS

We obtained informed written consent from study participants prior to the start of the study. The protocol was approved by the ethics committee of the North Rhine Medical Association (Ärztekammer Nordrhein; protocol number: 2018253), and prospectively registered at the German Clinical Trials Register (DRKS, registration number: DRKS00015820). We conducted all procedures according to the Declaration of Helsinki (2013). Study participants were compensated for their participation. We performed the study as well as the medical screening at the Institute of Aerospace Medicine, German Aerospace Center (DLR) in Cologne, Germany.

Participants

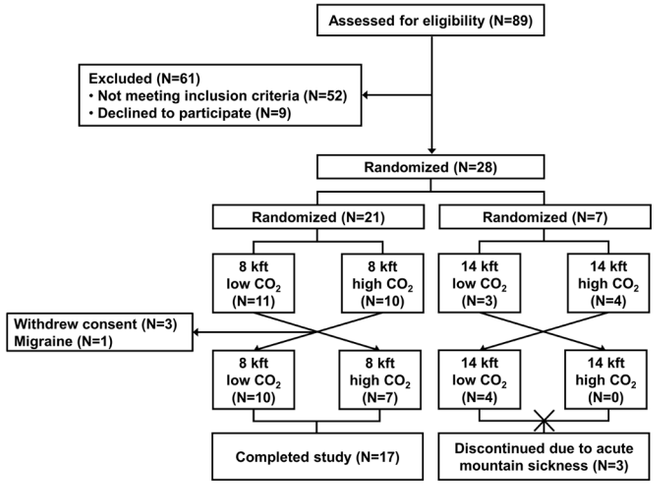
Participants were enrolled according to a predefined set of inclusion and exclusion criteria. In brief, participants were in good health as established by a physical examination, medical history, stress-electrocardiogram, routine blood, and urine testing. Men and women aged 18–40 years with a body mass index between 18 and 30 kg/m² were eligible. Participants reported no recent exposure to altitudes above 1,500 m within six weeks prior to the study, no regular practice of high-performance sports, and did not play wind instruments. Participants abstained from alcohol, nicotine, drugs, and caffeine for one week prior to each visit to the laboratory, which was verified by urine testing upon admission.

Study Design

Participants were randomized to one of two study arms – one simulating a cabin pressure equivalent to the air pressure at an altitude of ~8,000 ft and the second arm simulating a pressure equivalent to ~14,000 ft (Figure 1). In each study arm, participants underwent two ambient CO_2 conditions (1 hPa or 10 hPa) on separate days with a seven-day washout period, according to a randomized, double-blind, crossover design. Due to the occurrence of adverse events, the second study arm (~14,000 ft) was discontinued after 7 participants (see Results). During both conditions of the first study arm, participants spent 6 hours in a hypobaric chamber, which was depressurized to simulate an altitude of ~8,000 ft (753 hPa) corresponding to the maximum cabin altitude of an airliner as allowed during normal operation. The two conditions differed by the ambient CO_2 concentration in the hypobaric chamber, consisting of either a low or high CO_2 condition with normalized sea level (1000 hPa) equivalents of 0.1% (1 hPa) or 1.0% (10 hPa) CO_2. We randomly assigned participants 1:1 to the two sequences in which the high or low CO_2 conditions occurred. Participants were initially grouped (groups of 2) based on their availability. Subsequently, the order of treatment conditions was randomized across these groups using an R script that simulates a coin toss. Both the physician taking the measurements and the participants inside the chamber were blinded to the treatment conditions. In contrast, the study staff operating the chamber from outside were unblinded.

We collected baseline data every morning on each study day under normobaric and normoxic conditions. The experiment in the pressure chamber was divided into six test blocks, each lasting 60 minutes. A test block comprised the Environmental Symptoms Questionnaire (ESQ) for assessing Acute Mountain Sickness - Cerebral (AMS-C) (3 min), a cognitive test battery (22 min), physiological measurements (20 min), and a break (15 min). During each break, participants received hourly isocaloric snacks and had the opportunity to use the restroom within the altitude chamber. In addition, we performed capillary blood gas analysis (cBGA) 15 minutes and 6 hours after the start of hypoxia exposure.

FIGURE 1 Flow diagram of the study.



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Oxygenation and cognitive measurements

CAPILLARY BLOOD GAS ANALYSES (CBGA) We preferably collected capillary blood from the earlobe five minutes after application of a blood flow stimulating ointment (Finalgon, Sanofi-Aventis, Frankfurt, Germany). We used the fingertip as alternative sampling site. We analyzed samples using epoc Blood Analysis System (Siemens Healthineers, Erlangen, Germany) which involves electrodes to assess concentration of gases in the blood.

SPIROMETRY AND PULSE OXIMETRY We measured ventilation and gas exchange parameters (O_2 and CO_2) through an oral-nasal mask using a breath-by-breath spirometer (Inno0400, Innovision, Glamsbjerg, Denmark). We assessed blood oxygen saturation (SpO_2) through a finger pulse oximeter (Nonin, Plymouth, Minnesota). After conducting our analysis, we discovered that the spirometer encountered problems when exposed to high ambient CO_2 levels (10 hPa) under hypobaric conditions. After consulting with the manufacturer, we chose to exclude all data that depended on CO_2 measurements of the Innovision device referred to above. In the absence of end-expiratory CO_2 measurements, we were unable to calculate alveolar ventilation. Therefore, we focused on minute ventilation as a key mediator in optimizing oxygen saturation.

NEAR-INFRARED SPECTROSCOPY (NIRS) We placed sensors (Portalite and Portamon, Artinis Medical Systems, Elst, Netherlands) over the right frontal eminence of the skull and the belly of the right vastus lateralis muscle. We securely fixed both sensors with dark elastic tape to minimize artefacts from external light. The devices measured tissue concentration of oxygenated (HbO_2, µmol/L tissue) and deoxygenated haemoglobin (Hb, µmol/L tissue) at a sampling rate of 10 Hz. From these value total haemoglobin (tHb = HbO_2 + Hb, µmol/L tissue), and tissue oxygen saturation index (TSI; 100 x HbO_2/tHb) were determined. For the analysis, we averaged the 15-min recordings that were measured at hourly intervals.

COGNITIVE PERFORMANCE The cognitive test battery employed in this study comprised three components: a 10-min version of the psychomotor vigilance task (PVT)[13,14] to measure sustained attention, the N-back task with varying difficulty levels (1, 2, and 3-back load) to evaluate working memory,[15,16] and the adaptive tracking task to assess hand-eye coordination.[17,18] The selected measures of interest included PVT speed (ms^{-1}), PVT number of lapses (response time > 500 ms), 2-back number of omissions, and the mean score obtained from the adaptive tracking task. To mitigate the potential impact of training effects, each task was performed three times prior to the study.

Statistical analysis

A statistical power analysis for the first arm was performed assuming a moderate effect of f = 0.18 for the effect of ambient CO_2 on blood oxygen saturation during hypoxia exposure (high vs. low ambient CO_2). The analysis was based on a repeated measures analysis of variance (ANOVA) with an alpha = 0.05 and power = 0.80. The projected sample size needed was approximately n = 17 for the crossover design in each arm. We analysed the data using R, version 4.0.1 (R Foundation, Vienna, Austria). Numerical data are presented as means ± standard error of the means unless indicated otherwise. Repeatedly measured data were analysed with linear mixed-effects models with treatment, time, and treatment by time as fixed effects, and participant-specific intercepts. Measurements immediately preceding the hypoxia exposure served as baseline and were used as covariates in the models. In cases where the interaction term was not significant a simplified model excluding the interaction term is reported. Post-hoc comparisons were done on estimated marginal means. Data shown in the figures represent descriptive means. Normality was assessed using residual plots. An alpha value of 0.05 was considered significant, with correction for multiplicity performed using the Bonferroni-Holm method. The first five minutes of the spirometry and near-infrared spectroscopy (NIRS) recordings in each test block were excluded from the analysis to minimize artefacts due to application of the measurement equipment. The number of PVT lapses and 2-back omissions resulted in a considerable number of zeros. To account for the excess of zeros in the count variable PVT lapses, we used the zero-inflated negative binomial (ZINB) model.

For the 2-back omissions, the ZINB model returned no overdispersion and therefore a zero-inflated Poisson (ZIP) model was applied.

Missing data

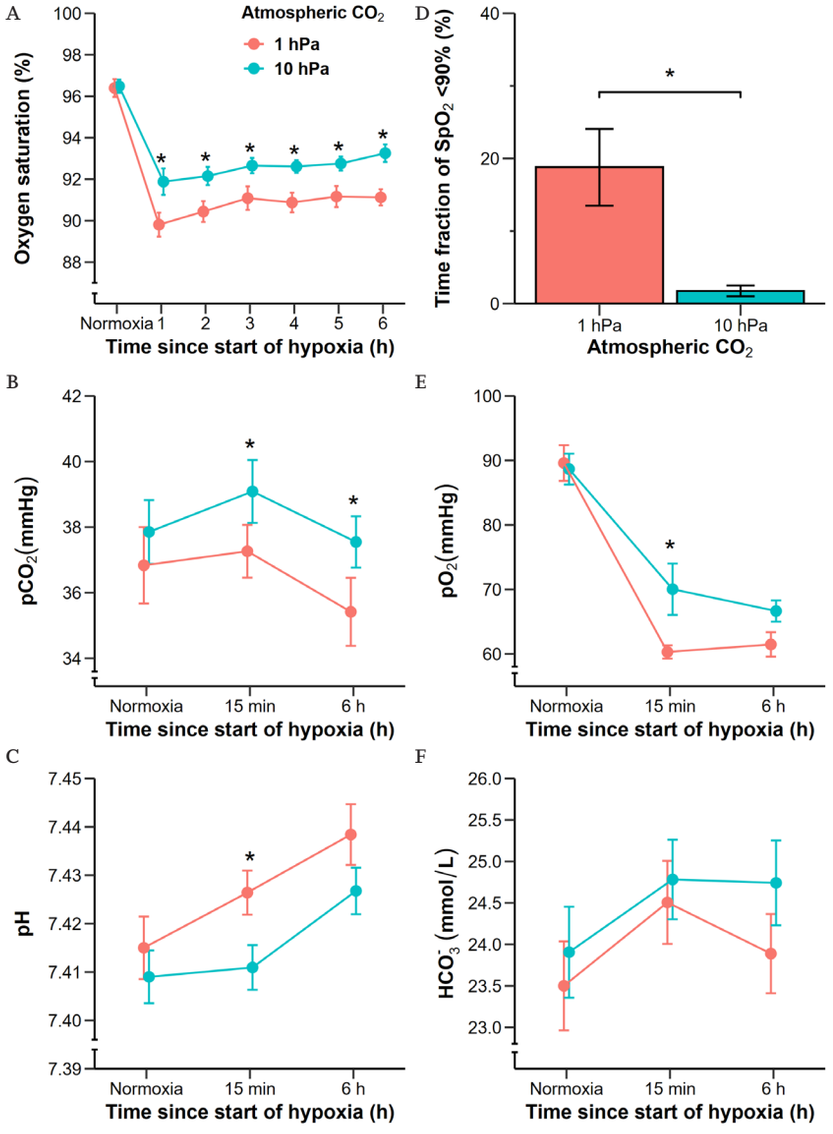
Two participants had missing SpO_2 data in a total of five test blocks and one baseline measurement due to a sensor connection failure with the spirometry device. As the average intra-individual baseline differences were negligible (0.1% SpO_2), we imputed the missing baseline value using the available baseline data from the same individual on the other experimental day. Additionally, for a third participant, all spirometry and SpO_2 data from one test block were missing due to device failure. Overall, the missing data accounted for 3% of our dataset, and their occurrence could reasonably be considered random.

RESULTS

Between December 2018 and July 2019, 89 interested volunteers entered a multi-step screening procedure, resulting in the selection of 28 healthy adults. The study was initially designed with two arms – one simulating a cabin pressure equivalent to the air pressure at an altitude of 8,000 ft and the second arm simulating a pressure equivalent to ~14,000 ft (Figure 1). However, due to signs of acute mountain sickness, we discontinued exposure to 14,000 ft after testing seven persons. Consequently, data collected from this arm was excluded from subsequent analyses. As a result, twenty-one participants were assigned to the 8,000 ft arm. Within this group, four participants did not continue the study after the first visit (low CO_2 condition in each case). Of those, three participants withdrew their consent, while one experienced migraine and was excluded. Consequently, the final dataset for the 8,000 ft arm included 17 complete datasets (8 women, mean age ± SD: 27.8 ± 4.2 years, mean BMI ± SD: 24.6 ± 3.2).

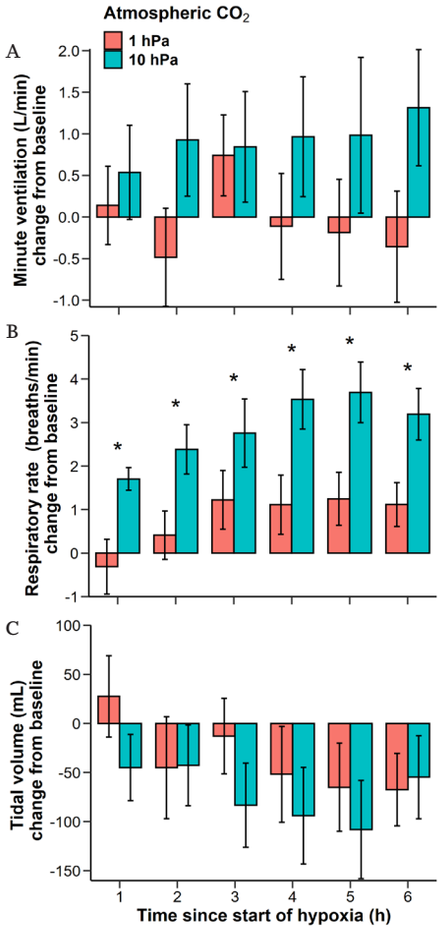
SpO_2 values were higher in high compared to low ambient CO_2 (Figure 2A, mixed model, $p < 0.001$). Time fraction of $SpO_2 < 90\%$ was lower in high compared to low ambient CO_2 (Figure 2B, paired t-test, $p = 0.005$). In hypoxia, high ambient CO_2 resulted in increased capillary blood levels of pCO_2 and pO_2 compared to low ambient CO_2 (Figure 2C-D, mixed model, factor treatment $p < 0.001$ in both cases), accompanied by a decrease in pH levels (Figure 2E, mixed model, $p = 0.006$), and a tendency toward increased bicarbonate levels (Figure 2F, mixed model, $p = 0.077$). There were no significant interactions between treatment and time for any variable.

FIGURE 2 Mean (± SEM) oxygen dynamics following 6 hours of hypobaric hypoxia exposure with low and high CO_2. (A) Mean blood oxygen saturation at hourly intervals during exposures. (B) Percentage of time fraction of $SpO_2 < 90\%$ during exposures, calculated as a fraction of the total time of oxygen saturation measurements. (C) Carbon dioxide partial pressure, (D) oxygen partial pressure, (E) pH and (F) bicarbonate levels in capillary blood at baseline, after 15 min, and 6 h of exposures. Asterisks indicate significant levels ($p < 0.05$) post Bonferroni-Holm correction. For results of mixed model analysis see Results section.



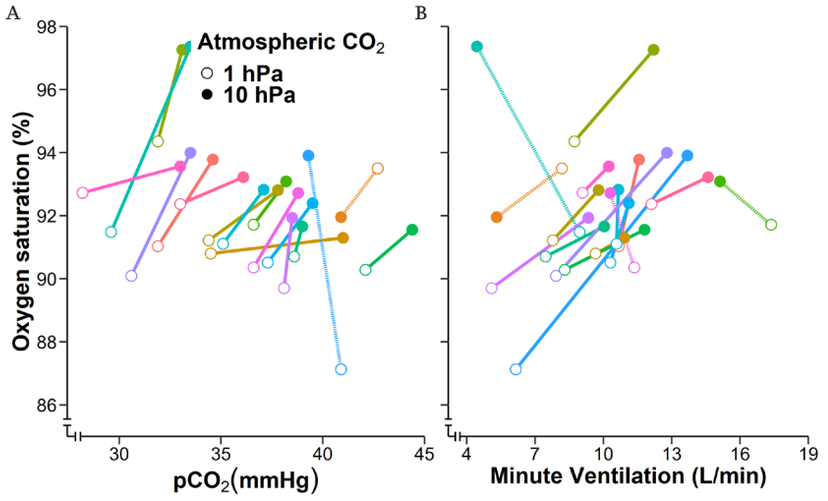
Changes from baseline in minute ventilation, tidal volume, and respiratory rate are shown in Figure 3. High ambient CO_2 levels resulted in increased minute ventilation and respiratory rate (factor treatment $p < 0.001$ in both cases), along with a tendency towards decreased tidal volume (factor treatment $p = 0.061$). There were no significant interactions between treatment and time for any variable.

FIGURE 3 Mean (± SEM) change from baseline for ventilation parameters during 6-h hypoxia with 1 hPa and 10 hPa ambient CO_2: (A) Minute ventilation (calculated as the product of tidal volume and respiratory rate) in L/min, (B) respiratory rate in breaths per minute and (C) tidal volume in mL. Asterisks indicate significant levels ($p < 0.05$) post Bonferroni-Holm correction. For results of mixed model analysis see Results section.



Individual trajectories between ambient CO_2 conditions are shown for SpO_2 and pCO_2 (Figure 4A), and for SpO_2 and minute ventilation (Figure 4B). Fifteen out of 17 participants (88%) showed an increase in SpO_2 with increasing pCO_2, and 13 participants (76%) showed an increase in SpO_2 with increasing minute ventilation.

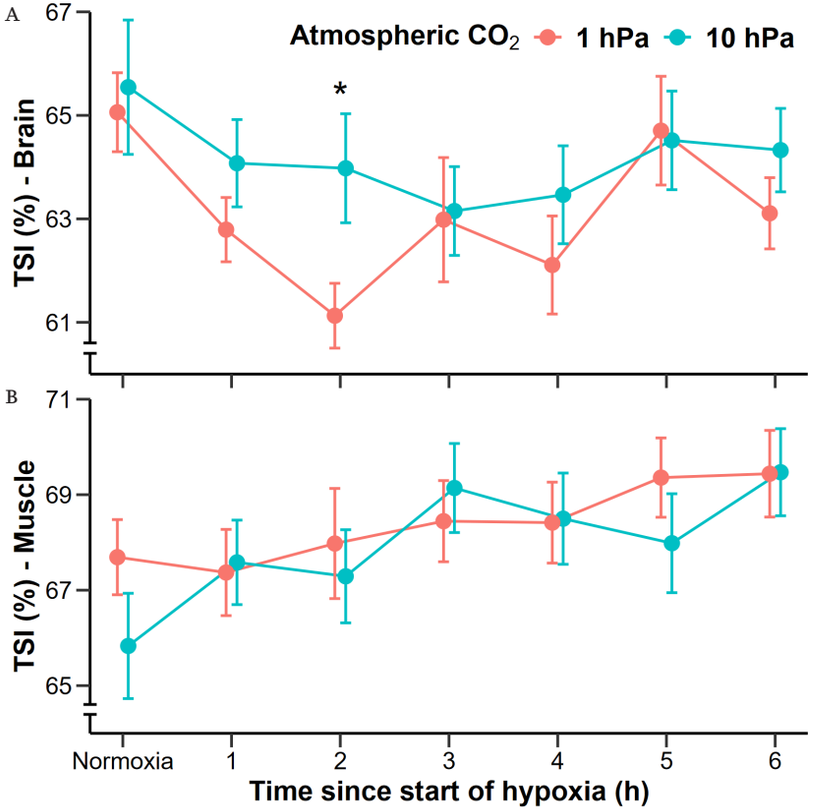
FIGURE 4 Individual trajectories of oxygen saturation between 1 hPa and 10 hPa ambient CO_2 as measured 6 h after hypoxia onset. Oxygen saturation plotted as (A) a function of pCO_2, and (B) as function of minute ventilation. Dashed lines highlight participants deviating from the predominant direction observed in the majority of subjects (solid lines).



High ambient CO_2 resulted in higher brain tissue saturation index (TSI) (Figure 5A, factor treatment $p = 0.003$), with the largest difference occurring 2 h after hypoxia onset. Muscle TSI showed no difference between treatments (Figure 5B). In both brain and muscle, TSI varied over time (factor time $p < 0.001$ in both cases). There were no significant interactions between treatment and time in either tissue. We observed no differences for tHb and HbO_2.

Cognitive performance measurements did not significantly differ between low and high ambient CO_2 (Figure S1). The time fraction of $SpO_2 < 90\%$ did not significantly correlate with cognitive performance measures. No significant differences in AMS-C scores were observed between treatments (Figure S2).

FIGURE 5 Mean (± SEM) tissue saturation index (TSI) of the (A) brain and (B) muscle during hypoxia exposure with 1 hPa and 10 hPa ambient CO_2. The measurement of TSI was performed hourly and is described in Material and Methods. Asterisks indicate significant levels ($p < 0.05$) post Bonferroni-Holm correction. For results of mixed model analysis see Results section.



DISCUSSION

In our study, moderate hypobaric hypoxia resembling conditions in an airliner cabin, CO_2 enrichment improved blood oxygenation. The intervention was well tolerated in healthy young individuals. High CO_2 elevated minute ventilation and respiratory rate. Consequently, CO_2 enrichment increased blood gas levels of pCO_2, pO_2, and SpO_2, while reducing the time fraction of SpO_2 < 90%. Furthermore, CO_2 increased tissue oxygenation in the brain but not in skeletal muscle. There was no cognitive impairment in both conditions compared to normoxia.

The minimum allowed effective oxygen level inside an airliner cabin at cruising altitude approximates 70% of that at sea level akin to conditions at 8,000 ft altitude. The altitude limit is a compromise between passengers' wellbeing and safety, and structural requirements of the aircraft. Numerous studies with healthy participants have reported a mean in-flight oxygen saturation ranging from 91% to 95%, which is considered safe for most people. However, in some passengers, especially in those with pre-existing respiratory conditions, oxygen saturation can decrease below 80%.[5,19–21] Moreover, during sleep under flight conditions, blood oxygenation can decrease below 90% for a sizable fraction of time even in healthy individuals.[3,6] Increased mortality risk has been observed in emergency admissions with SpO_2 levels between 86% and 89% compared to 90% or higher,[8] and postoperative outcomes worsen when desaturation occurs below 90%.[22] Moreover, cardiac and respiratory complaints are among the most serious in-flight medical events.[7] Most guidelines define hypoxemia as an oxygen saturation level below 90% that should be avoided.[23] However, existing atmospheric conditions in the cabin fail to effectively prevent hypoxemia in all passengers.

Subtle fluctuations in SpO_2 levels contribute to a higher frequency of discomfort—such as altitude-related malaise, muscular discomfort, and fatigue—reported by unacclimatized participants after 3 to 9 hours.[5] This suggests that the slight increase in brain oxygenation observed in our study may help mitigate fatigue during flights at 8,000 ft.

We investigated whether ambient CO_2's physiological properties could be leveraged to maintain oxygen saturation levels of airline passengers above 90%. While acknowledging potential adverse effects of ambient CO_2, we recognize the need for a nuanced discussion. Increased inhaled ambient CO_2 can lead to adverse effects.[24,25] However, aviation authorities currently mandate a cabin CO_2 limit of 0.5%.[26] Under hypoxic conditions and with a controlled ambient CO_2 concentration of 1%, we did not observe impairments in cognitive function or acute well-being. By providing a comprehensive perspective, our data align with the proposal to use 1% ambient CO_2 to maintain passenger well-being during flights. Nonetheless, it is important to recognize that the current investigations need to be extended to individuals with medical conditions and those of older age.

A practical implementation of the approach outlined in this paper would be reducing cabin ventilation to leverage the CO_2 generated by passengers. On the one hand, reducing cabin ventilation lessens the need for air sourced from the aircraft's engines (so called bleed air) for

ventilation purposes, as currently proposed by the European Union's Horizon 2020 research and innovation programme.[27] Positive effects would include lower fuel consumption, increased engine thrust, and reduced risk of toxic fume events.[9,28]

On the other hand, it might lead to concerns about increased odors and the heightened risk of spreading infectious diseases due to lower air exchange rates.[29] However, these challenges can be overcome with straightforward technological adaptations to the aircraft's air conditioning systems, such as the integration of CO_2 sensors and specialized air filters.[30,31]

Harvey *et al.* proposed the inhalation of 3% CO_2 as a potential therapy for mountain sickness, reporting symptom relief and an increase in arterial oxygen partial pressure ranging between 24 and 40%.[32] A follow-up study confirmed these findings, although to a lesser degree.[33] Our research aligns with these previous studies that employed a similar approach of increasing ambient CO_2, further supporting the validity and effectiveness off this method. It should be kept in mind however, that these previous studies were carries out in climbers at considerably higher altitudes.

Previous studies have reported a favorable impact of ambient CO_2 on cerebral oxygen saturation in the presence of hypoxia and normoxia.[34,35] Our NIRS measurements on brain tissue support these findings, suggesting a temporary vasodilatory effect and/or the increased respiration due to high CO_2.

During the 6-hour exposure at ~8,000 ft under both CO_2 conditions, there were no significant impairments observed in cognitive performance when compared to baseline under normobaric normoxia. Moreover, the addition of 1% ambient CO_2 to the aircraft cabin did not demonstrate cognitive impairments, indicating no apparent risk to cognition in healthy individuals. Furthermore, there was no correlation found between the time fraction of SpO_2 < 90% and performance in any of the cognitive tasks. A recent review revealed only minor impairments in some cognitive domains at oxygen levels between 80-89% SpO_2.[36]

An important limitation of our study is that we only included younger and healthy participants, which limits the generalizability of our study. Our findings cannot be simply extrapolated to older individuals and those with medical conditions. However, it is precisely these older and diseased individuals who are at a higher risk of experiencing in-flight hypoxemia, and who may benefit the most from in-flight measures to improve oxygenation. Passengers with respiratory diseases (e.g., chronic obstructive pulmonary disease, sleep apnea) and cardiovascular diseases

(e.g., cardiomyopathy), in particular, are more vulnerable to the dangers of in-flight hypoxemia which could prevent them from flying at all.[37–39] This increased vulnerability underscores the importance of studying their capacity to tolerate elevated ambient CO_2 levels. In clinical settings, inhalation of CO_2 at concentrations up to 3% has shown successful therapeutic outcomes for conditions such as Cheyne-Stokes respiration and central sleep apnea.[40] For passengers with respiratory diseases, the effects of increased ambient CO_2 are likely to vary depending on the underlying pathophysiology of their specific condition. Thus, it is crucial to not only evaluate the potential benefits of 1% inspiratory CO_2 in increasing oxygen saturation but also consider its potential to exacerbate the condition of these passengers. Another limitation is the absence of end-expiratory CO_2 measurements, which prevented us from accurately calculating alveolar ventilation in addition to minute ventilation.

STATEMENT OF SIGNIFICANCE

With the popularity of air travel as a major transportation mode, atmospheric conditions within aircraft cabins should be optimized to ensure the well-being of all passengers. Our study challenges existing aviation regulations by showing that an increase to up to 1% in cabin CO_2 concentration improved mean blood oxygenation in the majority of the healthy individuals studied here. Notably, our findings demonstrate that a higher cabin CO_2 concentration effectively prevents mean blood oxygen saturation from dropping below the clinical threshold of 90%. However, considering the diverse and changing demographics of airline passengers – including more individuals who are older and/or have medical conditions –, safety implications of 1% CO_2 concentration should be thoroughly investigated across different groups of passengers.

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SUPPLEMENTARY MATERIALS

FIGURE S1 Mean (± SEM) cognitive performance of (A) PVT speed, (B) PVT lapses, (C) 2-Back omissions, (D) Tracker mean score during hypoxia exposure with 1 hPa and 10 hPa ambient CO_2. No significant differences were found between treatments (mixed model, zero-inflated negative binominal regression model was used in the case of PVT lapses and zero-inflated Poison model was used in the case of 2-back omission.

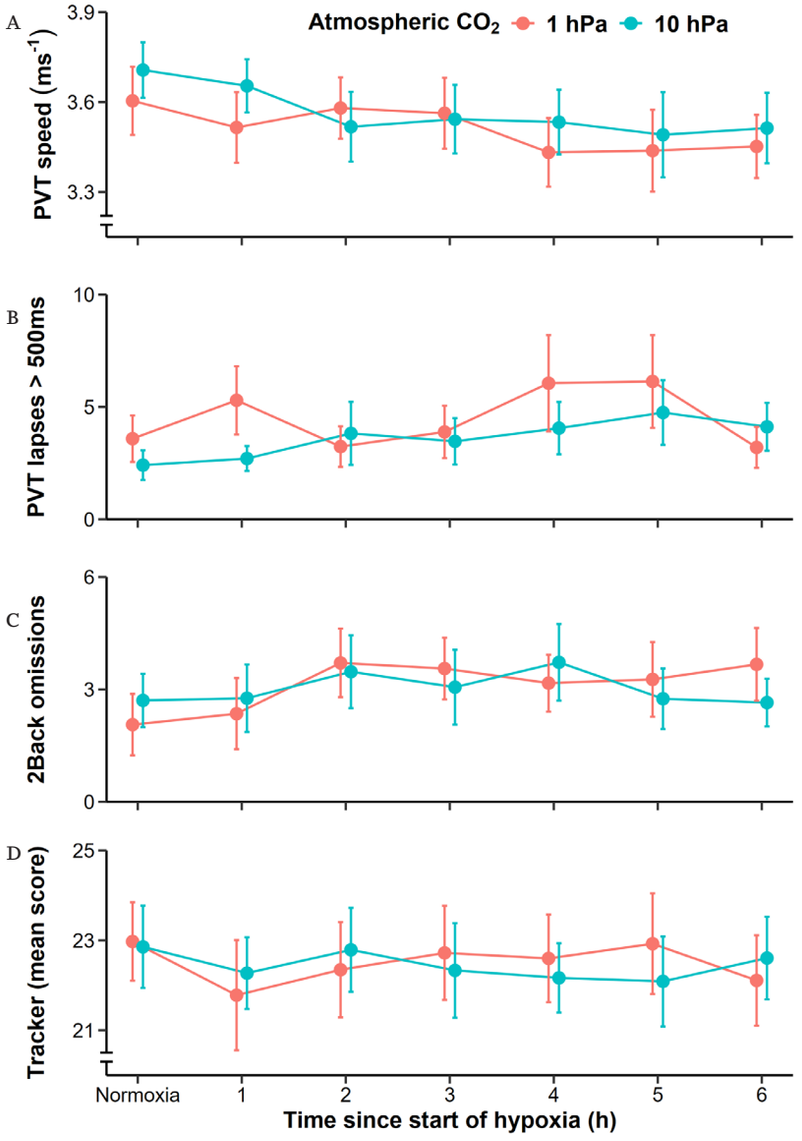
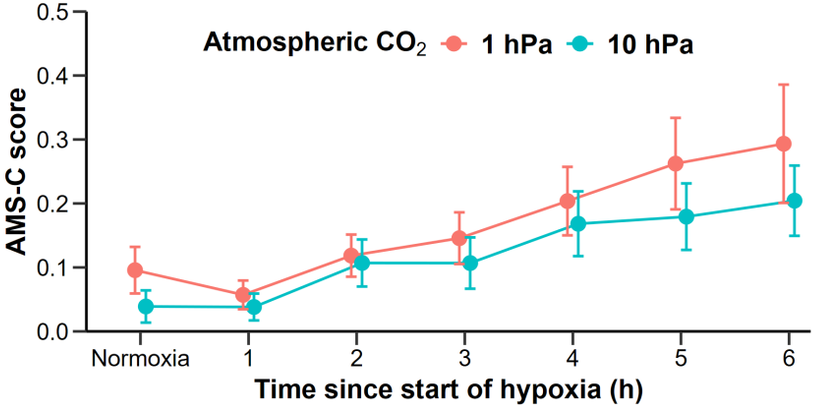


FIGURE S2 Mean (± SEM) AMS-C score during hypoxia exposure with 1 hPa and 10 hPa ambient CO_2. No significant differences were found between treatments (mixed model).



CHAPTER IV | SENSITIVITY OF COGNITIVE FUNCTION TESTS TO ACUTE HYPOXIA IN HEALTHY SUBJECTS: A SYSTEMATIC LITERATURE REVIEW

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ABSTRACT

Acute exposure to hypoxia can lead to cognitive impairment. Therefore, hypoxia may become a safety concern for occupational or recreational settings at altitude. Cognitive tests are used as a tool to assess the degree to which hypoxia affects cognitive performance. However, so many different cognitive tests are used that comparing studies is challenging. This structured literature evaluation provides an overview of the different cognitive tests used to assess the effects of acute hypoxia on cognitive performance in healthy volunteers. Less frequently used similar cognitive tests were clustered and classified into domains. Subsequently, the different cognitive test clusters were compared for sensitivity to different levels of oxygen saturation. A total of 38 articles complied with the selection criteria, covering 86 different cognitive tests. The tests and clusters showed that the most consistent effects of acute hypoxia were found with the Stroop test (where 42% of studies demonstrated significant abnormalities). The most sensitive clusters were auditory/verbal memory: delayed recognition (83%); evoked potentials (60%); visual/spatial delayed recognition (50%); and sustained attention (47%). Attention tasks were not particularly sensitive to acute hypoxia (impairments in 0-47% of studies). A significant hypoxia level-response relationship was found for the Stroop test ($p = 0.001$), as well as three clusters in the executive domain: inhibition ($p = 0.034$), reasoning/association ($p = 0.019$), and working memory ($p = 0.024$). This relationship shows a higher test sensitivity at more severe levels of hypoxia, predominantly below 80% saturation. No significant influence of barometric pressure could be identified in the limited number of studies where this was varied. This review suggests that complex and executive functions are particularly sensitive to hypoxia. Moreover, this literature evaluation provides the first step towards standardization of cognitive testing, which is crucial for a better understanding of the effects of acute hypoxia on cognition.

INTRODUCTION

Hypoxemia is defined as low oxygen levels in the blood.[1] It can occur in obstructive sleep apnea (OSA), in various pulmonary diseases such as COPD and COVID-19, in neuromuscular disorders like Guillain-Barré syndrome, Pompe's disease or myasthenia gravis, and in central nervous system (CNS) conditions like Alzheimer's.[2–6] Hypoxemia may also develop at high altitudes, where less oxygen is available due to the low atmospheric pressure.[7]

Hypoxia research is divided into three arbitrary exposure designs: chronic, intermittent and acute hypoxia. Research on chronic hypoxia has shown the ability of the human body to adapt to prolonged exposure to low oxygen levels or pressures.[8] Hence, individuals residing at high altitudes or those who have had recent altitude exposure are frequently ineligible to participate in clinical trials investigating the impacts of hypoxia. In recent years, intermittent hypoxia (IH) has been studied, attempting to use the human body's adaptability to IH as a therapeutic effect for various diseases.[9] This review will focus only on acute non-intermittent hypoxia.

Studying acute hypoxia is of particular importance in assessing the safety of occupational or recreational settings that involve acute exposure to high altitude, e.g. pilots, military personnel, mountaineers etc. Hypoxia facilities can induce either hypobaric hypoxia by reducing barometric pressure or normobaric hypoxia by diluting oxygen fraction with nitrogen.[10] There has long been debate as to whether there is a difference in physiological response to isolated changes in barometric pressure, with normal levels of oxygen and carbon dioxide.[11] More recent studies suggest that there is indeed a significant impact of pressure alone.[12–16]

One of the first symptoms of hypoxia exposure is impaired cognitive functioning. The level of oxygen saturation is considered to be the key predictor in determining the extend of cognitive impairment caused by hypoxia.[14] During high-altitude activities, cognitive performance plays a critical role in tasks that require attention, decision-making and memorization of protocols, as it can mitigate the risk of potential disasters. Over the years, numerous cognitive tests have been developed to evaluate cognitive performance. However, due to the wide range of cognitive tests used in studies examining cognitive performance during acute hypoxia exposure, it is challenging to compare results across different studies.

A useful test for the cognitive effects of acute hypoxia can be characterized as any response measure that shows a clear, consistent response

to meaningful hypoxia levels, across studies from a sufficient number of different research groups. A hypoxia level-response relationship and a plausible relationship between the function test and the physiological response to hypoxia are indications that the test reflects physiological activity.

Previously, similar criteria were used to evaluate the usefulness of CNS-tests for the effects of antipsychotic drugs,[17] benzodiazepines,[18] selective serotonin re-uptake inhibitors (SSRIs),[19] 3,4-methyleendioxymethamfetamine (MDMA),[20] D9-tetrahydrocannabinol (THC)[21] and alcohol[22] in healthy subjects. In general, these systematic reviews showed that only a small number of tests actually display proper characteristics for a meaningful effect biomarker, and that these tests differ between drug classes.[23]

This review aims to provide an overview of the wide range of cognitive tests used to assess cognitive performance during acute hypoxia in healthy volunteers, to evaluate the differences in test sensitivity to different levels of hypoxia, to explore the role of barometric pressure, and to ultimately identify which tests best meets the criteria to serve as a meaningful test of the functional effects of hypoxia.

METHODS

Structured literature evaluation

A literature search was performed up to 9 of November 2022 using Pubmed, Embase, Web of Science, Cochrane Library, Emcare, PsycINFO and Academic Search Premier. Key words used in the searches were combinations of 'hypoxia', 'cognitive function', 'cognition', 'neuropsychological tests', 'biomarkers', 'cognitive dysfunction' and 'mental deterioration'. The searches were limited to healthy adults and articles in English. The resulting studies were subject to several selection criteria. Reviews, studies in experimental animals or patients, ≤ 5 healthy subjects and confounding factors like exercise, acclimatization, sleep, brain stimulation and breathing exercise were excluded. The review was restricted to the effects of hypoxia exposure of < 8 hours and the presence of a normoxia condition, served as baseline or control group. Studies that contained a confounding factor but included both a normoxia and hypoxia group in the study design were eligible for analysis. Controlling the end-tidal CO_2 can impact oxygen saturation, making isocapnia a potential confounding factor. Nevertheless, studies with isocapnic hypoxia were included when the oxygen saturation was documented.

Mapping of results

Most studies compared hypoxia and normoxia groups, using one or more cognitive tests. In addition, some studies examined different exposure durations and/or severities. The normoxia condition represented either a placebo-controlled group or served as a baseline measurement of the group that would later be exposed to the hypoxia condition. Each primary test parameter for each cognitive test used in a study was considered a unique data point. Thus, a single study usually accounted for multiple data points. In some cases, there was no significant difference between groups for the primary effect parameter, while there was a significant difference for a secondary effect parameter. However, including these secondary effects would result in an overestimation of the cluster sensitivity. Hence, only primary effect parameters were considered.

All data points were collected in a Microsoft Excel® database and recorded as a significant impairment or decrease (-), as no significant effect (=) or as a significant improvement or increase (+) of the test parameter during the hypoxia condition compared to the normoxia condition. The absolute values of the test effects or the levels of significant impairment were not included in the analysis. Consequently, this approach addresses the likelihood that a study with a hypoxia condition produces a statistically significant effect on a given cognitive test.

Study characteristics that were recorded in the database were: effect (+ (improvement/increase) / = (no change) / - (deterioration/reduction)), test domain, test cluster, barometric pressure (hypobaric or normobaric), hypoxia intensity ('dose', displayed as FiO_2/altitude), dose normalization (four levels), oxygen saturation, exposure duration (including test duration), test duration, oxygen administration (mask/chamber/altitude), blinding (open/single-blind/double blind), randomization (randomized/non-randomized), control (baseline/control group), design (parallel/crossover), number of subjects, sex and age. Although blood CO_2 levels are relevant for hypoxia research, they were not included in the database, as most studies did not document this information.

Analysis of individual tests and test clusters

Cognitive tests with at least five data points (i.e. five different results from different experiments) were included in the analysis of the individual test results. Cognitive tests that were alternate versions of the original tests, or which tested the same cognitive functionality, were clustered to increase the number of evaluable data points. The clusters were divided into six domains for a better overview. The allocation of domains and clustering

of cognitive tests was based on "A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary",[24] adapted according to comparable systemic biomarker reviews that we performed previously for drug classes.[17–22] The sensitivity of a test was expressed as the percentage of statistically significant outcomes, relative to the total number of times that the test was used in the literature. The percentages of tests that showed cognitive impairment were calculated for each cluster.

Level-response relationships

While analysis of individual tests and test clusters provides an overview of the sensitivity of different neuropsychological functions to hypoxia, this does not include information on the relationship between the level of hypoxia and the effect on cognitive test performance. This relationship was studied by first applying a four-level dose normalization to oxygen saturation. These four levels represent the severity of hypoxia as described by Castor and Borgvall[25] and based on Woodrow and Webb.[26] The oxygen saturation levels are ≥ 90%, 89-80%, 79-70% and ≤ 69%. For studies that did not provide oxygen saturation, the fraction of inspired oxygen or altitude was used to estimate oxygen saturation.[25,26] Only tests or clusters containing at least 10 data points were included in this analysis. Relationships were tested with simple linear regression analysis. Finally, a comparison was made between all similar tests/clusters that were measured both in normobaric hypoxia and hypobaric hypoxia. Similarly, these results were divided over the four levels of dose normalization.

RESULTS

Study design

The literature search yielded 38 different studies on acute hypoxia that met all criteria, published between 1993 and 31 August 2022. The number of participants ranged from 6 to 50 and ages from 22 to 41 years (range of mean ages between different studies). In 40% of studies only healthy men were included, and 5% of studies included only women. Fifty-five percent of studies included men and women. A summary of the study characteristics is reported in Table 1.

Forty-seven percent of the reviewed studies had an open design; 37% were single-blinded; 5% were double blinded and for 11% the blinding was unknown. In addition, a majority of the studies had a crossover design (89%) and 11% had a parallel design. Normoxia served in 61% of studies as a control group and in 39% as a baseline measurement.

TABLE 1 Summary of general study characteristics.

Author	Participants (sex)	(Corresponding) altitude (NH/HH)	Exposure duration including test time (test time)	SpO_2 in % (SD, SEM, 95% CI)*	Design
Asmaro *et al.* (2013)[27]	34 (M/F)	5334 m (HH)	30 min (5 min)	NA	baseline controlled
		7620 m (HH)	5 min (5 min)		
Beer *et al.* (2017)[28]	12 (M/F)	5486 m (HH)	18 min (continuous)	NA	baseline controlled
	11 (M/F)	7620 m (HH)	5 min (continuous)		
Blacker *et al.* (2021)[29]	26 (M/F)	6096 m (NH)	10 min (10 min)	79.8 ± 6.9% (SD)	baseline
Blacker *et al.* (2022)[30]	30 (M/F)	6096 m (NH)	14.5 min (14 min)	~75 ± 1.5% (SEM)	placebo crossover
Caldwell *et al.* (2018)[31]	10 (M)	NA	25 min (NA)	79 ± 3% (SD)	placebo crossover
				88 ± 1% (SD)	
Chroboczek *et al.* (2022)[32]	32 (M)	3800 m (NH)	35-36 min (5-6 min)	~78 ± 4% (SD)	placebo crossover
Davranche *et al.* (2016)[33]	11 (M)	4350 m (HH)	3-5 h (~24 min)	83 ± 1.2% (SEM)	baseline controlled
Decroix *et al.* (2018)[34]	20 (M/F)	4000 m (NH)	57 min (27 min)	83.8 ± 2.1% (SD)	placebo crossover
Falla *et al.* (2022)[35]	48 (M/F)	3000 m (HH)	10 min (5 min)	93.6 ± 2% (SD)	placebo crossover
		5000 m (HH)		79.2 ± 4.8% (SD)	
Feeback *et al.* (2017)[36]	6 (M) (CAU)	4400 m (NH)	< 32 min (< 2 min)	~81 ± 2.3%	placebo crossover
			< 62 min (< 2 min)	79.5 ± 4.8% (SD)	
			< 92 min (< 2 min)	~83 ± 9% (SD)	
			< 117 min (< 2 min)	~83 ± 4.2% (SD)	
	6 (M) (AA)		< 32 min (< 2 min)	~86 ± 4.7% (SD)	
			< 62 min (< 2 min)	~86 ± 4.6% (SD)	
			< 92 min (< 2 min)	~83 ± 4% (SD)	
			< 117 min (< 2 min)	~85 ± 4.7% (SD)	

[Continuation Table 1]

Author	Participants (sex)	(Corresponding) altitude (NH/HH)	Exposure duration including test time (test time)	SpO_2 in % (SD, SEM, 95% CI)*	Design
Gerhart *et al.* (2019)[37]	10 (M)	3800 m (NH)	60 min (5 min)	~85 ± 3%	placebo parallel
Hewett *et al.* (2010)[38]	50 (M/F)	2438 m (NH)	45 min (30 min)	~95 ± 1% (SEM)	placebo crossover
		3048 m (NH)		~92 ± 1% (SEM)	
		3658 m (NH)		~88 ± 1% (SEM)	
		4267 m (NH)		~84 ± 1% (SEM)	
Kammerer *et al.* (2018)[39]	11 (M/F)	3883 m (HH)	~53 min (~8 min)	84.8 ± 4.9% (SD)	baseline controlled
	7 (M/F)	3883 m (NH)		82.9 ± 5.8% (SD)	
Kerr *et al.* (2022)[40]	21 (M/F)	14% (NH)	60 min (5 min)	91 ± 3% (SD)	placebo crossover
			90 min (5 min)	90 ± 3% (SD)	
Kim *et al* (2015)[41]	8 (M)	4300 m (NH)	< 32 min (< 2 min)	87 ± 2% (SEM)	baseline controlled
Kourtidou-Papadeli *et al.* (2008)[42]	10 (M/F)	2438 m (NH)	16 min (16 min)	92 ± 4.3% (SD)	baseline controlled
Lefferts *et al.* (2016)[43]	20 (M)	4600 m (NH)	165 min (60 min)	75 ± 6% (SD)	baseline controlled
Legg *et al.* (2012)[44]	25 (M)	2438 m (NH)	30 min (NA)	91 ± 2% (SD)	placebo crossover
			90 min (NA)		
Legg *et al.* (2016)[45]	36 (M)	2438 m (HH)	39 min (12-15 min)	95 ± 3% (SD)	placebo crossover
		3658 m (HH)		88 ± 3% (SD)	
Lei *et al.* (2019)[46]	30 (F)	4000 m (NH)	12 min (2 min)	87 ± 6% (SD)	placebo crossover
Loprinzi *et al.* (2019)[47]	21 (M/F)	4000 m (NH)	30 min (NA)	85 ± 1% (95% CI)	placebo crossover
Malle *et al.* (2016)[48]	45 (M)	10000m (NH)	3 min (3 min)	76±0.8% (SEM)	placebo parallel
Nakata *et al.* (2017)[49]	15 (M/F)	4400 m (NH)	40 min (5 min)	~80±10% (SD)	placebo crossover
Noble *et al.* (1993)[50]	24 (M)	5850 m (NH)	30 min (25 min)	78±2.9% (SD)	placebo parallel
Ochi *et al* (2018)[51]	14 (M/F)	3500 m (NH)	16.5 min (6.5 min)	86.2±1.2% (SEM)	placebo crossover
Ogoh *et al.* (2018)[52]	14 (M/F)	4400 m (NH)	45 min (5 min)	80%±10% (SD)	baseline controlled
Reményi *et al.* (2018)[53]	33 (M/F)	4000 m (HH)	20 min (15 min)	~86±4.5% (SD)	baseline controlled

[Continuation Table 1]

Author	Participants (sex)	(Corresponding) altitude (NH/HH)	Exposure duration including test time (test time)	SpO_2 in % (SD, SEM, 95% CI)*	Design
Rossetti *et al.* (2021)[54]	24 (M/F)	4500 m (NH)	210 min (90 min)	82 ± 2% (95% CI)	placebo crossover
Seech *et al.* (2020)[55]	39 (M/F)	5400 m (NH)	1-9 min (continuous)	~81 ± 0.8% (SEM)	placebo crossover
			10-18 min (continuous)	~74 ± 1% (SEM)	
			19-27 min (continuous)	~73 ± 1% (SEM)	
Seo *et al.* (2015)[56]	16 (M)	4300 (NH)	60 min (5 min)	~82 ± 2.5%	baseline
Seo *et al.* (2017)[57]	15 (F)	4300 m (NH)	30 min (NA)	83.3 ± 5.1% (SD)	baseline controlled
			60 min (NA)	82.2 ± 5.1% (SD)	
Stepanek *et al.* (2013)[58]	25 (M/F)	7101 m (NH)	< 5 min (< 2 min)	75.8 ± 8.3% (SD)	baseline controlled
Turner *et al.* (2015)[59]	15 (M/F)	5850 m (NH)	90 min (75 min)	80 ± 10% (SD)	placebo crossover
Turner *et al.* (2015)[60]	22 (M/F)	5850 m (NH)	90 min (40 min)	75 ± 1% (SEM)	placebo parallel
Valk *et al.* (2016)[61]	24 (M)	2438 m (HH)	1-6 h (20 min)	93% (range 85–95%)	baseline controlled
Van der Post *et al.* (2002)[62]	12 (M/F)	NA	130 min (100 min)	80.3 ± 1.2% (SD)	placebo crossover
				90±0.9% (SD)	
Wang *et al.* (2013)[63]	10 (M)	3560 m (HH)	6.5 h (30 min)	NA	baseline controlled
Williams *et al.* (2019)[64]	12(M)	4500 m (NH)	60 min (< 5 min)	~81 ± 4% (SD)	placebo crossover
		3000 m (NH)		~90 ± 1.5% (SD)	
		1600 m (NH)		~94 ± 1.3 (SD)	

M = male, F = female, M/F = male and female, NH = normobaric hypoxia, HH = hypobaric hypoxia, SpO_2 = oxygen saturation, CAU = Caucasian individuals, AA = African-American
*SpO_2 levels with the '~' symbol, were estimated from a graphical representation.

Atmospheric conditioning of hypoxia

Eighteen studies were performed in a hypoxic chamber,[27–30,32,34–37,40,41,43,45,53,55–57,61,64] 16 with a breathing mask that induced hypoxia,[31,38,42,44,46–52,58–60,65] two studies were performed at altitude,[33,63] one both at altitude and in a chamber,[39] and one both in a chamber and with a breathing mask.[54] The exposure duration ranged from 10 minutes to 6.5 hours with 92% of studies using durations of < 3 hours. The data points obtained were divided into exposure to normobaric hypoxia (133; 67%) and hypobaric hypoxia (66; 33%). The mean altitude and mean oxygen saturation for normobaric and hypobaric hypoxia were 4500 m and 84.4%, and 3300 m and 86.9%, respectively. Only two studies were performed with isocapnic hypoxia, so this condition was not analysed separately.[31,65]

Tests, clusters and domains

A total of 86 different tests were used. Only six tests (including comparable variants) (7%) were used five times or more and consequently produced five or more data points for the analysis of hypoxia on individual tests. These most frequently used tests were the Trail making test A (14), Trail making test B (14), Stroop test (12), Finger tapping (5), Go/No-go (5) and Digit symbol substitution test (DSST) (5), shown in Table 2. Overall, the Stroop test and Finger tapping test showed the highest sensitivity to acute hypoxia (which produced significant effects in 42% and 40% of experiments, resp.), followed by Trail making B (29%), Trail making A (21%) and the Go/No-go task (20%). The DSST did not show any significant cognitive impairment.

A cluster analysis was performed because of the small number of tests that were performed frequently enough to allow an individual test analysis. Tests used only incidentally were clustered together with other tests that require the same cognitive functionality. This allowed us to increase the sample size and thus perform a more reliable analysis on how hypoxia affects different cognitive functions. The tests were grouped into 32 functional clusters, covering six main neurocognitive domains (Table 3). Fourteen of the 32 clusters contained at least five data points and were used for further analysis of overall hypoxia effects (Table 4). Both delayed recognition clusters auditory/verbal memory (83%) and visual/spatial memory (50%), and the evoked potential cluster (60%) had the highest sensitivity to acute hypoxia (irrespective of level). In contrast, immediate recognition tests showed virtually no significant effects (auditory/verbal memory tests (20%); visual/spatial memory tests (0%)). In addition to delayed recognition and evoked potential, the clusters of sustained attention (47%), motor control (40%) and divided attention (36%) showed a higher sensitivity for hypoxia. The clusters inhibition (26%), shifting (23%) and reaction time (19%) rarely yielded significant results, whereas in the literature, tests from these clusters were used most frequently (inhibition 23/182, shifting 31/182, and reaction time 21/182 tests).

TABLE 2 **A summary of the most frequently used tests (≥ 5 times) for measuring the effects of acute hypoxia on cognitive performance. The table shows the number of times tests were, and in brackets, the number of studies from which these data points were collected. The test performance is indicated by significant impairment or decrease (-), no significant effect (=) or significant improvement or increase (+). Test sensitivity was calculated as the number of times a test showed a significant impairment out of the total number of times the test was taken.**

Test name	Number of times taken (number of studies)	Test performance hypoxia vs normoxia			Test sensitivity
		-	=	+	
Stroop	12 (9)	5	7	0	42%
Finger tapping	5 (4)	2	3	0	40%
Trail making B	14 (5)	4	10	0	29%
Trail making A	14 (5)	3	11	0	21%
Go/No-go	5 (5)	1	4	0	20%
DSST	5 (4)	0	5	0	0%

TABLE 3 **An overview of the 86 tests condensed into domains and clusters.**

Domain	Cluster	Test
Attention	Divided Attention	Auditory monitoring task, Combined distributive attention test, Divided attention test, Recourse management task, System monitoring, Visual monitoring task
	DSST-like	Digit symbol substitution test
	Focused/selective attention	Shifting attention test
	Reaction time	Choice reaction time, Deary–Liewald reaction time task, Go/No-go task, Simple auditory and visual reaction times, Simple unprepared reaction time, Sorted reaction test, Target reaction test, The binary choice task
	Sustained attention	Behavioural tracking task, Continuous performance test, Motion detection task, Paced auditory serial addition task 1.2s, Paced auditory serial addition task 1.6s, Psychomotor vigilance task, Tracking task, Vigilance and tracking task
Executive	Inhibition	Eriksen flanker test, Go/No-go task, Simon task, Stroop test, Verbal interference, Visual interference
	Language	King-Devick test
	Planning	Tower puzzle

[Continuation Table 3]

Domain	Cluster	Test
	Reasoning/association	Abstract matching, Complex logical reasoning task, Logical relations, Math task, Mathematical processing, Pathfinder combined, Symbol digit coding, SYNWIN math task
	Reward	Balloon analogue risk test
	Shifting	Switching of attention, The Wisconsin card sorting task, Trail making test A, Trail making test B
	Spatial orientation	Letter rotation test, Line orientation test, Manikin test
	Working memory	Corsi block-tapping task, Digit span backward test, Digit span forward test, Maze, N-back, Operation span task, Visual sequence comparison
Memory	Auditory/verbal memory: delayed recall	Free recall test, Memory interference task, Multiple memory task
	Auditory/verbal memory: delayed recognition	Emotion recognition task, Memory recognition task, Verbal memory test, Serial recognition of words
	Auditory/verbal memory: immediate recognition	Memory search, Running memory continuous performance task
	Visual/spatial memory: delayed recall	Digital tachistoscopy
	Visual/spatial memory: delayed recognition	Memory task, Serial recognition of figures, Sternberg short-term memory task, Visual memory task, Visual object learning test
	Visual/spatial memory: immediate recognition	Matching to sample, Visual searching task
Motor	Motor control	Finger tapping test
	Visuo-motor control	Motor praxis test, Gridshot
Neuro-physiological	Evoked potential	Auditory N100, Auditory P200, Auditory P50, Electroencephalogram, N140, P300, Visual P100
Subjective experience	Scale anger	Mood test
	Scale anxiety	Mood test
	Scale depression	Mood test
	Scale fatigue	Mood test
	Scale happiness	Mood test
	Scale restlessness	Mood test
	Scale sleepiness	Mood test, Stanford sleepiness scale
	Scale vigilance	Mood test, Level of vigilance visual analogue scale
	Scale vigour	Mood test
	Total mood	Profile of mood state, Mood test

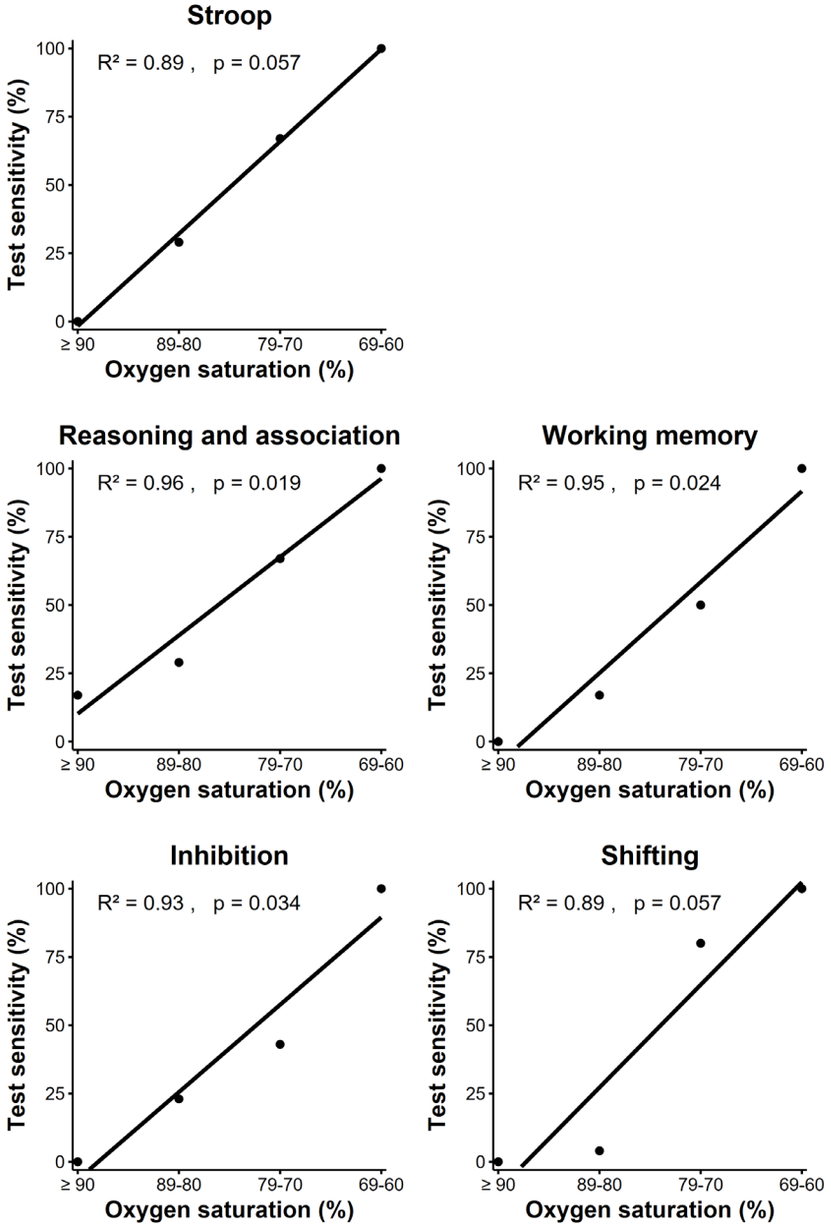
TABLE 4 **An overview of clusters in which at least five times a test was taken. The table shows the number of times tests were taken in each cluster and, in brackets, the number of studies from which these tests were collected. The test performance is indicated by significant impairment or decrease (-), no significant effect (=) or significant improvement or increase (+). Cluster sensitivity was calculated as the number of times a test showed a significant impairment out of the total number of times the test was taken.**

Domain	Cluster	Number of times taken (number of studies)	Test performance hypoxia vs normoxia			Cluster sensitivity
			-	=	+	
Attention	Sustained attention	17 (12)	8	9	0	47%
	Divided attention	11 (5)	4	7	0	36%
	Reaction time	21 (9)	4	16	1	19%
	DSST-like	5 (4)	0	5	0	0%
Executive	Reasoning/association	17 (8)	6	11	0	35%
	Working memory	17 (7)	5	12	0	29%
	Inhibition	23 (16)	6	16	1	26%
	Shifting	31 (7)	7	24	0	23%
Memory	Auditory/verbal memory: delayed recognition	6 (4)	5	1	0	83%
	Visual/spatial memory: delayed recognition	8 (6)	4	4	0	50%
	Auditory/verbal memory: immediate recognition	5 (3)	1	4	0	20%
	Visual/spatial memory: immediate recognition	6 (2)	0	6	0	0%
Motor	Motor control	5 (4)	2	3	0	40%
Neuro-physiological	Evoked potential	10 (4)	6	4	0	60%

Level-response relationship

Individual tests and clusters with ≥ 10 datapoints were inspected for potential level-response relationships (Table 5 and 6). (Near) significant associations were found for the Stroop test, Trail making A, Trail making B and 25% of clusters. The Stroop test ($p = 0.001$, $R^2 = 1.00$) and clusters within the executive domain showed a significant relationship reasoning/association $p = 0.019$, $R^2 = 0.96$; working memory $p = 0.024$, $R^2 = 0.95$; and inhibition $p = 0.035$, $R^2 = 0.93$) or trend (shifting $p = 0.057$, $R^2 = 0.89$) between the four defined levels of oxygen saturation and test sensitivity, with lower saturations more often leading to a higher test sensitivity (Figure 1). The Trail making A, Trail making B and clusters in the attention and neurophysiological domain did not show a level-response relationship between oxygen saturation and test sensitivity.

FIGURE 1 Relationship between test sensitivity and oxygen saturation of Stroop test and clusters within the executive domain using simple linear regression analysis.



Since sustained- and divided attention were sensitive to hypoxia, while also playing a role in executive functioning and memory, the contribution of attention deficits to reduced cognitive functioning was explored in more detail. At an oxygen saturation level of ≥ 90%, tests measuring sustained- and divided attention demonstrated sensitivity ranging from 0% to 25%, respectively, while the executive domain showed sensitivity between 0% and 17%. At an oxygen saturation level of 80-90%, sustained attention showed a test sensitivity of 60%, whereas divided attention did not show any sensitivity, in contrast to the executive domain where sensitivity ranged from 4% to 29%. At an oxygen saturation level of 70-80%, the sensitivity of sustained attention was 40%, while divided attention showed a 50% sensitivity, compared to the executive domain which ranged from 43% to 80%. Although sustained attention was not measured between an oxygen saturation level of 69%-60%, divided attention and all clusters within the executive domain demonstrated 100% sensitivity. There appears to be a cautious relationship between hypoxia-related declines in divided attention and executive performance ($p = 0.059$).

TABLE 5 The level-response relationship between oxygen saturation and sensitivity for tests used ≥ 10 times. Oxygen saturation is normalized into four groups: ≥ 90%, 89-80%, 79-70% and 69-60%. The table shows the number of times tests were taken and, in brackets, the number of studies from which these tests were collected. The test performance is indicated by significant impairment or decrease (-), no significant effect (=) or significant improvement or increase (+). Test sensitivity was calculated as the number of times a test showed a significant impairment out of the total number of times the test was taken.

Test name	SpO_2 normalization	Tests (studies)	Test performance hypoxia vs normoxia			Test sensitivity
			-	=	+	
Stroop	Total	12 (9)	5	7	0	42%
	≥ 90%	1 (1)	0	1	0	0%
	89-80%	7 (6)	2	5	0	29%
	79-70%	3 (3)	2	1	0	67%
	69-60%	1 (1)	1	0	0	100%
Trail making B	Total	14 (5)	4	10	0	29%
	≥ 90%	0 (0)	0	0	0	0%
	89-80%	11 (3)	0	11	0	0%
	79-70%	2 (2)	2	0	0	100%
	69-60%	1 (1)	1	0	0	100%
Trail making A	Total	14 (5)	3	11	0	21%
	≥ 90%	0 (0)	0	0	0	0%
	89-80%	11 (3)	1	10	0	9%
	79-70%	2 (2)	2	0	0	100%
	69-60%	1 (1)	1	0	0	100%

TABLE 6 The level-response relationship between oxygen saturation and sensitivity of clusters tested ≥ 10 times. Oxygen saturation is normalized into four groups: ≥ 90%, 89-80%, 79-70% and 69-60%. The table shows the number of times tests were taken in each cluster and, in brackets, the number of studies from which these tests were collected. The test performance is indicated by significant impairment or decrease (-), no significant effect (=) or significant improvement or increase (+). Cluster sensitivity was calculated as the number of times a test showed a significant impairment out of the total number of times the test was taken.

Domain	Cluster	SpO_2 normalization	Tests (studies)*	Test performance hypoxia vs normoxia			Cluster sensitivity
				-	=	+	
Attention	Sustained attention	Total	17 (12)	8	9	0	47%
		≥ 90%	2 (2)	0	2	0	0%
		89-80%	10 (7)	6	4	0	60%
		79-70%	5 (5)	2	3	0	40%
	Divided attention	Total	11 (5)	4	7	0	36%
		≥ 90%	4 (3)	1	3	0	25%
		89-80%	3 (2)	0	3	0	0%
		79-70%	2 (1)	1	1	0	50%
		69-60%	2 (1)	2	0	0	100%
	Reaction time	Total	21 (9)	4	16	1	19%
		≥ 90%	6 (2)	1	5	0	17%
		89-80%	12 (7)	2	9	1	17%
		79-70%	3 (2)	1	2	0	33%
Executive	Reasoning/association	Total	17 (8)	6	11	0	35%
		≥ 90%	6 (3)	1	5	0	17%
		89-80%	7 (5)	2	5	0	29%
		79-70%	3 (3)	2	1	0	67%
		69-60%	1 (1)	1	0	0	100%
	Working memory	Total	17 (7)	5	12	0	29%
		≥ 90%	5 (3)	0	5	0	0%
		89-80%	6 (5)	1	5	0	17%
		79-70%	4 (2)	2	2	0	50%
		69-60%	2 (1)	2	0	0	100%
	Inhibition	Total	23 (16)	6	16	1	26%
		≥ 90%	3 (2)	0	3	0	0%
		89-80%	13 (12)	3	10	0	23%
		79-70%	7 (5)	3	3	1	43%
		69-60%	1 (1)	1	0	0	100%
	Shifting	Total	31 (7)	7	24	0	23%
		≥ 90%	1 (1)	0	1	0	0%
		89-80%	23 (4)	1	22	0	4%
		79-70%	5 (3)	4	1	0	80%
		69-60%	2 (1)	2	0	0	100%
Neuro-physio-logical	Evoked potential	Total	10 (4)	6	4	0	60%
		89-80%	6 (3)	5	1	0	83%
		79-70%	4 (1)	1	3	0	25%

*Some studies included multiple SpO_2 levels in their experimental design.

The effect of barometric pressure

Table 7 presents the effects of normobaric and hypobaric hypoxia on overall cognitive performance for the four levels of oxygen saturation. At oxygen saturation above 80%, almost no differences in sensitivity were observed between normobaric and hypobaric hypoxia (4% and 5% at SpO_2 ≥ 90% and 27% and 27% at SpO_2 89-80%, respectively). At an oxygen saturation between 79-70%, hypobaric hypoxia affected cognitive performance more often (67%) than normobaric hypoxia (55%), although not significantly. At an oxygen saturation between 69-60%, no data were reported for normobaric hypoxia.

TABLE 7 Effects of barometric pressure on test sensitivity in all clusters. The table shows the number of times tests were taken and, in brackets, the number of studies from whish these tests were collected. Oxygen saturation is normalized into four groups: ≥ 90%, 89-80%, 79-70%, and 69-60%. The number of times a significant effect was found is indicated by impairment or decrease (-), no significant effect (=) or improvement or increase (+).

Barometric pressure	SpO_2 normalization	Tests (studies)*	Test performance hypoxia vs normoxia			Test sensitivity
			-	=	+	
Normobaric	Total	133 (28)	39	92	2	29%
	≥ 90%	23 (5)	1	22	0	4%
	89-80%	79 (18)	21	57	1	27%
	79-70%	31 (7)	17	13	1	55%
Hypobaric	Total	66 (8)	25	41	0	38%
	≥ 90%	19 (3)	1	18	0	5%
	89-80%	26 (5)	7	19	0	27%
	79-70%	12 (3)	8	4	0	67%
	69-60%	9 (2)	9	0	0	100%

*Some studies included multiple SpO_2 levels in their experimental design.

Given that a level-response relationship between oxygen saturation and test sensitivity occurred solely in clusters belonging to the executive domain, the comparison between normobaric hypoxia and hypobaric hypoxia was narrowed to this particular domain (Table 8). Oxygen saturation levels of ≥ 90% and between 89-80% did not yield significantly different effects on cognitive performance between normobaric hypoxia and hypobaric hypoxia. The largest difference was found at an oxygen saturation between 79-70% (50% and 86%, respectively), but this difference was not statistically significant.

TABLE 8 Effects of normobaric and hypobaric hypoxia on test sensitivity in the executive domain. The table shows the number of times tests were taken and, in brackets, the number of studies from whish these tests were collected. Oxygen saturation is normalized into four groups: ≥ 90%, 89-80%, 79-70% and 69-60%. The number of times a significant effect was found is indicated by impairment or decrease (-), no significant effect (=) or improvement or increase (+).

Barometric pressure	SpO_2 normalization	Tests (studies)*	Test performance hypoxia vs normoxia			Test sensitivity
			-	=	+	
Normobaric	Total	62 (19)	11	50	1	18%
	≥ 90%	10 (3)	0	10	0	0%
	89-80%	40 (14)	5	35	0	13%
	79-70%	12 (4)	6	5	1	50%
Hypobaric	Total	28 (7)	14	14	0	50%
	≥ 90%	6 (2)	1	5	0	17%
	89-80%	9 (4)	1	8	0	11%
	79-70%	7 (3)	6	1	0	86%
	69-60%	6 (2)	6	0	0	100%

Some studies included multiple SpO_2 levels in their experimental design.

DISCUSSION

A large number of tests were used in the literature to measure the acute CNS effects of hypoxia in healthy adults. As with similar reviews for drug classes,[17–22] there were more tests than studies: 86 in 38 studies. Only six individual tests, including their variants, (7%) were used five times or more and consequently provided sufficient data points for our individual test analysis. This wide variety of tests limits cross-study comparison. This limitation not only hampers the current review, but a virtually unrestricted use of a large number of distinct neurocognitive tests also thwarts the field of hypoxia research as a whole. Although it may be useful to study different distinct areas of cognitive performance, interpretations and insights would strongly benefit from standardization within cognitive domains and test clusters. Moreover, standardization of exposure protocols would also improve comparisons between studies. Given this suboptimal context, we grouped tests into test clusters and functional domains, to obtain more insights into potentially meaningful hypoxia CNS-effects and protocols. Prior reviews demonstrated that this approach is useful for assessing function tests or biomarkers for drug effects.[17–22] While this methodology inevitably results in the loss of some information, it ultimately yields a structured and comprehensive overview of the chance of measuring significant CNS effects of acute hypoxia in studies with healthy adults.

Attention is an important functional domain that underlies most of the other CNS-performance tasks. Hypoxia has an effect on attention, particularly, at levels below 80%, which caused significant abnormalities of divided or sustained attention in 36% and 47% of studies, respectively. This effect of hypoxia on more demanding (particularly, divided) attention tests may also have influenced other complex or multifunctional tasks, particularly those in the executive domain. These clusters represent aspects of cognition with a direct effect on safety for military personnel and pilots. Especially, impairments in sustained attention, which were already frequently observed at an oxygen saturation between 89-80% (60%), could lead to serious safety risks when military personnel have to stand guard at altitude (2438 m - 4572 m) on hostile territory. Therefore, this aspect of cognition should be carefully considered during training and preparation for a mission or flight. The effect of hypoxia on reaction time seems limited, even under more severe hypoxic conditions (SpO_2 < 80%) a low-test sensitivity is observed (33%). However, it should be noted that in some cognitive tests, secondary effects on reaction time were observed, which were not included in the analysis to prevent bias.

In the executive domain, the different test clusters rarely showed an effect of mild hypoxia (SpO_2 ≥ 80%). However, when oxygen saturation fell below 80%, the test sensitivity increased. The level-response relationship between oxygen saturation and test sensitivity could indicate that cognitive testing of the executive domain gives the most accurate representation of the physiological response of hypoxia. Although the mechanism causing the impairment of executive function by hypoxia is not clearly understood, this could be related to impaired concentration or (divided) attention, which are required during performance of any executive task. It is also possible that hypoxia-induced executive impairment is related more directly to decreased neural activity of the prefrontal cortex.[66–68] A review by Beebe and Gozal linked executive impairment of OSA patients to prefrontal cortex dysfunction.[69] To our knowledge, however, only one study has examined the role of the prefrontal cortex in reducing executive function after hypoxia exposure in healthy individuals.[51] Although this study by Ochi *et al.* found a statistically significant relationship between impaired Stroop test performance and lower dorsolateral prefrontal cortex activation after a combined intervention of exercise and hypoxia, this relationship was not significant in hypoxia at rest. However, the oxygen saturation was also lower in the combined exercise and hypoxia

intervention than in the hypoxia at rest intervention, which is expected as, exercise acutely decreases the oxygen saturation both combined with and independent of hypoxia.[70] Therefore, a similar effect on the lower dorsolateral prefrontal cortex might be found for more severe hypoxia. Further research into the interaction between hypoxia, prefrontal cortex activation and executive function could help to get a better understanding of the physiological response to hypoxia.

Within the inhibition cluster, the Stroop test more often showed impairments (42%) than when other inhibition tests were used (mean of 26%). This may reflect the dependence of the performance of the Stroop test on accurate colour discrimination. Although this systematic review did not specifically search for visual function studies, the retina has been reported to be highly sensitive to hypoxia.[71] Based on this observation we recommend choosing the Stroop test over other inhibition tasks.

In the memory domain, delayed recognition was the most sensitive to hypoxia. Delayed auditory/verbal recognition tests showed the highest sensitivity among all clusters (83%). Delayed recognition is an aspect that can be highly relevant while navigating through new terrain. Therefore, this should be considered especially for exploratory expeditions at altitude.

Although the number of studies using neurophysiological tests during acute hypoxia was limited, our data suggest that tests within the neurophysiological domain could be a particularly sensitive tool to assess CNS functions during hypoxia. At an oxygen saturation between 89-80%, the evoked potential cluster showed higher sensitivity than any other cluster. This is in line with previous research by Tsarouchas *et al.* which concluded that evoked brain responses can be used for early detection of cognitive alterations during exposure to moderate hypobaric hypoxia.[72]

The impact of barometric pressure on the severity of hypoxia is a subject of debate.[11-16] In light of this, a secondary objective of this review was to explore the effects of barometric pressure on cognitive function. No significant difference was found in test sensitivity due to barometric pressure in the overall analysis at all oxygen saturation levels. Similarly, when only the (complex) attention or executive function domains were considered, no significant difference was found. Although the number of studies was too small to be conclusive, this review does not provide indications that barometric pressure has a large impact on hypoxia sensitivity. This supports the traditional consensus that normobaric and hypobaric hypoxia can be used interchangeably.[11] However, more recent findings of several studies indicate that normobaric and hypobaric hypoxia have different physiological effects.[12-16] Coppel *et al.* suggested that the traditional view might be based on the barometric pressure only showing effects for longer exposure times (> 3 hours).[13] This could explain why this review of acute hypoxia showed no effect of barometric pressure, as only 8% of our studies used exposure times of more than 3 hours, and only one of these tested the executive domain.

One limitation of this study was its reliance on characterizing the literature based on inspired oxygen fraction or altitude levels. This approach is probably hiding relatively large interindividual SpO_2 variability and therefore mixing within single studies participants with very different hypoxemic levels. This limitation is inherent to experiments where oxygen saturations are not individually controlled, which constituted the vast majority of the studies in this review. To address this limitation and provide a clearer understanding of the results, we expressed the variability in Table 1 within each study by providing measures such as standard deviation (SD), standard error of the mean (SEM), or 95% confidence interval (95% CI).

An associated limitation of this review may be the variation in the distribution and heterogeneity of SpO_2 levels between different tests or clusters, which were derived from a heterogeneity of different studies. This disparity in SpO_2 levels may account for the observed differences in sensitivity to hypoxia among these clusters. For instance, clusters with studies encompassing a wide range of hypoxic levels may have an increased likelihood of demonstrating higher sensitivity to hypoxia severity, whereas clusters with studies investigating a narrow range of hypoxic levels may have reduced chances of showing hypoxia sensitivity. Consequently, this effect could introduce bias in the analysis of the hypoxic-dose response.

A third limitation of this study is that the majority of included studies were not blinded. This lack of blinding may introduce bias, as participants and researchers being aware of the hypoxic or normoxic exposure could inadvertently influence cognitive performance assessments.

All these factors reflect variabilities and disparities between hypoxia studies, which limits the conclusions that can be reached from the literature, and the selection of the most sensitive and reliable tests of cognitive effects of acute hypoxia. This emphasizes the need for more standardisation of methodologies, to improve the comparability and generalizability of hypoxia experiments.

CONCLUSION

A large variety of cognitive tests were used in the literature to assess the effects of acute hypoxia on cognition in healthy adults. This huge methodological diversity is a major detriment to the investigation of the CNS effects of hypoxia. With these limitations, some suggestions can be made. The Stroop test as well as the clusters of sustained attention, auditory/verbal: delayed recognition, visual/spatial: delayed recognition, and evoked potential showed higher sensitivity than other tests and clusters. All clusters within the executive domain with more than 10 data points showed a clear level-response relationship or trend, with more frequent impairments at more severe levels of hypoxia. The data in our review showed no different physiological effect between hypobaric hypoxia and normobaric hypoxia. To further improve our understanding of the effects of acute hypoxia on cognition, standardization of exposure protocols and cognitive testing is crucial.

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CHAPTER V | ORAL FRUCTOSE INTAKE DOES NOT IMPROVE EXERCISE, VISUAL, OR COGNITIVE PERFORMANCE DURING ACUTE NORMOBARIC HYPOXIA IN HEALTHY HUMANS

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ABSTRACT

The ability to metabolize fructose to bypass the glucose pathway in near-anaerobic conditions appears to contribute to the extreme hypoxia tolerance of the naked-mole rats. Therefore, we hypothesized that exogenous fructose could improve endurance capacity and cognitive performance in humans exposed to hypoxia. In a randomized, double-blind, crossover study, 26 healthy adults (9 women, 17 men; 28.8 ± 8.1 (SD) years) ingested 75 g fructose, 82.5g glucose, or placebo during acute hypoxia exposure (13% oxygen in a normobaric hypoxia chamber, corresponding to oxygen partial pressure at altitude of ~3,800 m) on separate days. We measured exercise duration, heart rate, SpO_2, blood gasses, and perceived exertion during a 30-min incremental load test followed by Farnsworth-Munsell 100 Hue (FM-100) color vision testing and the unstable tracking task (UTT) to probe eye-hand coordination performance. Exercise duration in hypoxia was 21.13 ± 0.29 (SEM) min on fructose, 21.35 ± 0.29 min on glucose, and 21.35 ± 0.29 min on placebo ($p = 0.86$). Heart rate responses and perceived exertion did not differ between treatments. Total error score (TES) during the FM-100 was 47.1 ± 8.0 on fructose, 45.6 ± 7.6 on glucose and 53.3 ± 9.6 on placebo ($p = 0.35$) and root mean square error (RMSE) during the UTT was 15.1 ± 1.0, 15.1 ± 1.0 and 15.3 ± 0.9 ($p = 0.87$). We conclude that oral fructose intake in non-acclimatized healthy humans does not acutely improve exercise performance and cognitive performance during moderate hypoxia. Thus, hypoxia tolerance in naked mole-rats resulting from oxygen-conserving fructose utilization, cannot be easily reproduced in humans.

INTRODUCTION

African naked mole-rats are unique animals which can withstand pain, cancer and even survive up to 18 min of anoxia without complications.[1–3] Recent studies delineated the metabolic mechanism that explains how these mammals survive in habitats characterized by deep, crowded burrows with very low oxygen and high carbon dioxide levels.[1,4–6] One of the mechanisms enabling naked mole-rats to tolerate extreme hypoxia appears to be the ability to obtain energy from fructose during oxygen deprivation, bypassing the usual glucose pathway that requires oxygen. Under anaerobic conditions, glycolysis is blocked by feedback inhibition of phosphofructokinase via low pH, citrate, and allosteric binding of adenosine triphosphate. Fructose is metabolized through a pathway that bypasses the metabolic block at phosphofructokinase supporting viability. The metabolic rewiring of glycolysis avoids the usual lethal effects of oxygen deprivation, a mechanism that could be utilized to minimize hypoxic damage in human disease.[1,7] For example, such rewiring could be therapeutically exploited in patients with conditions resulting from systemic or organ-specific oxygen deprivation as in stroke and myocardial infarction.[8,9] Recent evidence suggests that hypoxia-inducible factor 1α (HIF-1α) impacts fructose metabolism in the context of cardiac pathologic stress-induced hypertrophic growth.[10] Therefore, we tested the hypothesis that exogenous fructose improves acute hypoxia tolerance in humans. Given the limited evidence regarding human fructose metabolism, we first assessed the systemic availability and metabolic response following oral fructose loading. Then, we determined whether fructose ingestion acutely improves endurance capacity and visual and cognitive performance in humans exposed to acute hypoxia in a randomized, double-blind, placebo-controlled, and crossover study.

METHODS

Participants

The study comprised two substudies. In the first substudy, we assessed systemic availability and metabolic effects of oral fructose. Because the main goal of this study was to determine to what extent oral fructose reaches the systemic circulation, the experiment was conducted in normoxic conditions. In the second substudy, we compared influences of placebo, fructose, and glucose ingestion on physical, visual and cognitive

performance in normobaric hypoxia. In both substudies, we included healthy persons following screening comprising a physical exam and routine laboratory testing. Men and women aged 18-45 years with a body mass index between 18 and 25 kg/m² were eligible. We excluded participants with fructose malabsorption determined by fructose malabsorption testing (Gastro+™Gastrolyzer®, Bedfont Scientific, UK). In substudy 2, additional inclusion criteria were being recreationally active defined as biking > 2 h or running > 1 h per week and no color blindness (24-plates Ishihara). We obtained written informed consent prior to the start of the study. Both substudies were approved by the North Rhine Medical Association (Ärztekammer Nordrhein) and prospectively registered at the German Clinical Trials Register (DRKS, registration numbers: DRKS00028599 and DRKS00028644). We conducted all procedures according to the Declaration of Helsinki. Study participants were compensated for their participation. We performed medical screening and all experiments at the German Aerospace Center (DLR) in Cologne, Germany.

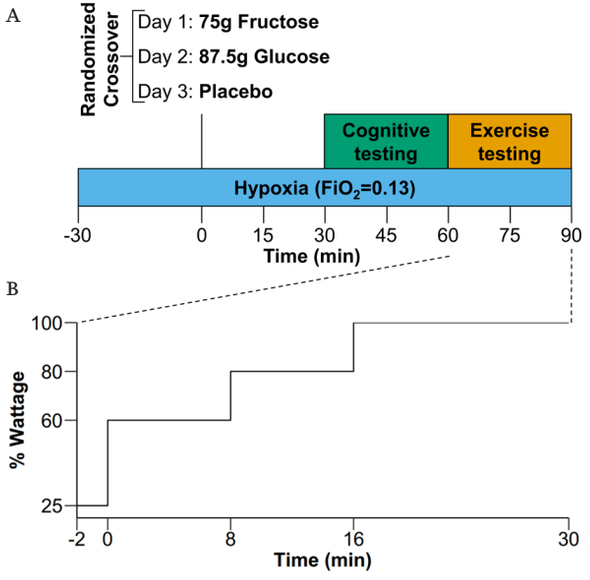
Substudy 1 – systemic fructose availability and metabolic actions

We included 8 healthy participants (4 women, 4 men; mean age ± SD: 25.5 ± 3.6 y). Participants abstained from intense physical activity for one day prior to the study day and maintained a food diary for three days prior to the study day. After a 10 h fast, they reported to the laboratory. A venous catheter was placed in the forearm and participants remained in a supine position for 10 min before being placed under the respiratory hood and initiation of the assessment. Following baseline measurements, participants ingested 75 g fructose dissolved in 300 mL water. We obtained venous blood for fructose, glucose, insulin, free fatty acids (FFA), and triglyceride measurements before and 15, 30, 45, 90, 120, 150, 180 min following fructose ingestion. Plasma fructose concentrations were determined using a manual colorimetric assay (Abnova). Glucose and triglyceride levels were assessed using the ADVIA XPT clinical chemical system (Siemens Healthineers, Eschborn, Germany). Serum insulin was determined using an immunoassay on an ADVIA Centaur XPT system (Siemens Healthineers, Eschborn, Germany). Plasma concentrations of total FFA were measured with an enzymatic method (WAKO Chemicals, Neuss, Germany) on the latter instrument. We assessed resting metabolic rate and substrate oxidation (using the Weir equation) through indirect calorimetry (Quark RMR, COSMED).[11] After a 30-min baseline, changes in substrate oxidation were assessed in three different intervals 5-45 min, 65-80 min and 95-115 min after fructose ingestion.

Substudy 2 – randomized, crossover comparison of placebo, glucose, and fructose in hypoxia

We included 26 healthy participants (9 women, 17 men; mean age ± SD: 28.8 ± 8.1 y). The substudy comprised a familiarization period followed by a randomized, double-blind, crossover comparison of placebo, glucose, and fructose in a normobaric hypoxia chamber (Figure 1A). In the familiarization period, participants practiced exercise, visual and cognitive tasks in normobaric hypoxia (FiO_2 = 13.0%, equivalent to a simulated altitude of 3,800 m) on two separate days.

FIGURE 1 **(A) Study design of the randomized, crossover comparison of effects of placebo, glucose, and fructose in hypoxia. Blue bar indicates the 2 h hypoxia exposure (FiO_2 = 0.13). Green panel shows the visual and cognitive test and orange panel the exercise testing. (B) Exercise protocol of the increasing load test in % of the estimated maximum exercise capacity using normative values for maximal workload (see Methods section).**



Six to nineteen days following the familiarization period, we randomized participants to one of six groups (fructose-glucose-placebo, fructose-placebo-glucose, glucose-fructose-placebo, glucose-placebo-fructose, placebo-fructose-glucose and placebo-glucose-fructose). Thus, we examined each participant on three separate study days with a three-day

recovery between visits under identical atmosphere but following ingestion of 75 g fructose (Euro OTC Pharma GmbH, Bönen, Germany), 82.5 g glucose (isocaloric to fructose) (Caelo, Hilden, Germany), or placebo (non-caloric, 200 mg saccharin / Buxtrade, Buxtehude, Germany) dissolved in 300 mL water.

One week before each study day, participants abstained from recreational drugs, caffeine, alcohol, and nicotine, which was verified by urine testing on study days. Subjects also abstained from intense physical activity for 24 h before study days. To limit influences of sleep deprivation and circadian misalignment on our measurements, participants maintained regular bedtimes and rise times (time in bed: 8 h) during the three days prior to each study day, confirmed with wrist-actigraphy (Actiwatch-L; Philips/Respironics). In each participant, all three study days commenced at the same time, but varied among participants between 8 AM and noon. Participants arrived after 10 h overnight fasting and entered the hypoxia module. We placed a venous catheter in the forearm and participants remained in a seated position before and between exercise and cognitive testing. After 30 min of hypoxia exposure participants ingested fructose, glucose, or placebo within 1 min. Thirty minutes later, participants performed the visual and cognitive tasks, which took approximately 30 min to complete and that were followed by the incremental load exercise test. Thus, exercise testing coincided with expected systemic fructose and glucose peak concentrations following oral ingestion. During the experiment in hypoxia, participants were limited to drink only water.

Normobaric hypoxia

We achieved hypoxia by nitrogen dilution through the air conditioning system in the atmospheric self-sustaining hypoxia chamber under normobaric conditions (1,013 hPa). Nitrogen was supplied by an external tank. Participants were exposed to normobaric hypoxia ($FiO_2 = 0.13$) for 2 h without pre-acclimatization. The normobaric hypoxia condition with an oxygen fraction of 13% corresponds to the partial oxygen pressure present at an altitude of approximately 3,800 m. We chose this altitude and exposure time so that the selected visual, cognitive and exercise tests were sensitive to measure a performance impairment, while simultaneously being performed safely and without symptoms of high mountain sickness developed.[12–15]

Color vision and cognitive testing

We tested color vision with the FarnsworthMunsell 100-Hue (FM-100) test (Luneau, Paris). Test illuminance, color temperature of the light source, procedure, and data processing adhered to the original Farnsworth instructions.[16] The test consists of 4 sub-sectors: sector 1 (caps 76–12), sector 2 (caps 13–33), sector 3 (caps 34–54), and sector 4 (caps 55–75). The total error score (TES, sum of scores for each four sectors), total number of errors in each sector and in the red-green (caps 13-33 and 55-75) and blue-yellow axis (caps 1–12, 34–54, and 76–85) were calculated.[17] The unstable tracking task (UTT) is an eye-hand coordination test, in which a horizontally and continuously moving cursor has to be centered within a marked target located in the middle of the screen by moving a lever to the left or right with the dominant hand. The task duration is 3 min according to the recommendation of the AGARD Handbook and was shown to be sensitive to the stressors. Performance was measured as a root mean square error (RMSE) of the distance of the cursor to the center.[18–20]

Exercise testing

Exercise testing was done on an ergometer bike (COSMED). We estimated participants' exercise capacity using normative values for maximal workload of the average population (in watts) normalized for age, sex, and body weight.[21] Our population was more athletic but performed exercise in hypoxia, so we tried three different protocols prior to the start of the study (40-60-80%, 50-60-90% and 60-80-100%, for 8-8-14 min, respectively). The latter proved to be the most suitable for our population. The exercise protocol consisted of a 2-min warm-up at 25% of the estimated maximal exercise capacity followed by a 30-min incremental load test with 8 min at 60%, 8 min at 80%, and up to 14 min at 100% of estimated maximal exercise capacity (Figure 1B). We instructed participants to maintain a cadence between 70 and 90 rpm and to exercise as long as tolerated in case they could not complete the 30-min test. We measured heart rate (Philips ECG IntelliVue MX40) and blood oxygenation (finger pulse oximeter) at 1-min intervals throughout the exercise test. Moreover, we assessed venous blood gasses (ABL90 FLEX analyzer, Radiometer Medicals ApS, Denmark) and perceived exertion using the BORG scale before and during the bike test at the end of each incremental step (the last measurement was taken when the test was stopped at voluntary exhaustion).

Study endpoints and sample size justification

Substudy 1 was an exploratory investigation with the primary goal to test whether oral fructose ingestion increases systemic fructose availability. We did not conduct a formal sample size estimation for this substudy. In substudy 2, the primary endpoint was the difference in exercise performance between fructose, glucose and placebo, measured as bike duration till voluntary exhaustion (min) and heart rate (bpm) during the increasing load exercise test. Secondary outcomes of the exercise tests were blood oxygen content, blood lactate concentrations and BORG score measured at the end of each incremental step. TES during the FM-100 and RMSE during the UTT were also considered secondary outcomes. A statistical power analysis (GPower 3.1 software) was performed for sample size estimation, assuming a moderate effect of fructose ingestion on bike duration and heart rate during an increasing load exercise test, according to Cohen (1988) with 0.296 effect size. We chose a more cautious effect size because there was no clear evidence as to whether humans benefit from exogenous fructose under hypoxic conditions. We used alpha = 0.05 and power of 0.80, applied in three groups (fructose, glucose and placebo) analyzing the results through an analysis of variance (ANOVA) with repeated measures and post-hoc comparison test for a comparative analysis within groups. The projected sample size needed with this effect size was approximately n = 20 for a crossover design.

Statistical analysis

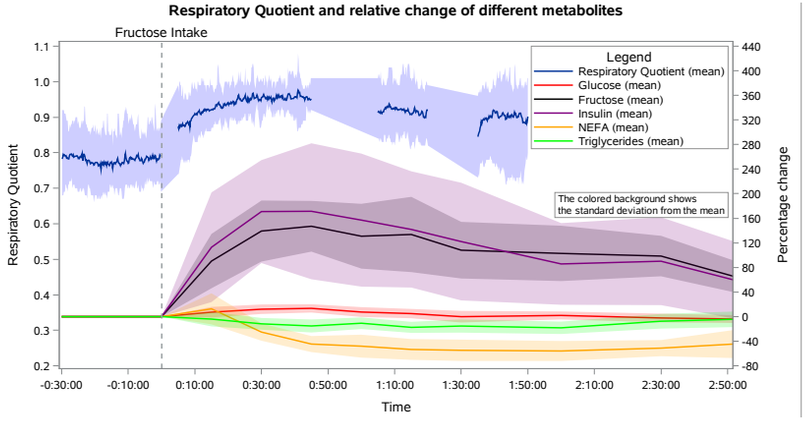
Statistical analysis was performed using RStudio software (version 2022.07.1). If not otherwise indicated, data of substudy 1 are reported as mean ± SD and substudy 2 mean ± SEM. Kaplan-Meier (KM) curve was used to analyze and compare the survival differences between the three treatment conditions during the increasing load test. The other outcomes were analyzed using a linear mixed model with time and condition as fixed factor, and participant as random factor.

RESULTS

Substudy 1 - systemic fructose availability and metabolic actions

Figure 2 illustrates the time course of fructose, glucose, insulin, non-esterified fatty acids, triglycerides and the respiratory quotient following fructose ingestion. Mean plasma fructose concentration was 61.4 ± 7.4 µg/mL at baseline and rapidly increased to 145.5 ± 27.9 µg/mL and 150.4 ± 23.2 µg/mL 30 and 45 min after fructose ingestion, respectively. Three hours after fructose ingestion, plasma fructose concentration was 93.0 ± 9.5 µg/mL. Insulin increased from 53.1 ± 22.2 pmol/L at baseline to 132.7 ± 46.2 pmoL/L at 30 min and 128.2 ± 44.4 pmol/L at 45 min after fructose ingestion. Three hours after fructose ingestion, insulin concentration was 71.7 ± 26.5 pmol/L. Triglycerides levels slightly decreased from 74.9 ± 35.6 mg/dL at baseline to 60.1 ± 27.6 mg/dL 120 min after fructose ingestion. Non-esterified fatty acids levels decreased from 697.9 ± 242.2 µmol/g at baseline to 297.4 ± 129.9 µmol/g 120 min after fructose ingestion. Glucose concentration was 88.5 ± 5.7 mg/dl at baseline and 100.2 ± 4.5 mg/dL 45 min after fructose ingestion. Mean fasting respiratory quotient was 0.79 ± 0.09 at baseline and increased to 0.96 ± 0.07 30 min and 0.96 ± 0.07 45 min after fructose ingestion consistent with a relative shift from fatty acid to carbohydrate oxidation.

FIGURE 2 **Relative time profiles of blood metabolites and respiratory quotient following 75 g oral fructose ingestion. Data represents means ± SD (n = 8)**



Substudy 2 – randomized, crossover comparison of placebo, glucose, and fructose in hypoxia

Four participants were excluded from the study due to missing data on one of the three visits (two participants developed a COVID infection and two vasovagal syncope related to multiple venipuncture attempts on visit one or two and we decided to discontinue the study). Two subjects

developed vasovagal syncope during the exercise test on their last visit and were only excluded for the exercise analysis. This resulted in 22 complete datasets for the cognitive performance analysis and 20 complete datasets for the exercise performance analysis.

None of the measurements of the FM-100 and UTT test showed significant differences between the three conditions (Figure 4). All participants completed 11 min of bike exercise before the first participants finished the test at voluntary exhaustion (after minute 3 at 80% of maximal workload). Four participants completed the entire 30-min period (Figure 3). We observed no differences between conditions regarding the time to exhaustion. At the start of the exercise test, 1.5 h into hypoxia exposure, heart rate was 89.7 ± 2.6 bpm on placebo, 87.4 ± 3.9 bpm on fructose, and 96 ± 3.4 bpm on glucose. At the end of the exercise test, heart rate was 159.8 ± 2.6 bpm on placebo, 162.8 ± 2.4 bpm on fructose, and 161.8 ± 3.1 bpm on glucose. At this time perceived exertion according to the BORG scale was 19.4 ± 0.3, 19.3 ± 0.2, and 19.4 ± 0.3 with placebo, fructose, and glucose, respectively (Figure 4). No significant differences between the three treatments were observed for heart rate, BORG scale and SpO_2. Lactate and pO_2 increased and pCO_2 decreased significantly during the exercise test but did not show significant differences between the three conditions (Figure 5).

FIGURE 3 Effect of oral fructose, glucose or placebo intake on color vision and cognitive performance: Mean (SEM) of (A) total error score (FM-100), (B) lapses and (C) tracking deviation (UTT) of the three treatment conditions.

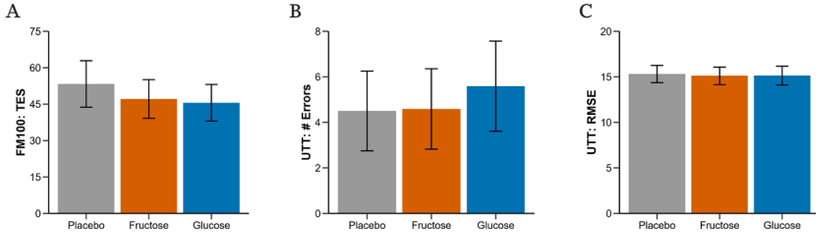


FIGURE 4 Kaplan-Meier plot of the duration of exercise to exhaustion in minutes during the exercise test in hypoxia.

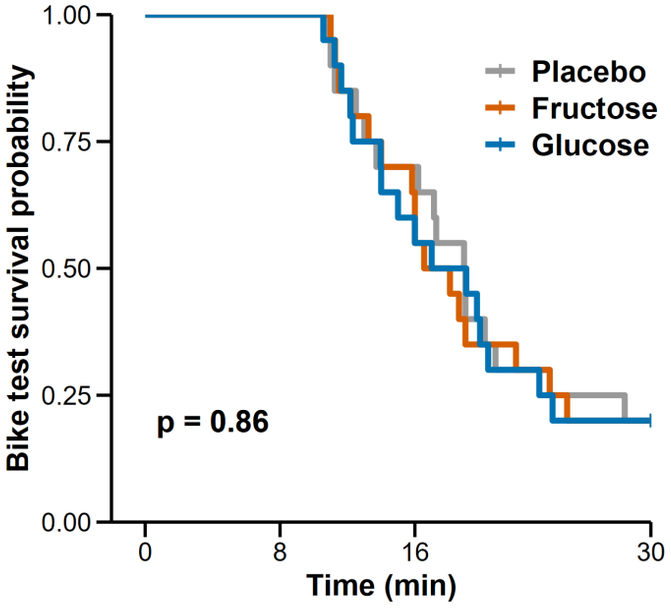
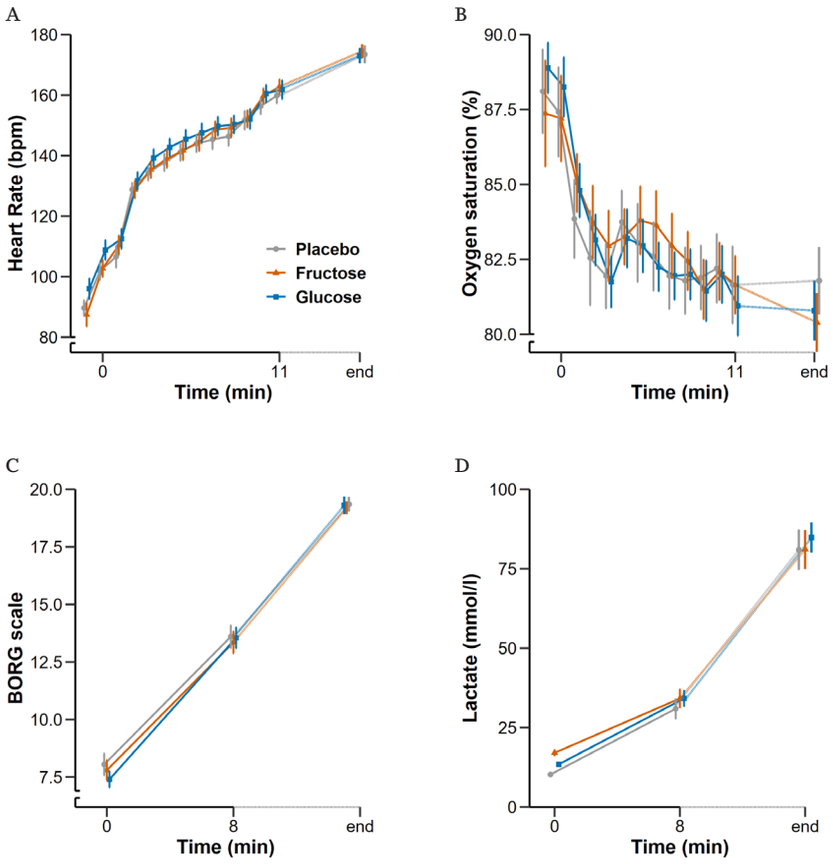


FIGURE 5 Effect of oral fructose, glucose or placebo intake on exercise performance during acute hypoxia: Mean (SEM) of (A) heart rate (bpm) and (B) oxygen saturation (%) in minute intervals from baseline to 11 min and at the end of the exercise test. (C) BORG scale and (D) blood lactate concentration (mmol/L) at baseline, 8 min and at the end of the exercise test.



DISCUSSION

We showed here that acute ingestion of fructose increases its systemic availability in healthy humans, but does not augment physical, visual or cognitive performance during exposure to moderate hypoxia. Indeed, time to exhaustion, perceived exertion, heart rate, oxygen saturation, and blood gasses during physical exercise in hypoxia were virtually identical following ingestion of fructose, glucose, or placebo. Similarly, fructose ingestion had no effect on color vision or cognitive performance in hypoxia. Thus, the remarkable hypoxia tolerance in naked mole-rats, which is achieved in part through metabolic rewiring toward reduced oxygen demand by fructose utilization, cannot be reproduced in humans through fructose administration.

While fructose utilization plays a significant role, the hypoxia tolerance of naked mole rats is not solely reliant on this mechanism. These animals possess a range of additional coping mechanisms that collectively contribute to their remarkable survival in low-oxygen environments. These mechanisms include efficient oxygen utilization due to their lower metabolic rate, enhanced oxygen binding capacity through high hemoglobin-oxygen affinity, tolerance to high carbon dioxide levels, resistance to acidosis facilitated by improved buffering systems and efficient acid removal, as well as antioxidant defenses protecting brain cells from oxidative stress and reducing metabolic demands.[1,22–24] Together, these adaptations highlight the complex and multifaceted nature of their hypoxia tolerance. These observations also suggest that in humans metabolic rewiring toward fructose metabolism may not suffice to enhance performance under hypoxic conditions.

We determined established hypoxia-sensitive responses in a randomized, double-blind, placebo-controlled, and crossover fashion, which is a strength of our study. Performance during endurance exercise testing decreases substantially in hypoxia with concomitant increases in perceived exertion and heart rate, and reductions in oxygen saturation. A systematic review highlighted ergolytics effect of acute hypoxia exposure on time to exertion during physical exercise in relation to hypoxia severity and test duration.[25] A level of 13% of oxygen exacerbates peripheral fatigue of limb locomotor muscles, which likely contributes to early termination of exercise.[26] In our study, most participants did not complete the full exercise protocol leaving room for potential improvements on treatment. We chose visual and cognitive tests known to be sensitive to 13% O_2.

Both tests are sensitive at an altitude of 3,000 m, reflected in an increase in total number of errors in the FM-100, and an increase in tracking deviations in the UTT.[12,13] Reduced oxygen saturation during exercise in normobaric hypoxia ($FiO_2 = 0.135$) contributes to exercise-induced cognitive fatigue.[27] To exclude a possible interaction effect of hypoxia and exercise we therefore scheduled visual and cognitive testing to occur prior to the exercise test.

Fructose is rapidly cleared by the intestines and liver and is catabolized for energetic purposes, converted into glucose, and stored as glycogen, or converted into fatty acids and stored as triglycerides.[28] Dietary fructose (75 g) acutely increases systemic serum fructose levels and elicits acute metabolic and endocrine responses in humans.[29] Thus, fructose at a dose applied in our study in addition to resulting in significant increases in systemic fructose availability elicited changes in insulin release and carbohydrate metabolism. In previous studies, changes in systemic glucose following fructose ingestion were rather small.[30] There may have been a small increase in glucose in our study. We speculate that the increase in circulating glucose may have been attenuated by increased glucose disposition as we studied relatively young and insulin sensitive persons. We selected a 75 g fructose dose for experiments under hypoxia because the dose is sufficient to increase systemic fructose availability (substudy 1), challenges the metabolic system substudy 1 and,[29] surpasses typical dietary intake, is well tolerated, and could be reasonably applied in subsequent interventions studies in healthy persons or patients. Because fructose concentrations and associated metabolic changes reached a peaked approximately 30 min after fructose ingestion, we tested cognitive and visual performance in hypoxia in this period.

Because performance could be affected by a placebo effect, we decided to use saccharin as a placebo to mimic the sweetness of glucose and fructose. It is known that artificial sweeteners, including saccharin, can impact brain activity.[31] However, no association has been found between the intake of artificial sweeteners in various forms and cognitive performance, as measured by an array of tests.[32] Furthermore, several studies suggest that when compared to saccharin, glucose or sucrose (glucose-fructose) elicit more pronounced brain responses.[33–35]

Compared with placebo and glucose ingestion, fructose ingestion was not associated with changes in hypoxia tolerance. Possibly, the negative findings of our study could be explained by fructose dosing, the level of hypoxia, or species differences between naked mole-rats and other

mammals including humans. While we showed in our pilot study that orally ingested fructose reaches the systemic circulation, first pass metabolism may nevertheless have limited peripheral fructose availability.[36,37] Moreover, the administration of a single fructose dose may not have allowed sufficient time and doses to observe a metabolic shift in humans. Furthermore, high fructose concentrations could lead to substrate inhibition of its transporters and enzymes, limiting fructose absorption, metabolism, and utilization.

The naked mole-rat has developed the ability to use fructose to fuel vital organs such as the brain and heart under near-anaerobic conditions. This metabolic rewiring supplies metabolically active organs with transporters and enzymes that are required to metabolize fructose to lactate. Fructose metabolism relies on ketohexokinase enzymes and GLUT-5 transporters, which in most mammals studied so far appear to be present exclusively in the liver and kidney.[38] GLUT-5 has been identified in smaller quantities in several adult human tissues, including the kidney, brain, muscle, and adipose tissue.[39,40] However, the specific physiological relevance of GLUT-5 in these tissues is currently unknown. In contrast, the naked mole-rat showed significant presence of these two key factors even in heart and brain tissue.[1]

Limitations

No dietary questionnaire was conducted to determine whether the recruited subjects were accustomed to eating more or less fructose. Moreover, we excluded participants with fructose malabsorption based on a 25 g fructose dose. In a dose-response study of fructose absorption during a breath test in healthy subjects, 10% of individuals tested positive for malabsorption with a 25 g dose of fructose, while 80% tested positive with a 50 g dose, which could have confounded our study.[41] Yet, we are confident that we exclude participants with more severe fructose malabsorption and we reached a substantial increase in systemic fructose availability with a 75 g fructose dose. Another potential limitation is that we conducted the study on systemic fructose availability following oral ingestion under normoxic conditions. We cannot exclude that hypoxia may have changed the response. Furthermore, in substudy 2 we estimated exercise capacity rather than determining it individually by VO_2 max measurement at baseline. The rational was that we attempted to limit the number of study visits during a peak period of the COVID pandemic. Moreover, although FM-100 and UTT are known to be sensitive

to hypoxia exposure, the tests may not suffice to detect subtle changes in visual or cognitive function. It is important to note that our study lacked baseline measurements in normoxia, which limits our ability to assess individual hypoxia sensitivity. Yet, acute fructose or glucose ingestion did not improve performance in normoxia.[42] Although the sensitivity of the cognitive and visual test is supported by the literature, there are differences in the protocol used in those studies. Consequently, we cannot be certain that our protocol produces changes in cognitive and visual performance. Thus, our findings cannot be simply extrapolated to other populations. Whether administering a single fructose dose is sufficient to improve cognitive and physical performance in hypoxia is uncertain. In terms of hypoxia tolerance, multiple fructose doses ingested over several days may be more effective. We dare to speculate that multiple fructose doses could induce metabolic adaptation necessary for fructose metabolism. However, potential improvements in hypoxia tolerance have to be weighed against adverse effects on cardiovascular and metabolic risk with long-term exposure to increased dietary fructose. To achieve better results, a longer period of acclimatization to hypoxia through HIF-dependent expression of transporters and enzymes, as well as to fructose, would likely be necessary.

CONCLUSIONS

Oral intake of a single 75 g fructose dose in non-acclimatized healthy humans compared to glucose and placebo does not improve visual, cognitive or exercise performance during moderate normobaric hypoxia. While oral fructose ingestion may be ineffective in improving hypoxia tolerance in humans, the metabolic pathways could nevertheless have physiological and clinical relevance. It is tempting to speculate that fructose-metabolizing pathways could be upregulated to ameliorate consequences of systemic or localized hypoxia. A switch to fructolysis under hypoxic stress has been associated with heart failure, metabolic syndrome and malignant cancer. This hints at a potential link between the metabolic adaptation of the naked mole-rat to chronic hypoxia and its resistance to cancer. It is therefore important to understand whether, in humans, chronic adaptation to hypoxia is required to induce metabolic changes necessary to benefit from fructose. Future studies in humans are needed to rule out this question.

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CHAPTER VI | RESETTING OF THE HUMAN CIRCADIAN MELATONIN RHYTHM BY AMBIENT HYPOXIA

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ABSTRACT

Circadian clocks in the body drive daily cycles in physiology and behavior. A master clock in the brain maintains synchrony with the environmental day-night cycle and uses internal signals to keep clocks in other tissues aligned. Work in cell cultures uncovered cyclic changes in tissue oxygenation that may serve to reset and synchronize circadian clocks. Here we show in healthy humans, following a randomized controlled single-blind counterbalanced crossover study design, that one-time exposure to moderate ambient hypoxia (FiO_2 = ~15%, normobaric) for ~6.5 h during the early night advances the dim-light onset of melatonin secretion by 9 min (95% CI: 1-16 min). Exposure to moderate hypoxia may thus be strong enough to entrain circadian clocks to a 24-h cycle in the absence of other entraining cues. Together, the results provide direct evidence for an interaction between the body's hypoxia-sensing pathway and circadian clocks. The finding offers a mechanism through which behaviors that change tissue oxygenation (e.g., exercise and fasting/eating) can affect circadian timing, and through which hypoxia-related diseases (e.g., obstructive sleep apnea, chronic obstructive pulmonary disease) can result in circadian misalignment and associated pathologies.

INTRODUCTION

The circadian timing system drives daily cycles in physiology and behavior of many organisms. In mammals, this system consists of a central "clock" located in the suprachiasmatic nucleus (SCN) of the hypothalamus and cell-autonomous oscillators in most other tissues, referred to as peripheral clocks.[1,2] The central circadian clock uses light as the main entraining cue to maintain synchrony with the environmental day-night cycle, and internal signals to uphold phase coherence of peripheral clocks with itself. Misalignment between the central circadian clock and daily behaviors like sleeping and eating – such as occurring in shift work – has been linked to detrimental effects on health and performance, including cardiometabolic disease, sleep disruption, depression, cognitive impairment, and accidents.[3–7] Such misalignment may also cause *internal* circadian misalignment, an adverse condition in which some peripheral clocks remain synchronized to the central clock whereas others entrain to competing time cues, e.g., the fasting/eating cycle.[7] Insight into the nature of internal resetting pathways is thus of great importance both clinically as well as from a basic circadian science perspective.

Several internal factors have been implicated in resetting peripheral clocks, including temperature, glucocorticoids, a cell's redox state, and tissue oxygenation, but whether or not these factors constitute a universal entraining mechanism is not yet clear.[8–10] Emerging evidence indicates extensive molecular and functional interactions between oxygen-sensing and circadian pathways.[11] Measurements in rodents revealed daily rhythms in blood and tissue oxygenation, and oxygen cycles within the physiological range were shown to synchronize circadian clocks in cell cultures in a hypoxia-inducible factor-1α (HIF-1α) -dependent manner. Furthermore, one-time exposure to moderate hypoxia was reported to accelerate adaptation of rodents' rest-activity rhythm following an advance of the light-dark cycle, suggesting that responsiveness to hypoxia is not limited to peripheral circadian clocks.[12]

It has long been suggested that the human circadian system may be responsive to hypoxia.[13] Although changes in the melatonin and core body temperature rhythms during and after exposure to hypoxia have been reported, phase shifting of human circadian clocks under controlled conditions, however, has not been demonstrated.[14–16] Here we present results from a study in healthy humans in which we compared the circadian timing of melatonin secretion before and after exposure to

moderate hypoxia and normoxia (control) under strictly controlled conditions (continuous dim light, constant posture, time-free environment). Given the suspected role of the transcription factor HIF-1 in circadian phase resetting,[11] we explored in the blood whether exposure to moderate hypoxia activates transcription of HIF-1A and/or several target genes of the HIF-1 protein.

MATERIALS AND METHODS

The protocol was approved by the ethics board of the North Rhine Medical Association (Ärztekammer Nordrhein; protocol number: 2020075), and registered at the German Clinical Trials Register (www.drks.de, registration number: DRKS00023387). Informed written consent was obtained from study participants prior to the start of the study. All procedures were conducted according to the Declaration of Helsinki (2013). Study participants were compensated for their participation. The study was carried out in the :envihab research facility (www.dlr.de/envihab/) of the German Aerospace Center (DLR) in Cologne, Germany, which features private rooms equipped for sleep recordings, around-the-clock blood sampling, and various atmospheric conditions including normobaric hypoxia.

Study Participants and Screening Procedures

Between September 2020 and July 2022, 198 interested volunteers entered a multi-step screening procedure, resulting in the selection of 22 healthy adults (12 females; mean age ± SD: 25.18 ± 2.68 years; range: 20-30 years). Study participants were of intermediate chronotype (Munich ChronoType Questionnaire, MCTQ; MSFsc = 3:00-5:00 h), and were in good health as established by Pittsburgh Sleep Quality Index, PSQI < 8, medical history, physical examination, routine blood and urine testing, and overnight polysomnography (apnea-hypopnea Index, AHI < 10, periodic leg movements in sleep, PLMS < 15). Participants reported no night shifts for three months prior to the study and no air travel across multiple time zones within the past month. Participants were asked to abstain from use of drugs, caffeine, alcohol and nicotine one week prior to each admission to the laboratory, which was verified by urine testing upon admission.

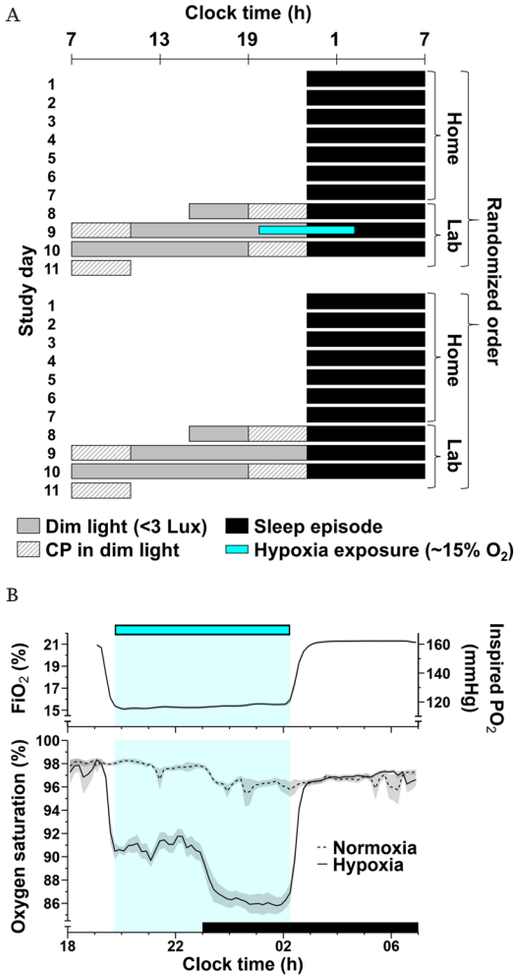
Study Protocol

We used a single-blind counterbalanced crossover design with two conditions, each of which was 11 days in duration and included a 4-day laboratory visit (Figures 1A and S1), scheduled in block-randomized order (blocks of 6-8). In the active condition participants were exposed to normobaric hypoxia (FiO_2 = ~15%) for ~6.5 h centered at 23:00 h on day 9, whereas no such exposure occurred in the control condition (normobaric normoxia, FiO_2 = 21%). During the first seven days at home as well as during the laboratory visit of each condition participants followed a fixed sleep schedule (23:00-7:00 h). Prior to the laboratory visits wrist actigraphy (Actiwatch-L; Philips/Respironics) and daily call-ins into a voicemail system were used to check compliance to the sleep instructions. The 4-day laboratory visits were 10 to 11 days apart. During the visits, participants remained in private rooms in dim light during scheduled wakefulness and in complete darkness during scheduled sleep episodes (Figure 1A). LED-based ceiling lights covered with dark window film were the only light source in the rooms (< 3 lux, < 0.006 W/m², < 0.9 melanopic IDE[17] in the angle of gaze, color temperature 2900-2990 K; measurements obtained at study start (spectrometer: BLACK-Comet-CXR-SR-50, StellarNet Inc.), illuminance checked again at study end). Room temperature was maintained at ~22.2°C. Participants were monitored by cameras inside their rooms to allow staff to enter and if needed prevent them from falling asleep outside the scheduled sleep episodes. Participants strictly adhered to meal times at 8:00, 12:00, 16:30, and 20:30 h (snack) and had 30 min to consume the meals. The meals were of mixed content; the macronutrient content and caloric intake were not controlled. Participants had no access to television, video, and electronic devices, and remained uninformed about the time of day.

Atmospheric conditions

Hypoxia was achieved by nitrogen dilution through the air handling system in the atmospheric self-sustained hypoxia chamber under normobaric conditions (~760 mmHg). Nitrogen was supplied by an external tank, resulting in an oxygen fraction of ~15% and an oxygen partial pressure (pO_2) of ~115 mm Hg. Oxygen availability thus approximated the maximum cabin altitude (8000 ft or 2438 m) as allowed during normal operation of an airliner. The target oxygen concentration was reached 30 min after the start of nitrogen dilution (Figure 2A). We measured blood oxygenation (SpO_2) levels continuously using a finger pulse oximeter (OEM Oximetry module, Nonin), starting on day 9 at 18:00 h (n = 14) or 23:00 h (unintended late start of recording, n = 8), and ending on day 10 at 7:00 h.

FIGURE 1 Representation of the study design, atmospheric conditions, and participants' blood oxygenation. (A) Raster plot of the study protocol. The protocol consisted of two conditions that were 11 days long and each included a 4-day laboratory visit during which participants were exposed either to normobaric hypoxia or normoxia (control). Black bars indicate the 23:00–07:00 h scheduled sleep episode in darkness. Gray bars denote dim room light (< 3 lux in the angle of gaze; see *Materials and Methods*). Striped bars show the constant posture (CP) procedures during wakefulness in dim room light. The blue bar denotes the scheduled hypoxia exposure (19:45–02:15 h). Participants were randomized to the order of atmospheric condition (crossover, counterbalanced, single-blind). (B) Atmospheric conditioning and blood oxygen saturation levels during hypoxia exposure and control. (*Upper panel*) Fraction of inspired oxygen and corresponding oxygen partial pressure during 6.5 h of normobaric hypoxia exposure (blue bar and shaded area). (*Lower panel*) Mean (± SEM) blood oxygen saturation (SpO_2) profiles during hypoxia exposure and control. The black bar denotes the scheduled sleep episode. n = 14 due to accidental late start of the SpO_2 recordings in 8 participants.



Constant Posture Procedures

Constant Posture (CP) procedures occurred before (beginning on day 8), and after (beginning on day 10) the treatment night of each visit, for the assessment of circadian phase of the melatonin rhythm (Figure 1A). Participants remained for a 16-h period in bed in a semi-recumbent posture with minimal activity for 4 h before and 4 h after the 8-h sleep episode, and were required to sleep in the same position. Room temperature and dim light conditions remained constant during the CP except for the sleep episodes that took place in complete darkness.

Plasma melatonin

Hourly blood samples were collected during CPs via an indwelling forearm intravenous catheter before and after treatment nights for measurement of plasma melatonin levels. During the sleep episode, samples were taken from outside the rooms through a port hole without disturbing the participants' sleep. Samples were collected and then frozen (–80°C) for subsequent assay. Plasma melatonin samples were assayed (Chrono@Work, Groningen, the Netherlands) using liquid chromatography in combination with mass spectrometry (LCMS/MS), which has a functional sensitivity of 2.3 pg/mL and an analytical sensitivity of 1.9 pg/mL, an intraassay precision of 3.5–8.9% for low to high concentrations, and interassay precision of 4.1–9.5%.

Circadian phase assessment

The dim light melatonin onset (DLMO) served as marker of central clock timing. Circadian phase of $DLMO_{25\%}$ was calculated as the time at which levels of melatonin rose above 25% of the peak-to-trough amplitude during the 17-h sampling interval: $DLMO_{25\%}$ = (melatonin amplitude - daytime melatonin level) x 25/100 + daytime melatonin level.[18] The melatonin amplitude was calculated as the difference between the maximum and the daytime melatonin levels. To avoid inflation by a local maximum, the median of the three highest melatonin values during the 17-h interval was chosen as the maximum melatonin value. To determine the daytime melatonin level, the median of the three lowest melatonin values was chosen. A linear interpolation between the melatonin values just below and just above the 25% threshold was used to identify the time of $DLMO_{25\%}$. First, phase shifts were calculated as the difference in the time of $DLMO_{25\%}$ before (pre) and after (post) treatment (hypoxia or control).

A potential phase shift due to hypoxia exposure (primary outcome) was assessed by subtracting in each individual the phase shift in the control condition (corresponding to the circadian drift) from the phase shift in the hypoxia condition. One participant was excluded from analysis of DLMO timing due to an inability to draw blood during a period of one of the CP procedures. Therefore, hypoxia-induced phase shifting was determined in 21 participants.

In addition to using $DLMO_{25\%}$, we examined a potential phase shifting effect of hypoxia on the basis of $DLMO_{10}$, i.e. the time at which plasma melatonin crosses the absolute threshold of 10 pg/mL.[19,20]

RNA isolation and real-time PCR of HIF, HIF target genes and clock genes

Transcription levels of HIF-1α and HIF-2α (*HIF1A* and *HIF2A*), HIF target genes (glucose transporter type 1 *SLC2A1*, vascular endothelial growth factor *VEGF*, adrenomedullin *ADM*, pyruvate dehydrogenase kinase 1 *PDK1*, prolyl hydroxylases 1, 2, and 3 *PHD1*, *PHD2* and *PHD3*), and clock genes (aryl hydrocarbon receptor nuclear translocator-like *ARNTL*, circadian locomotor output cycles kaput *CLOCK*, cryptochrome circadian regulator 1 and 2 *CRY1* and *CRY2*, albumin gene D-site binding protein (*DBP*), nuclear receptor subfamily 1, group D, member 1 *NR1D1*, period circadian regulator 1, 2, and 3 *PER1*, *PER2*, and *PER3*, and retinoic acid-related orphan receptor A *RORA*) were assessed once before and twice during exposure to hypoxia and normoxia (i.e., 3.5 h and 6.25 h after beginning of treatment). Total RNA from whole blood was directly stabilized using PAXgene Blood RNA Tubes (PreAnalytiX GmbH) and then frozen (-20°C). RNA was isolated according to the PAXgene® Blood RNA Kit (PreAnalytiX GmbH) and reverse-transcribed using M-MLV reverse Transcriptase (Promega GmbH, Walldorf, Germany). Real-time polymerase chain reaction (RT-PCR) was performed with the Biozym Blue S'Green qPCR-Kit (Biozym Scientific GmbH, Oldendorf, Germany) on a Bio-Rad's CFX96™ real-time system (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). We reverse-transcribed 200 ng of total RNA into *c*DNA, which was amplified with gene-specific primers (Table S1) and normalized to *ACTB* (ß-actin). Primer specificity was checked by Primer-BLAST and confirmed by size analysis of the PCR amplicons.[21] Expression was calculated with the $2^{-\Delta CT}$ method for statistical analysis. A list of the primer sequences used for qRT-PCR analysis is provided in Table S1.

Statistical analysis

We hypothesized that the pre-post difference in DLMO timing (primary outcome) would differ between hypoxia and control. Accordingly, sample size was estimated for the primary outcome. Given the absence of previous data, a two-stage approach was employed. After stage one, an interim analysis of $DLMO_{25\%}$ was conducted with the data from the first 7 participants (3 females; one additional participant was excluded due to incomplete data during one of the CPs). Conditional power considerations based on a two-sided t-test with alpha of 0.05 and a mean of the DLMO-difference (hypoxia-normoxia) of 8.2 min with SD of 13.9 min achieved a power above 80% for a total of 21 participants. The second stage of the study involved an additional 14 participants (8 females). The overall results of both stages, before and after the interim analysis, were combined using the inverse normal p-value combination method.[22] The study was not intended to or had any specific hypotheses to test for potential effects of sex on the primary outcome and thus was not powered accordingly.

The RT-PCR sets were analyzed for Gaussian distribution using the D'Agostino–Pearson, Anderson-Darling and Shapiro-Wilk test. Gaussian distributed data were analyzed via mixed-effects analysis for repeated measurements. Non-Gaussian distributed data sets were either transformed to Gaussian distributed data and analyzed accordingly or analyzed via Friedman test for repeated measurements. Statistical analyses were performed using the R language and environment for statistical computing (version 2022.07.1) and GraphPad Prism® version 8.0.2 from GraphPad Software, Inc.. P values < 0.05 were considered statistically significant.[23]

RESULTS

Ambient hypoxia lowers blood oxygen saturation, particularly during sleep

Exposure to normobaric hypoxia (Figure 1B) lowered SpO_2 during the interval of scheduled wakefulness (19:45-23:00 h) to an average of 90.9% (95% CI: 90.6-91.1%), and during the interval that overlapped with the sleep episode (23:00-02:15 h) to 86.5% (86.1-86.9%). In total, participants spent 247 min (231-263 min) below 90% SpO_2, and 49 min (38-60 min) below 85%. Exposure to normoxia resulted in an average SpO_2 of 97.8% (97.7-97.9%) during scheduled wakefulness, and 96.3% (96.1-96.5%) during the interval that overlapped with the sleep episode. In total, participants spent 5 min (1-8 min) below 90% SpO_2, and 2 min (0-4 min) below 85%.

Early-night exposure to hypoxia advances the timing of dim light melatonin onset

The time course of mean plasma melatonin levels on the days before and after hypoxia exposure and on corresponding days in the control condition (normoxia) are illustrated in Figure 2. In the control condition, the timing of the $DLMO_{25\%}$ was delayed on average by 24 min (9-38 min, $p = 0.009$) between pre- and post-exposure (Figure 2C), reflecting the intrinsic period-dependent phase drift in dim light. In contrast, no significant delay of $DLMO_{25\%}$ was observed after hypoxia exposure (15 min, 0-30 min, $p = 0.062$, Figure 2D). Linear regression of individual post-exposure $DLMO_{25\%}$ time with pre-exposure $DLMO_{25\%}$ time indicated a phase advance due to hypoxia as illustrated by a decrease in the y-intercept in the hypoxia condition compared to control (Figure 3A). In order to correct for each individual's circadian phase drift in dim light, the pre-post difference in the timing of $DLMO_{25\%}$ in the control condition was subtracted from the pre-post difference in the timing of $DLMO_{25\%}$ in the hypoxia condition. The result revealed an average phase advance of 9 min (1-16 min, $p = 0.036$) due to hypoxia exposure (Figure 3B). Sixteen out of 21 participants (76%) showed a phase advance attributed to hypoxia exposure. Similar results were obtained for $DLMO_{10}$: in this case the mean hypoxia induced phase advance was 11 min (1-21 min, $p = 0.037$).

The magnitude of phase shifting did not correlate with the individual circadian phase of hypoxia exposure (Figure 3B; Spearman $r = -0.21$, $p = 0.36$). Due to the study design, however, the range of individual phases of exposure was quite narrow (mean difference between estimated $DLMO_{25\%}$ and midpoint of hypoxia exposure: 1.98 h, 95% CI: 1.59-2.38 h).

Hypoxia exposure did not induce a significant phase shift of $DLMOff_{25\%}$ (4 min, -14-21 min, $p > 0.4$). Moreover, neither the duration of the nocturnal melatonin profile ($p > 0.3$) nor its amplitude ($p > 0.5$) was affected by exposure to hypoxia. Table 1 provides an overview of the timing of $DLMO_{25\%}$ and $DLMOff_{25}$%, as well as the duration and amplitude observed during each CP.

We explored whether the duration of an individual's hypoxemia (i.e., the total time during which SpO_2 fell below 90% or below 85%) was a predictor of the magnitude of the hypoxia induced phase shift of $DLMO_{25\%}$. However, in neither case was there a significant correlation (below 90%: Spearman $r = -0.20$, $p = 0.37$; below 85%: $r = -0.21$, $p = 0.35$).

FIGURE 2 Average plasma melatonin profiles and individual dim light melatonin onset (DLMO) times during nights before and after hypoxia exposure and control. Data in (A) and (B) show mean (± SEM) plasma melatonin levels in the control (n = 22) and hypoxia (n = 21) condition, respectively. Time series were split and aligned relative to mean times of $DLMO_{25\%}$ and dim light melatonin offset ($DLMOff_{25\%}$). The black bars indicates the scheduled sleep episodes. The blue bar denotes the scheduled hypoxia exposure (19:45–02:15 h) during the night in between melatonin measurements. Data in (C) and (D) show individual time points of the $DLMO_{25\%}$ and group averages (95% CI) in the two conditions. *p* Value indicates significant drift of $DLMO_{25\%}$ between pre- and post-normoxia exposure (*t* test).

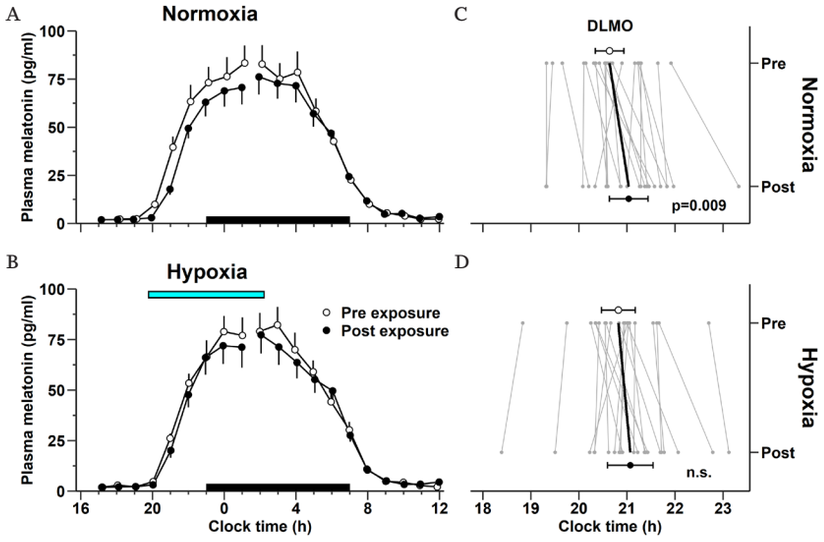
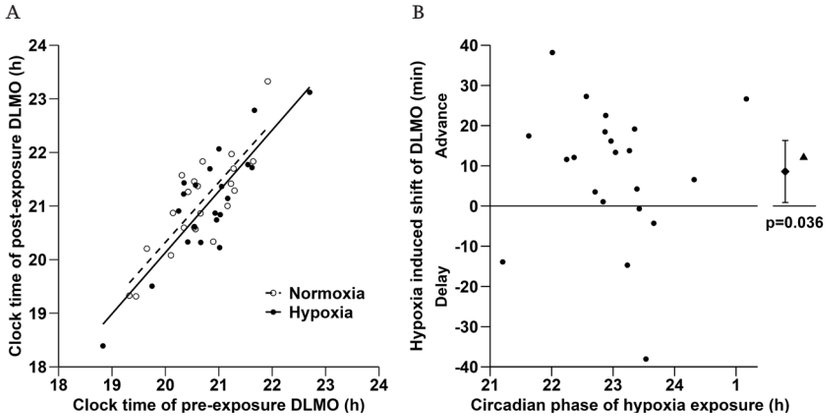


TABLE 1 Timing, duration, and amplitude of the plasma melatonin profiles before and after exposure to hypoxia and normoxia.

	Pre-normoxia	Post-normoxia	Pre-hypoxia	Post-hypoxia
$DLMO_{25\%}$ (h:min)	20:41	21:05*	20:49	21:04#
	(20:23-20:59)	(20:40-21:29)	(20:28-21:10)	(20:36-21:33)
$DLMOff_{25\%}$ (h:min)	07:06	07:16	07:14	07:21
	(06:38-07:33)	(06:48-07:44)	(06:48-07:40)	(06:47-07:55)
Duration (h)	10.41	10.19	10.42	10.28
	(10.25-10.58)	(10.14-10.24)	(10.34-10.50)	(10.19-10.37)
Amplitude (pg/ml)	85.4	76.7	81.1	78.7
	(64.8-106.1)	(58.9-94.5)	(63.5-98.8)	(59.7-97.6)

*Note: The times for dim light melatonin onset ($DLMO_{25\%}$) and dim light melatonin offset ($DLMOff_{25\%}$) refer to clock time. Duration is the difference between the times of $DLMO_{25\%}$ and $DLMOff_{25\%}$. Values are expressed as means (n=21) with 95% CI shown in parentheses. For $DLMOff_{25\%}$, duration, and amplitude no pre–post difference, or difference of pre–post difference between hypoxia and normoxia conditions was observed. *$p < 0.05$ for pre–post difference (t test). #$p < 0.05$ for difference of pre–post difference between hypoxia and normoxia conditions (t test).*

FIGURE 3 Resetting of individual dim light melatonin onset (DLMO) by ambient hypoxia. (A) Post-exposure $DLMO_{25\%}$ times are plotted against pre-exposure $DLMO_{25\%}$ times for the hypoxia and control condition (n = 21). A decrease of the y-intercept of the regression line illustrates an overall advance of DLMO after hypoxia exposure compared to control. (B) Individual phase shifts due to hypoxia (filled dots) are plotted against the circadian phase of the midpoint of the hypoxia interval, with 21 h defined as the time of estimated DLMO during the exposure night. Phase shifts were corrected for each individual's circadian drift during dim light control in normoxia (see Figure 2C). Mean phase shift with 95% CI (filled diamond) and median (filled triangle) are shown on the right (n=21); *p* value indicates significant difference from 0 (*t* test).



No changes in HIF and HIF-dependent gene transcription in leukocytes during hypoxia exposure

In whole blood, mRNA levels of HIFs (HIF1A and HIF2A), HIF target genes (SLC2A1, VEGF, ADM, PDK1, PHD1, PHD2 and PHD3) and clock genes (ARNTL, CLOCK, CRY1, CRY2, DBP, NR1D1, PER1, PER2, PER3 and RORA) were not different during hypoxia and normoxia compared to the pre-exposure measurement, or between the two conditions (Figures S2 and S3).

DISCUSSION

We found that exposure of healthy adults to a 6.5-h interval of normobaric hypoxia early in the night advanced $DLMO_{25\%}$ by 9 min and $DLMO_{10}$ by 11 min. To our knowledge, this is the first controlled study demonstrating that hypoxia may act as a zeitgeber of the central circadian clock in humans. A phase advance of 9 min may seem small but, remarkably, it matches the extent to which the intrinsic circadian period of healthy adults (24 h 9 min; females: 24 h 5 min, males: 24 h 11 min)[24] deviates from 24 h, when measured in an environment with minimal confounding influences. Thus, exposure to moderate hypoxia early in the night may be strong enough to entrain circadian clocks to a 24-h cycle in the absence of other time cues.

Despite the fact that hypoxia induced a phase advance of DLMO while the duration of the melatonin profile did not differ from the control condition, we did not find a significant advance of DLMOff. This discrepancy can be explained by the larger interindividual variance of DLMOff compared to DLMO due the contribution of other, likely non-circadian factors, including differences in melatonin's clearance from the blood. In general, DLMO is a more reliable marker of central clock timing than DLMOff[25] and considered the "gold standard" in circadian phase-assessment in humans. In contrast to a previous report,[15] we did not find a decrease in the melatonin amplitude after hypoxia exposure. The former observation likely represents an acute after-effect of hypoxia – possibly due to sympathetic activation – and not a circadian effect, given that hypoxia exposure ended only ~6 h prior to melatonin secretion, whereas in the current study it did so ~22 h prior to the onset of secretion.

The hypoxia exposure in the current study is considered moderate. However, one should keep in mind that the co-occurrence with sleep – likely due to the associated change in breathing pattern – resulted in considerable oxygen desaturation similar to previous findings,[26] such that participants spent > 4 h below the clinical hypoxia threshold of 90% SpO_2 and even ~50 min below 85%. Such desaturation is comparable to observations in patients with obstructive sleep apnea (OSA).[27] This disorder is associated with disrupted respiration, fragmented sleep and cardiometabolic dysregulation. Internal circadian misalignment due to intermittent hypoxia has been implicated as a contributing factor of OSA sequelae in a recent study.[28] While that study used a mouse model and exposure to severe hypoxia (6%), our data provide direct human evidence that even moderate hypoxemia may interfere with circadian time keeping. Consistent with this finding, a recent exploratory analysis observed internal misalignment between the circadian rhythms of blood pressure and melatonin in OSA patients.[29] Apart from OSA, our results are relevant for chronic lung diseases, since circadian clock disruption appears to play an important role in those pathologies as well.[30]

We propose that changes in tissue oxygenation may serve as a mediator of various non-photic phase-shifting stimuli:

1 Exercise is known to shift circadian rhythms in humans[31,32] and to affect oxygenation of various tissues including the brain. Cerebral oxygenation showed a quadratic response to incremental exercise, increasing with moderate intensities and decreasing at high intensity.[33] In mice, strenuous exercise induced clock- and HIF-1α target genes and this response also depended on time of day.[34]

2 Eating at night can shift peripheral circadian rhythms and even uncouple them from central clock control.[7] Eating/nutrient processing is associated with changes in tissue-specific blood flow and oxygen consumption that in addition to the rest-activity rhythm likely contribute to the observed daily rhythms in tissue oxygenation. Conversely, oxygen cycles within the physiological range can synchronize cellular clocks in a HIF-1α-dependent manner.[12] It should be noted, however, that recent work in mice also suggested a role for carbon dioxide in feeding-mediated clock resetting.[35]

3 Exposure to high altitude, which is associated with reduced oxygen partial pressure in the air, alters time-of-day dependent variations in the human blood transcriptome.[36] Moreover, changes in daily physiological rhythms were observed in experiments in an altitude chamber.[37] Remarkably, human chronotype, which has been linked to the length of an individual's intrinsic circadian period,[24] was reported to vary between groups living at different altitudes.[38] Thus, it is possible that apart from acute effects, there may be long-term adaptive/genetic effects of hypoxia exposure on the human circadian system.

4 Trans-meridian flights cause circadian misalignment, which is associated with sleep disruption, fatigue, mood disturbance, and gastrointestinal symptoms, collectively referred to as jetlag. The present study indicates that the level of hypoxia typically experienced in an airliner at cruising altitude may play a role in jetlag. Accelerated adaptation to an advance of the light-dark cycle – corresponding to an eastbound flight – was reported after hypoxia exposure in a mouse model of jetlag.[12]

The hypoxia-induced phase advance observed in the present study did not appear to be mediated by changes at the level of HIF-1α gene transcription. The HIF pathway is known to be activated at the protein level through stabilization of HIF-1α under conditions of reduced oxygen availability.[39] HIF-1, which is composed of the subunits HIF-1α and HIF-1β, regulates the expression of genes including clock genes. Currently, there is no

reliable method established to measure HIF-1α protein in human peripheral leukocytes. Our approach was therefore to quantify transcription of HIF-1 target genes. Since we did not find hypoxia-induced changes in gene transcription, we conclude that the oxygen partial pressure in the blood was not low enough to elicit significant HIF-1 activation in the majority of circulating leukocytes. However, oxygen partial pressure varies considerably across tissues even under normoxia;[40] it has been reported to be lower in brain tissue – particularly in deeper layers – than in venous blood,[41,42] making activation of HIF-1 following a decrease in the inspiratory oxygen partial pressure more likely in the former than in the latter. Moreover, in mice, nuclear HIF-1α accumulation following hypoxia exposure differed between tissues and in some cases was scant, suggesting the involvement of other transcription factors in the response to hypoxia.[28]

Limitations of the study

In order to maximize statistical power, we chose to administer hypoxia only at one time of day in all participants. Thus, we were not able to examine possible time-of-day dependent (and ultimately circadian-phase dependent) effects of hypoxia. An exploratory analysis in which the magnitude of the hypoxia-induced phase shift was expressed relative to the time of each individual's initial DLMO did not reveal a significant relationship – possibly due to the limited interindividual variation in initial DLMO time. It remains to be investigated whether hypoxia exposure can induce phase delays, and ultimately whether its effect on the circadian system can be adequately described by a phase-response curve – similar to established entraining cues like light or melatonin. In the absence of a demonstrated phase delay, it cannot be excluded that hypoxia induces a general shortening of the intrinsic circadian period, similar e.g., to the action of small molecule inhibitors of glycogen synthase kinase 3 (GSK-3).[43] Notably, inhibition or depletion of GSK-3 can lead to HIF-1α induction, while GSK-3β overexpression reduces HIF-1α levels.[44] These findings hint at a promising link between the modulation of GSK-3 activity, the regulation of circadian rhythms, and hypoxia. Finally, whereas our study focused on melatonin as a marker of the central clock, and although central and peripheral clocks appear to share identical core molecular clockworks,[2] it needs to be established whether hypoxia exposure can reset peripheral clocks in humans, as reflected e.g., in glucose and insulin rhythms during a constant routine protocol.[7]

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SUPPLEMENTARY MATERIALS

FIGURE S1 Flow diagram of the study.

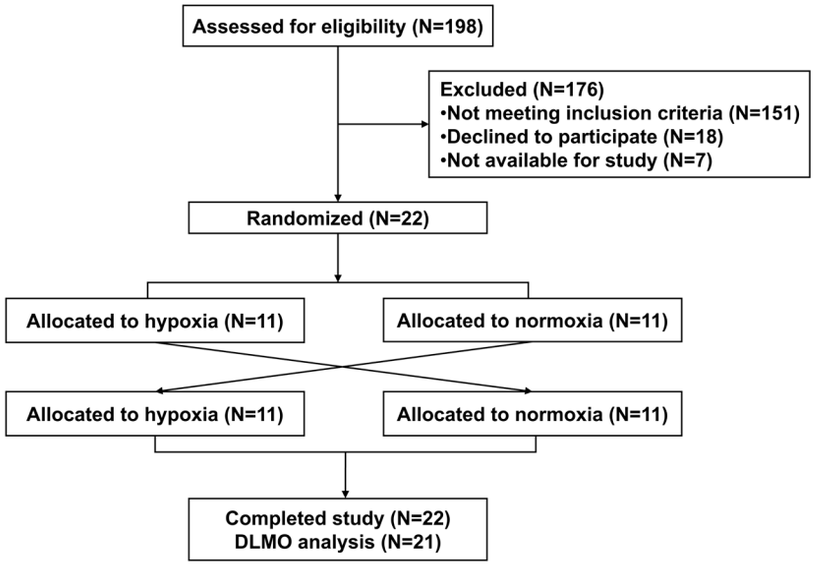


FIGURE S2 Expression of HIF1A, HIF2A and HIF target genes in leukocytes collected from participants before and during exposure to 6.5 hours of normobaric hypoxia ($FiO_2 = 15\%$) and normoxia ($FiO_2 = 21\%$). mRNA expression was analyzed for genes encoding for hypoxia-inducible factor 1 alpha (HIF1A), hypoxia-inducible factor 2 alpha (HIF2A), and typical HIF target genes including glucose transporter type 1 (SLC2A1), vascular endothelial growth factor (VEGF), adrenomedullin (ADM), pyruvate dehydrogenase kinase 1 (PDK1), and the prolyl hydroxylase domain-containing proteins 1, 2 and 3 (PHD1, PHD2, PHD3). Expression levels of genes were normalized to beta-actin (ACTB) and are presented as $2^{-\Delta CT}$ values (mean ± SD, n = 22). Gene expression was not different during hypoxia and normoxia compared to the pre-exposure measurement, or between the two conditions (mixed-effects analysis for repeated measures or Friedman test).

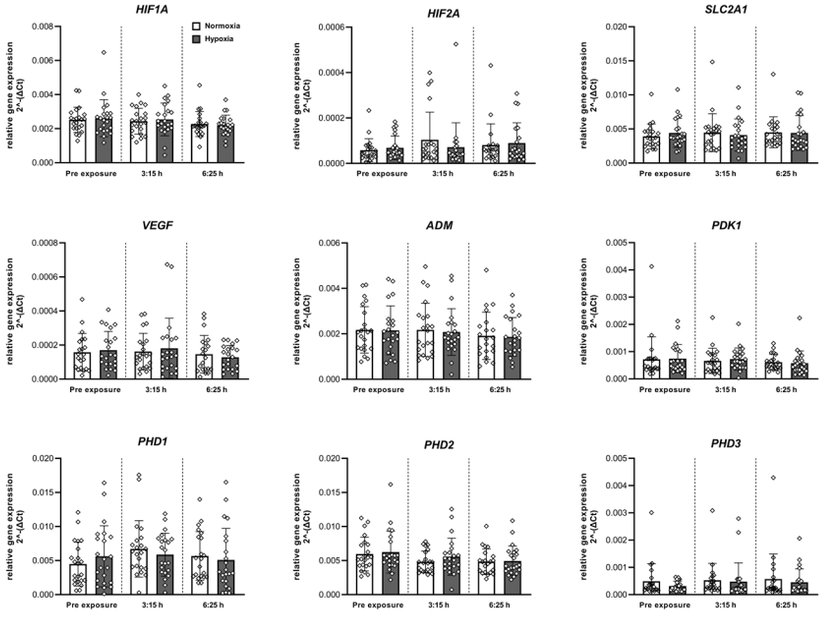


FIGURE S3 Expression of clock genes in leukocytes collected from participants before and during exposure to 6.5 hours of normobaric hypoxia (FiO_2 = 15%) and normoxia (FiO_2 = 21%). mRNA expression was analyzed for genes encoding for Aryl Hydrocarbon Receptor Nuclear Translocator-Like (ARNTL), Circadian Locomotor Output Cycles Kaput (CLOCK), Cryptochrome Circadian Regulator 1 and 2 (CRY1, CRY2), albumin gene D-site Binding Protein (DBP), Nuclear Receptor subfamily 1, group D, member 1 (NR1D1), Period Circadian Regulator 1, 2, and 3 (PER1, PER2, PER3), RAR Related Orphan Receptor A (RORA). Expression levels of genes were normalized to beta-actin (ACTB) and are presented as $2^{-\Delta CT}$ values (mean ± SD, n = 22). Gene expression was not different during hypoxia and normoxia compared to the pre-exposure measurement, or between the two conditions (mixed-effects analysis for repeated measures or Friedman test).

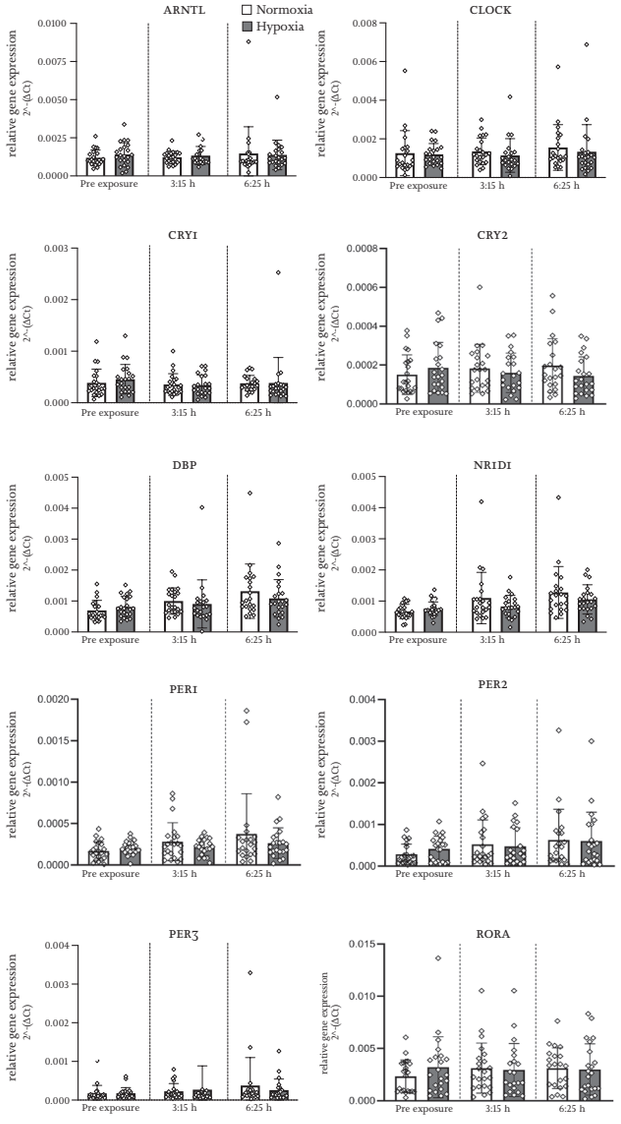


TABLE S1 Primer sequences of specific PCR products used for RNA quantification of human whole blood cells.

Gene	Primer	Sequence
ACTB	forward	CAGCGGAACCGCTCATTGCCAATGG
	reverse	TCACCCACACTGTGCCCATCTACGA
ADM	forward	AGTCGTGGGAAGAGGGAACT
	reverse	ATCCGGACTGCTGTCTTCGG
ARNTL	forward	GTGGTGCTGGCTAGAGTGTA
	reverse	TTTCAGGCGGTCAGCTTCTT
CLOCK	forward	GGCGCTCGGTTTCTCTTCTT
	reverse	GCCAGAGCCAACTCCAGAAA
CRY1	forward	TTGGAAAGGAACGAGACGCAG
	reverse	CGGTTGTCCACCATTGAGTT
CRY2	forward	TCCCAAGGCTGTTCAAGGAAT
	reverse	TGCATCCCGTTCTTTCCCAAA
DBP	forward	CGACCGCTTGATCTGGACAC
	reverse	GCTGCAAATCCTAGGAGCGA
HIF1A	forward	CTCCATTACCCACCGCTGAA
	reverse	TCACTGGGACTATTAGGCTCAGGT
HIF2A	forward	CGGAGGTGTTCTATGAGCTGG
	reverse	AGCTTGTGTGTTCGCAGGAA
NR1D1	forward	TGGACTCCAACAACAACACAG
	reverse	GATGGTGGGAAGTAGGTGGG
PER1	forward	AGTCCGTCTTCTGCCGTATCA
	reverse	AGCTTCGTAACCCGAATGGAT
PER2	forward	CTTCAGCGATGCCAAGTTTGT
	reverse	CGGATTTCATTCTCGTGGCTTT
PER3	forward	GCAGGTCTATGCCAGTGTGA
	reverse	CCACCACCATTCGGTTCTGT
PHD1	forward	TGGCCCTGGACTATATCGTG
	reverse	GGCACCAATGCTTCGACAG
PHD2	forward	GCACGACACCGGGAAGTT
	reverse	CCAGCTTCCCGTTACAGT
PHD3	forward	CACAGCGAGGGAATGAACCT
	reverse	TCCTGCTGTTAAGGCTTCCG
PDK1	forward	TGAACGGATGGTGTCCTGAG
	reverse	GGCCAGGTGGACTTCTACG
RORA	forward	CACGACGACCTCAGTAACTACA
	reverse	TGGTGAACGAACAGTAGGGAA
SLC2A1	forward	CTAGCGCGATGGTCATGAGT
	reverse	TCTGGCATCAACGCTGTCTT
VEGF	forward	CCGCCTCGGCTTGTCACA
	reverse	GCAAGACAAGAAAATCCCTGTGGGCC

ACTB: *beta-actin*, ADM: *adrenomedullin*, ARNTL: *aryl hydrocarbon receptor nuclear translocator-like*, CLOCK: *circadian locomotor output cycles kaput*, CRY1/2: *Cryptochrome Circadian Regulator 1 and 2*, DBP: *albumin gene D-site binding protein*, HIF1A: *hypoxia-inducible factor 1 alpha*, HIF2A: *hypoxia-inducible factor 2 alpha*, NR1D1: *nuclear receptor subfamily 1, group D, member 1*, PER1/2/3: *Period Circadian Regulator 1, 2 and 3*, PHD1/2/3: *pyruvate dehydrogenase kinase 1, 2 and 3*, PDK1: *pyruvate dehydrogenase kinase 1*, RORA: *RAR Related Orphan Receptor A*, SLC2A1: *glucose transporter type 1*, VEGF: *vascular endothelial growth factor*.

CHAPTER VII | GENERAL DISCUSSION

The thesis is structured to methodically explore the complex and multifaceted role of hypoxia in human biology, focusing on implications for disease, potential measures to enhance human performance in hypoxia, and potential applications of human hypoxia models in drug development. Central to all these investigations are highly standardized human hypoxia models. The significance of this research is highlighted by the 2019 Nobel Prize for the discovery of the HIF gene, a crucial regulator in oxygen sensing. Additionally, the thesis covers the interaction between the body's hypoxia-sensing pathways and circadian rhythms, a connection highlighted by the work of Adamovich *et al.* and further supported by our findings in humans. This expands upon the foundational discoveries recognized by the 2017 Nobel Prize for the discovery of the clock gene. Unique animal models, such as the naked mole rat, informed the development of human hypoxia models. The thesis examined how elevated carbon dioxide (CO_2) levels might improve oxygenation during air travel and explored the effects of hypoxia on cognitive, metabolic, and circadian responses.

Human hypoxia models, originally developed for aerospace medicine, are essential for studying our body's response to low oxygen levels (**Chapter 2**). These models are invaluable, as they allow researchers to replicate hypoxic conditions and closely monitor physiological responses in a controlled environment. However, their full potential remains unexploited. To improve their effectiveness, it is recommended to leverage existing aerospace medicine infrastructure to promote collaborative projects and expand their application. By studying how the body adapts to low oxygen in extreme environments like high altitudes or space, we can discover new ways to improve health and performance. Additionally, standardizing and validating these models is important to ensure they provide accurate and reliable insights, which can help advance our understanding of human physiology and the development of new treatments.

Focusing on the practical implications of hypoxia, **Chapter 3** explored the effects of elevated CO_2 levels in airplane cabins as a strategy to counteract hypoxemia risks. Despite pressurization of airliner cabins, there remains a risk that some passengers may still suffer from reduced oxygen saturation, increasing the risk of hypoxemia-related health issues. The work presented in **Chapter 3** demonstrated that increased ambient CO_2 can lead to higher levels of capillary CO_2 and blood oxygen saturation (SpO_2), along with increased minute ventilation, without negatively impacting cognitive functions. These observations suggest that maintaining

a cabin environment with a 1% CO_2 concentration could help prevent critical SpO_2 reductions. Moreover, these findings may drive technological advancements to refine cabin conditions, challenging current aviation practices to balance passenger health, operational efficiency, and environmental sustainability. This research underscores the importance of integrating health considerations into aviation, ensuring the well-being of all travelers—especially those with medical conditions or age-related vulnerabilities. Impaired cognitive functioning is among the first signs of hypoxia, raising important safety concerns for activities at high altitudes. **Chapter 4** reviewed existing studies, emphasizing the challenge of comparing results due to the widespread use of different cognitive tests. The Stroop test emerged as the most sensitive test, consistently detecting significant cognitive impairment under hypoxic conditions. The cognitive clusters most affected by hypoxia included auditory/verbal memory, evoked potentials, visual/spatial delayed recognition and sustained attention. In contrast, tasks that solely assessed attention showed lower sensitivity to hypoxia. A clear correlation was found between different levels of hypoxia and cognitive performance, particularly in tests measuring executive functions, which demonstrated increased vulnerability under severe hypoxic conditions. Interestingly, changes in barometric pressure seemed to have no effect on hypoxia sensitivity. This review underscores the particular risk hypoxia poses to complex and executive cognitive functions, highlighting the need for standardized cognitive testing in future research.

The exceptional hypoxia tolerance observed in the naked mole-rat provides insights into potential adaptations that could benefit humans. **Chapter 5** explored fructose' potential to similarly boost endurance and cognitive function in humans under hypoxic conditions. However, a single oral administration of fructose to non-acclimatized, healthy individuals did not improve visual, cognitive, or physical performance under moderate hypoxia, suggesting that fructose consumption may not enhance human hypoxia tolerance. Yet, exploring these metabolic pathways remains valuable due to their physiological and clinical potential. Boosting fructose metabolism to combat systemic and localized hypoxia remains a promising line of research. Additionally, the connection between fructose metabolism in hypoxia and conditions like heart failure, metabolic syndrome, and cancer suggests a complex relationship between metabolic adaptation to prolonged hypoxia and disease resistance. The naked mole-rat's metabolic resilience, particularly its resistance to cancer, highlights the importance

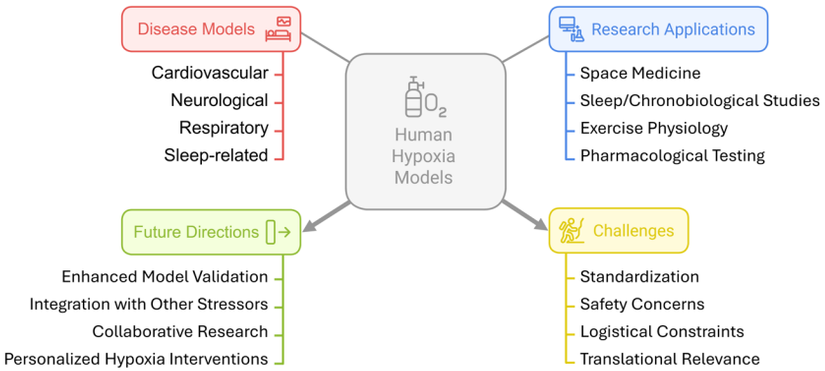
for further research to determine whether humans might benefit from similar adaptations in fructose metabolism under chronic hypoxia. While replicating the hypoxia tolerance mechanisms of the naked mole-rat in humans presents challenges, ongoing investigations into these unique species are crucial for identifying novel approaches that could leverage these biological adaptations to improve human health.

The discovery that hypoxia exposure can reset the human circadian clock deepens our understanding of circadian rhythm regulation (**Chapter 6**). Even a single pulse of hypoxia can significantly shift the timing of melatonin release, a key indicator of circadian phase, suggesting that oxygen availability acts as a time cue, or zeitgeber, for the central circadian clock. This effect, consistent with findings in animal models, emphasizes the role of oxygen cycles in synchronizing circadian rhythms through hypoxia-sensing pathways. Our study suggests that daily activities like exercise, diet, and altitude exposure, which influence tissue oxygenation, may serve as non-photic signals that reset circadian clocks. These findings have broad implications, deepening our understanding of circadian biology and opening up new possibilities for treating circadian-related disorders. By clarifying the link between oxygen cycles and circadian timing, we propose a connection between lifestyle factors, hypoxia-related diseases, and circadian misalignment. This highlights the potential of oxygen therapy as a tool for managing circadian health and related conditions.

While **Chapter 2** introduced the conceptual framework of human hypoxia models (Figure 1), **Chapter 3-6** examined their practical applications and identified biomarkers associated with hypoxia responses in cardiovascular, neurological, respiratory, and sleep-related conditions (Table 1). Despite their potential, human hypoxia models face several challenges that must be addressed to enhance their effectiveness. Standardization remains a key issue, as variability in exposure protocols, biomarker selection, and cognitive testing methods limits the reproducibility of studies. Developing standardized methodologies will improve data comparability and ensure consistency across research efforts. Safety concerns must also be considered, as prolonged or severe hypoxia can pose risks to vulnerable populations. Ethical guidelines and safety protocols should be established to protect study participants while enabling meaningful research. Logistical constraints remain a barrier, as hypoxia research requires specialized facilities, trained personnel, and controlled environments, which can limit accessibility and scalability. Translational relevance is another

important consideration, as findings from experimental models must be validated in real-world clinical settings to ensure their applicability. Cross-comparisons with studies of CNS-active compounds can create a functionally meaningful framework, for both various hypoxia models (compared with prototype CNS depressants or stimulants), and conversely for novel compounds (relative to hypoxaemic effects). Hypoxia models may also serve as challenge tests for compounds that potentially mitigate cognitive impairment.

FIGURE 1 **Potential applications, future directions and challenges of human hypoxia models described in Chapter 2.**



To maximize the impact of human hypoxia models, several future directions should be explored. Further validation of biomarkers identified in this thesis is essential to confirm their diagnostic and prognostic value in clinical populations. Hypoxia rarely occurs in isolation, and future research should integrate hypoxia studies with other physiological stressors, such as metabolic stress, inflammation, and environmental factors, to provide a more comprehensive understanding of adaptive responses. Expanding interdisciplinary collaborations between physicians, physiologists, and aerospace medicine experts will enhance the translational scope of hypoxia research. Personalized hypoxia interventions represent another promising area, where individualized protocols based on genetic and metabolic responses could optimize the use of hypoxia-based therapies for rehabilitation, high-altitude training, and disease treatment.

The findings presented in this thesis demonstrate the potential of human hypoxia models for advancing medical science, particularly in understanding and treating hypoxia-related diseases. By integrating

biomarker discovery, cognitive assessments, metabolic adaptations, and circadian rhythm research, this work provides a more complete picture of how human hypoxia models can be deployed. Moving forward, efforts to standardize models, validate biomarkers, and bridge research with clinical practice will be key to unlocking the full potential of hypoxia-based diagnostics, therapies, and performance optimization strategies.

TABLE 1 **Summary of hypoxia models, biomarkers, diseases and conditions in Chapter 3-6.**

Study	CHAPTER 3 Hypoxia & CO_2	CHAPTER 4 Cognitive Sensitivity	CHAPTER 5 Fructose Metabolism	CHAPTER 6 Circadian Phase Shift
Hypoxia Model				
DURATION	6 h	10 min – 6.5 h	2 h	6.5 h
EXPOSURE	15% O_2+1% CO_2	6.3-17.6% O_2	13% O_2	15% O_2
PRESSURE	HH	NH/HH	NH	NH
METHOD	Chamber	Chamber, mask, altitude	Chamber	Chamber
Biomarkers	SpO_2, NIRS-TSI, AMS-C	SpO_2	Plasma fructose, blood lactate	DLMO
	pCO_2, pO_2, pH, HCO_3^-	Cognitive function	SpO_2, pO_2, pCO_2	SpO_2
	MV, RR, TV		FM-100, UTT	HIF-1 target genes
	PVT, N-back, tracking		Exercise duration, BORG	Clock genes
Disease/ condition	Air travel and at-risk passengers	Cognitive impairment in OSA, COPD, high-altitude scenarios	Stroke, myocardial infarction, metabolic syndrome and cancer	Circadian rhythm disruption in OSA, COPD, jetlag and shift work
Results	Ambient CO_2 improves oxygenation	Executive domain are most sensitive	Fructose metabolism alone does not replicate naked mole-rat hypoxia tolerance	Hypoxia may act as a zeitgeber

POTENTIËLE TOEPASSINGEN VOOR MENSELIJKE HYPOXIEMODELLEN

Het proefschrift onderzoekt het complexe en veelzijdige effect van hypoxie (een toestand van zuurstoftekort) op de menselijke biologie. Hierbij wordt aandacht besteed aan de gevolgen voor ziekten, mogelijke manieren om menselijke prestaties bij hypoxie te verbeteren, en de toepassing van hypoxiemodellen in de ontwikkeling van geneesmiddelen. Centraal in dit onderzoek staan gestandaardiseerde menselijke hypoxiemodellen. Het belang van dit onderzoek wordt onderstreept door de toekenning van de Nobelprijs in 2019 voor de ontdekking van het hypoxie-induceerbare factor (HIF)-gen, een cruciale regulator van zuurstofwaarneming. Daarnaast behandelt dit proefschrift de samenwerking tussen systemen in het lichaam die gevoelig zijn voor zuurstoftekort en het dag-nachtritme, zoals aangetoond door Adamovich *et al.* en bevestigd door ons eigen onderzoek bij mensen. Dit onderzoek bouwt voort op de ontdekkingen die in 2017 werden bekroond met de Nobelprijs voor de identificatie van het klokgen. Unieke diermodellen, zoals de naakte molrat, hebben bijgedragen aan een beter begrip van hoe het menselijk lichaam omgaat met zuurstoftekort. Dit proefschrift onderzoekt onder meer hoe verhoogde kooldioxide (CO_2)-waarden de zuurstofvoorziening tijdens vliegreizen kunnen verbeteren en wat de effecten van hypoxie zijn op cognitieve functies, stofwisseling en dag-nachtritme.

Menselijke hypoxiemodellen, oorspronkelijk ontwikkeld voor de lucht- en ruimtevaartgeneeskunde, helpen wetenschappers te begrijpen hoe het lichaam reageert op zuurstoftekort (**Hoofdstuk 1**). Deze modellen zijn waardevol omdat ze hypoxie in een gecontroleerde omgeving nabootsen, maar hun volledige potentieel wordt nog niet benut. Door samen te werken en gebruik te maken van bestaande lucht- en ruimtevaartfaciliteiten, kunnen deze modellen verder worden verbeterd. Door te bestuderen hoe het lichaam zich aanpast aan lage zuurstofniveaus in extreme omgevingen, zoals grote hoogten of de ruimte, kunnen er nieuwe strategieën ontwikkelt worden om de gezondheid en prestaties te verbeteren. Daarnaast is standaardisatie en validatie van deze modellen essentieel om ervoor te zorgen dat ze nauwkeurige en betrouwbare inzichten bieden. Een vergelijking van de effecten van hypoxie en van geneesmiddelen kan wederzijds nuttige informatie verschaffen over de functionele relevantie van cognitieve en functionele testen, die in beide velden veel worden gebruikt. Dit kan bijdragen aan een beter begrip van de menselijke fysiologie en de ontwikkeling van nieuwe behandelingen. **Hoofdstuk 2** onderzoekt hoe hogere CO_2-niveaus in vliegtuigcabines het risico op een te laag zuurstofgehalte in het bloed (hypoxemie) kunnen verminderen. Op zeeniveau bevat de lucht 21% zuurstof, maar in een vliegtuigcabine wordt het zuurstofgehalte ingesteld op ongeveer 15% om het drukverschil tussen binnen en buiten het vliegtuig zo klein mogelijk te houden. Een hoger zuurstofpercentage zou namelijk een te groot drukverschil veroorzaken op grote hoogte. Dit lagere zuurstofgehalte kan het zuurstofniveau in het bloed verlagen, maar zou geen directe negatieve invloed op de gezondheid van passagiers moeten hebben. Toch kunnen sommige passagiers nog steeds een te laag zuurstofgehalte in hun bloed krijgen, wat gezondheidsrisico's met zich meebrengt. Het onderzoek liet zien dat een verhoogd CO_2-niveau in de cabine zorgt voor een hoger zuurstofgehalte in het bloed en een snellere ademhaling, zonder negatieve effecten op de cognitieve functies. Dit suggereert dat een CO_2-niveau van 1% in de cabine kan helpen om te voorkomen dat het zuurstofgehalte te veel daalt. De bevindingen kunnen bijdragen aan verbeteringen in vliegtuigtechnologie, zodat de luchtvaart beter in balans komt met passagiersgezondheid, operationele efficiëntie en het milieu. Het onderzoek benadrukt het belang van het integreren van gezondheidsaspecten in de luchtvaart, vooral voor kwetsbare reizigers, zoals ouderen of mensen met medische aandoeningen.

Verminderd cognitief functioneren behoort tot de eerste tekenen van hypoxie, wat belangrijke veiligheidsrisico's met zich meebrengt voor activiteiten op grote hoogte. **Hoofdstuk 3** evalueerde bestaande studies en benadrukte de uitdaging om resultaten te vergelijken door het wijdverspreide gebruik van verschillende cognitieve tests. De Stroop-test bleek de meest gevoelige test, omdat deze consequent significante cognitieve beperkingen onder hypoxische omstandigheden detecteerde. De cognitieve domeinen die het sterkst werden beïnvloed door hypoxie omvatten onder andere auditief/verbaal geheugen, opgewekte potentialen, visueel/ruimtelijke vertraagde herkenning en volgehouden aandacht. Daarentegen bleken taken die uitsluitend aandacht meten minder gevoelig voor hypoxie. Er werd een duidelijke correlatie vastgesteld tussen verschillende niveaus van hypoxie en cognitieve prestaties, vooral in tests die executieve functies meten. Deze functies worden relatief sterk beinvloed onder ernstige hypoxische omstandigheden. Opvallend was dat veranderingen in luchtdruk geen effect leken te hebben op de gevoeligheid voor hypoxie. Deze review onderstreept het bijzondere risico dat hypoxie vormt voor

complexe en executieve cognitieve functies en benadrukt de noodzaak van gestandaardiseerde cognitieve tests in toekomstig onderzoek.

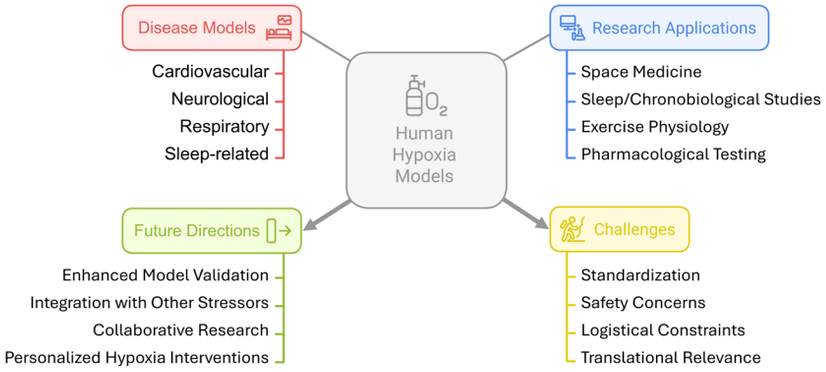
De uitzonderlijke hypoxietolerantie van de naakte molrat biedt waardevolle inzichten in mogelijke aanpassingen die ook voor mensen gunstig kunnen zijn. **Hoofdstuk 4** onderzocht het potentieel van fructose om het uithoudingsvermogen en de cognitieve functie bij mensen onder hypoxische omstandigheden te verbeteren. Echter, een eenmalige orale toediening van fructose aan niet-geacclimatiseerde, gezonde individuen had geen significante invloed op visuele, cognitieve of fysieke prestaties onder matige hypoxie. Dit suggereert dat fructoseconsumptie mogelijk geen directe verbetering van de menselijke hypoxietolerantie oplevert. Toch blijft het verkennen van deze metabole routes waardevol vanwege hun fysiologische en klinische potentieel. Het stimuleren van fructosemetabolisme als strategie om systemische en lokale hypoxie te bestrijden blijft een veelbelovende onderzoekslijn. Bovendien wijst de relatie tussen fructosemetabolisme in hypoxische omstandigheden en aandoeningen zoals hartfalen, metabool syndroom en kanker op een complexe wisselwerking tussen metabole aanpassingen aan langdurige hypoxie en ziekteresistentie. De metabole veerkracht van de naakte molrat, met name zijn uitzonderlijke weerstand tegen kanker en een voor knaagdieren hoge leeftijdsverwachting van 35 jaar, onderstreept het belang van verder onderzoek. Dit kan helpen vaststellen of mensen baat kunnen hebben bij vergelijkbare aanpassingen in fructosemetabolisme onder chronische hypoxie. Hoewel het repliceren van de hypoxietolerantiemechanismen van de naakte molrat bij mensen uitdagend is, blijven lopende studies naar deze unieke diersoort van groot belang.

De ontdekking dat hypoxie onze biologische klok kan resetten, geeft nieuwe inzichten in hoe onze interne ritmes worden geregeld (**Hoofdstuk 5**). Zelfs een korte blootstelling aan hypoxie kan de afgifte van melatonine, een belangrijke indicator van onze slaap-waakcyclus, merkbaar verschuiven. Dit suggereert dat zuurstofniveaus een rol spelen als natuurlijke tijdsaanduiding (*zeitgeber*) voor onze interne klok. Dit effect, dat ook in dierstudies is gevonden, laat zien dat schommelingen in zuurstofniveaus helpen om ons ritme te synchroniseren via mechanismen die zuurstoftekort waarnemen. Uit ons onderzoek blijkt dat dagelijkse activiteiten zoals bewegen, eten en verblijven op grote hoogte – die de zuurstoftoevoer in het lichaam beïnvloeden – kunnen helpen om onze biologische klok opnieuw af te stellen. Deze bevindingen hebben brede gevolgen: ze verdiepen ons begrip van hoe onze interne ritmes werken

en openen nieuwe mogelijkheden voor de behandeling van slaapstoornissen en andere ritme-gerelateerde aandoeningen. Door de link tussen zuurstofniveaus en onze biologische klok beter te begrijpen, leggen we een verband tussen leefstijl (onregelmatig werken en lange reizen), ziekten die verband houden met zuurstoftekort, en verstoringen in het dag-nachtritme. Dit roept bijvoorbeeld de vraag op of zuurstoftherapie zou kunnen bijdragen aan een evenwichtige biologische klok en daarmee de gezondheid zou kunnen ondersteunen.

Hoofdstuk 2 legt de basis uit van hypoxiemodellen bij mensen (Figuur 1). In **hoofdstukken 3-6** gaat het over hoe deze modellen in de praktijk worden gebruikt en welke biomarkers een rol spelen bij hypoxieresponsen in hart- en vaatziekten, neurologische aandoeningen, ademhalingsproblemen en slaapstoornissen (Tabel 1). Hoewel deze modellen veel potentie hebben, zijn er ook uitdagingen. Een groot probleem is dat er geen standaardmethode is: verschillen in blootstelling, biomarkerkeuze en cognitieve tests maken het lastig om studies goed te vergelijken. Een uniforme aanpak zou het onderzoek betrouwbaarder maken. Ook de veiligheid is belangrijk, want langdurige of zware hypoxie kan risico's hebben, vooral voor kwetsbare mensen. Daarom zijn duidelijke ethische richtlijnen en veiligheidsmaatregelen nodig. Daarnaast is hypoxieonderzoek niet altijd makkelijk uit te voeren, omdat het speciale apparatuur en goed getraind personeel vereist. Tot slot is het belangrijk dat de resultaten niet alleen in een laboratorium blijven, maar ook echt worden toegepast in de medische praktijk.

FIGUUR 1. Mogelijke toepassingen, toekomstige richtingen en uitdagingen van menselijke hypoxiemodellen uit hoofdstuk 2.



TABEL 1. Overzicht van hypoxiemodellen, biomarkers en ziektebeelden uit hoofdstuk 3-6.

Studie	HOOFDSTUK 3 Hypoxia & CO_2	HOOFDSTUK 4 Cognitive Gevoeligheid	HOOFDSTUK 5 Fructosemetabolisme	HOOFDSTUK 6 Circadiane Verschuiving
Hypoxiemodel				
DUUR	6 u	10 min – 6.5 u	2 u	6.5 u
BLOOTSTELLING	15% O_2+1% CO_2	6.3-17.6% O_2	13% O_2	15% O_2
DRUK	HH	NH/HH	NH	NH
METHODE	Kamer	Kamer, masker, hoogte	Kamer	Kamer
Biomarkers	SpO_2, NIRS-TSI, AMS-C	SpO_2	Plasma fructose, bloedlactaat	DLMO
	pCO_2, pO_2, pH, HCO_3^-	Cognitieve functie	SpO_2, pO_2, pCO_2	SpO_2
	MV, RR, TV		FM-100, UTT	HIF-1 doelfactoren
	PVT, N-back, tracking		Inspanningstijd, BORG	Klokgenen
Ziekte/ toestand	Luchtvaart en risico-passagiers	Cognitieve achteruitgang bij OSA, COPD, scenario's op grote hoogte	Beroerte, myocardinfarct, metabool syndroom en kanker	Verstoring van het circadiane ritme bij OSA, COPD, jetlag en ploegendienst
Resultaten	Omgevings-CO_2 verbetert de oxygenatie	Executieve domein zijn het meest gevoelig	Fructosemetabolisme alleen bootst de hypoxietolerantie van de naakte molrat niet na	Hypoxie kan werken als een *zeitgeber*

Om hypoxiemodellen optimaal te benutten, zijn er een paar belangrijke stappen nodig. Eerst moeten de gevonden biomarkers beter getest worden om zeker te weten dat ze bruikbaar zijn in de medische praktijk. Omdat hypoxie meestal niet op zichzelf staat, is het ook belangrijk om andere factoren zoals stofwisselingsstress, ontstekingen en omgevingsinvloeden mee te nemen in toekomstig onderzoek. Goede samenwerking tussen artsen, fysiologen en experts in lucht- en ruimtevaartgeneeskunde kan helpen om hypoxieonderzoek beter toepasbaar te maken. Een veelbelovende ontwikkeling is gepersonaliseerde hypoxiebehandeling, waarbij therapieën worden afgestemd op iemands genetische en stofwisselingskenmerken. Dit kan nuttig zijn voor revalidatie, hoogtetraining en de behandeling van bepaalde ziekten. Dit onderzoek laat zien dat hypoxiemodellen een belangrijke rol kunnen spelen in de medische wetenschap, vooral bij het begrijpen en behandelen van ziekten die met zuurstoftekort te maken hebben. Door biomarkers, cognitieve tests, stofwisselingsaanpassingen en biologische ritmes te combineren, krijgen we een completer beeld van hoe hypoxiemodellen ingezet kunnen worden. Om hun volledige potentie te benutten, is het belangrijk om standaarden te ontwikkelen, biomarkers te valideren en de stap te maken van onderzoek naar praktijk.

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CURRICULUM VITAE

Titiaan Post was born on October 16, 1990, in Hilversum and raised in Ankeveen. He graduated from Sint Vitus College in Bussum and Luzac College in Hilversum. He then pursued a bachelor's degree in Pharmaceutical Sciences at Vrije Universiteit Amsterdam. During his final undergraduate year, he undertook an internship in the Analytical Chemistry division, focusing on the stability of nortriptyline.

After completing his bachelor's degree, Titiaan advanced to a master's program in Bio-Pharmaceutical Sciences and Science-based Business at Leiden University. As part of his master's training, he completed two internships: the first at the Centre for Human Drug Research (CHDR), where he studied doping effects of erythropoietin in well-trained cyclists, and the second in Clinical Development and Business Development at Polyphor Ltd (now Spexis) in Basel, Switzerland.

After completing his master's degree, Titiaan began his PhD as a clinical scientist at the Department of Sleep and Human Factors Research within the Institute of Aerospace Medicine at the German Aerospace Center (DLR) in Cologne, Germany, in collaboration with CHDR. His research was supervised by Prof. Dr. Daniel Aeschbach, Prof. Dr. Jens Jordan, Prof. Dr. Adam Cohen, and Prof. Dr. Joop van Gerven.

In 2023, Titiaan assumed the position of Associate Clinical Study Manager at CHDR, where he continues to contribute to the field of clinical research.