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Randomised Clinical Trials with Hyperbaric Oxygen in COVID-19 and Long COVID: Transcriptomic Insights into Benefits and Harms

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Thesis for Doctoral Degree (Ph.D.)

By

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Thoughts on evolution and adaptation

I have always been intrigued by how different species use different epitopes for survival, and how mammals can operate in a span of more than 11000 meters, adapting to high and low partial pressures of oxygen. Even though we look so different, the basic mechanisms in cells are conserved through evolution. Mankind has evolved immensely from where it started but, in our genome, we have traces from the beginning of life that give us the ability to adapt. To understand how our cells work we need to understand how we have evolved from one cell to an astonishing 30-trillion cell organism by adding new smarter features and shutting down others over the past 3.5 billion years.

I started my working career as a submarine officer, fascinated by diving and residing under water and subsequently became an intensive care physician, specialising in hyperbaric medicine. I was initially not very interested in hyperbaric oxygen treatment (HBOT) but, since I was interested in diving, I embraced it and adapted my interests. I was captivated by the effects I saw on patients receiving HBOT and at the same time astonished by the "quasi-religious" divergence in beliefs pro and con. So I decided to find out for myself, scientifically… Many years later, I realize that I'm not far from where I started, still intrigued, with more questions than answers, simply puzzled on a higher level. I started this thesis just before the Coronavirus disease 2019 (COVID-19) pandemic that has so negatively affected the whole world for years, possibly decades to come, but here again, we will adapt. COVID-19 strongly affected my thesis with a change of course. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has given me and other researchers, a unique opportunity to study the mechanisms of hypoxic adaptation and dysregulation of the immune system that will help us understand many disease mechanisms way beyond viral infections.

To my Family and Friends

"*Was mich nicht umbringt, macht mich stärker*" -Friedrich Nietzsche (1888)

("*What does not kill us, makes us stronger*" -English translation)

POPULAR SCIENCE SUMMARY OF THE THESIS

The flow from DNA in genes through RNA, which translates into finished proteins, is a common foundation for all life. We constantly need to adapt to various types of stress, such as UV light, radiation, infections, physical and mental stress. Numerous protective systems against oxidative stress exist, allowing our cells to adapt, themselves and the organ or organ system they work for.

Hormesis involves adaptation to stress that does not kill but instead strengthens the cell and the organism. If the stress becomes overwhelming, the cell can choose either to die for the benefit of the organ or to enter senescence, i.e., "ageing." The ability of cells to enter senescence is vital for our development and immune system. However, if too many cells enter senescence simultaneously and the timing is wrong, this manifests itself as symptoms of disease. If the cells cannot restart, or die in a timely manner, the entire organism ages, ultimately leading to death.

Most people can relate to physical training; if we regularly use our muscles, we become stronger, improve breathing capacity, and do not experience as high a pulse during exercise—this is hormesis. In the long run, exercise has many positive effects, with a clear correlation between exercise and general health, including a lower risk of cancer and of several acute and chronic inflammatory diseases. This is because exercise simultaneously stimulates the immune system and those systems overseeing quality control in our protein factories. However, for hormesis dosage matters, and the dosage is individually different. Without effort, no change occurs; with moderate effort, we experience positive effects; overexertion triggers injuries that may take a long time to heal, and premature ageing occurs if our system cannot heal the damage.

The same principles apply to hyperbaric oxygen therapy (HBOT), administered in a pressure chamber by intermittently breathing 100% oxygen several times a week at an ambient pressure equivalent to 10–20 meters under water. The aim of this thesis was to evaluate potential harms of HBOT for novel indications and to explore biomarkers accounting for dosage in experimental and clinical trials in order to enable future precision medicine. In our initial study, involving ten healthy volunteers we aimed to investigate if similar effects could be observed in high intensity interval training (HIIT) and the equal amount of time with hyperbaric oxygen, by measuring oxygen radicals in the blood and assessing the expression of crucial genes affected by oxygen deficiency and inflammation in the blood's immune cells. In both interventions, we observed changes in genes regulating the quality control of the protein factory.

Then, in spring 2020, COVID-19 emerged, imposing a terrible burden on healthcare professionals and on patients suffering from severe COVID-19 pneumonia and oxygen deficiency. Based on the hypothesis of the first study, we initiated a study across three

hospitals where patients were randomly assigned to receive either HBOT and standard care or only standard care. We investigated whether we could reduce the risk of needing a ventilator and expedite patients' discharge from the hospital. Additionally, at Karolinska, we explored how patients' immune systems responded to HBOT. Although we could not definitively conclude whether it was beneficial to avoid intensive care, as only 34 of the planned 200 patients were included in the study, it did not appear harmful. Importantly, patients at Karolinska who received HBOT began to recover after just a few days (on average) and could leave the hospital in almost half the time compared to those in the other group who stayed nearly a month until the study ended. Interestingly, after a week of treatment, we observed significant differences in gene expression in blood immune cells in those who received HBOT, including genes regulating the protein factory's quality control.

In the summer of 2020, many cases of chronic fatigue syndrome emerged after COVID-19 in previously healthy individuals, especially in women who had a mild disease and did not require hospitalisation. They experienced significant physical and mental disability, with poor quality of life, seemingly aging prematurely. At that point, we commenced a study where we randomly assigned 80 previously healthy patients with physical disability after COVID-19 to receive either HBOT or placebo (sham treatment). We investigated whether we could improve patients' quality of life and physical function. We were also exploring if we could identify an explanation for the symptoms and any potential positive effects on protein factory quality control. We followed the first 20 patients for three months and demonstrated that HBOT did not appear to be dangerous, even though we observed many more side effects than typically expected; we can only speculate whether it was a matter of dosage or the disease severity. The answer to whether HBOT has a relevant clinical effect lies beyond this dissertation.

In summary, we have shown that HBOT stresses immune cells similarly to physical training, affecting the quality control of protein factories. The treatment does not seem harmful to severely ill COVID-19 patients, opening up possibilities to investigate its effects on severely ill patients with other inflammatory conditions in the future. We speculate that HBOT reduces senescence or exhaustion, allowing dysregulated cells to undergo programmed cell death, thereby interrupting inflammation in COVID-19 and stimulating faster recovery. The appropriate dosage for different medical conditions and how to tailor the treatment to individual patient profiles are topics for future research.

POPULÄRVETENSKAPLIG SAMMANFATTING

Flödet från DNA i gener via RNA som utgör översättningen till färdigtillverkade proteiner är en gemensam bas för allt liv. Vi behöver ständigt anpassa oss till olika typer av stress som UV-ljus, radioaktiv strålning, infektioner, fysisk och mental stress. Vi har mängder av skyddssystem mot oxidativ stress som gör att våra celler kan anpassa sig själva, och det organ eller organsystem som dom arbetar för.

Hormesis innebär anpassning till en stress som inte dödar men i stället stärker cellen och organismen. Blir stressen för svår kan cellen välja att dö för organets bästa eller att gå in i senescens dvs ”åldrande”. Cellers förmåga att gå in i senescens är livsviktigt för vår utveckling och immunsystem men om för många celler samtidigt går in i senescens vid fel tidpunkt märker vi det som symtom på sjukdom. Om cellerna inte kan starta i gång igen, eller dö kontrollerat, åldras hela organismen och det är därför via alla till slut dör.

De flesta kan relatera till fysik träning. Använder vi våra muskler regelbundet så blir vi starkare, mindre andfådda och får inte lika hög puls när vi tränar—det är hormesis. På lång sikt har träning många positiva effekter och det finns ett tydligt samband mellan träning och allmän hälsa, inklusive mindre risk för cancer och flera akuta och kroniska inflammatoriska sjukdomar, bland annat för att vi samtidigt motionerar immunsystemet och de system som sköter kvalitetskontrollen i våra proteinfabriker. Hormesis är dock en fråga om dos och dosen är individuell. Utan ansträngning sker ingen förändring, lagom ansträngning ger positiva effekter, överansträngning ger skador som kan ta lång tid att läka och vi åldras i förtid om inte skadan kan läkas.

Samma principer gäller för hyperbar syrgasbehandling (HBOT) som ges i tryckkammare genom att andas 100% syrgas intermittent, flera gånger i veckan i ett omgivningstryck som motsvarar 10-20 meter under vattnet. Det övergripande målet i denna avhandling var att utvärdera negativa effekter med HBOT för nya indikationer och utforska biomarkörer i experimentella och kliniska studier för att möjliggöra individanpassad behandling i framtiden. I vår första studie med tio friska frivilliga försökspersoner som fick träna högintensiva intervaller (HIIT) och lika lång tid hyperbar syrgas ville vi se om vi kunde se liknande effekter genom att mäta syreradikaler i blod och hur viktiga gener som påverkas av syrebrist och inflammation uttrycktes i blodets immunceller. Vi såg bland annat att gener som styr proteinfabrikens kvalitetskontroll förändrades på liknande sätt.

Våren 2020 kom COVID-19, det var en fruktansvärd belastning för oss inom sjukvården och patienterna som led av svår COVID-19 lunginflammation och syrebrist. Vi startade då en studie på tre sjukhus där patienter lottades till HBOT och standardvård eller bara standardvård. Vi undersökte om vi kunde minska risken att hamna i respirator och få patienterna från sjukhuset fortare. Dessutom undersökte vi på Karolinska hur patienternas immunförsvar reagerade på att få HBOT. Vi kunde inte säkert säga om det var bra för att

slippa intensivvård då vi bara fick in 34 av planerade 200 patienter i studien men det verkade inte vara farligt och patienter på Karolinska som fått HBOT började återhämta sig redan efter några dagar (medelvärde) och kunde lämna sjukhuset efter nästan halva tiden jämfört med de i andra gruppen som blev kvar nästan en månad då studien avslutades. Intressant nog kunde vi efter en veckas behandling se en stor skillnad på hur gener uttrycktes i immunceller i blodet hos de som fått HBOT, bland annat i gener som styr proteinfabrikens kvalitetskontroll.

Sommaren 2020 började det dyka upp många fall av kroniskt trötthetssyndrom efter COVID-19 hos tidigare helt friska, ffa kvinnor som haft en lindrig sjukdom och inte legat på sjukhus. Dom hade stor funktionsnedsättning fysiskt och psykiskt med mycket dålig livskvalitet, det verkade som om dom åldrats i förtid. Då startade vi en studie där vi lottade 80 tidigare friska patienter med fysisk funktionsnedsättning efter COVID-19 till HBOT eller placebo (fejkbehandling). Vi undersökte om vi kunde öka patienternas livskvalitet och fysiska funktion och undersöker även här om vi kan hitta en förklaring till symtomen och en eventuell positiv effekt i proteinfabrikernas kvalitetskontroll. Vi följde de första 20 patienterna i tre månader och visade att det inte verkar farligt trots att vi såg många fler biverkningar än vi normalt ser vid användning av HBOT; vi kan bara spekulera i om det är det en dosfråga eller sjukdomsgrad. Svaret på frågan om det har en relevant klinisk effekt ligger utanför den här avhandlingen.

Sammanfattningsvis har vi visat att HBOT stressar immunceller på ett liknande sätt som vid fysisk träning och det påverkar proteinfabrikernas kvalitetskontroll. Behandlingen verkar inte skadlig för svårt sjuka patienter med COVID-19 vilket öppnar för möjligheter att undersöka effekten på svårt sjuka patienter med andra inflammatoriska tillstånd i framtiden. Vi spekulerar i att HBOT minskar cellernas åldrande och låter trötta celler gå i programmerad självdöd och på så sätt avbryter vi inflammationen vid COVID-19 vilket leder till snabbare återhämtning. Vilken dos vi ska använda vid olika sjukdomstillstånd och hur vi ska kunna skräddarsy den individuellt är frågeställningar för framtida forskning.

ABSTRACT

The flow from transcription of genes through translation and processing of proteins is a common basis for all life. Redox homeostasis is crucial for the defence against oxidative stress. We adapt through hormesis; non-lethal stress regulates redox-sensitive systems to maintain homeostasis. If the stress is chronic or acutely overwhelming, the cells can either go into apoptosis or into senescence to maintain homeostasis. Similar effects have been seen with HBOT as with intermittent oxygen deprivation. Hyperbaric oxygen therapy (HBOT) is delivered in a pressure chamber by breathing 100% oxygen intermittently, several times a week, in an ambient pressure equivalent to 10-20 meters of seawater.

The aim of this thesis was to evaluate potential harms of HBOT for novel indications and to explore biomarkers in experimental and clinical trials in order to enable future precision medicine. We used methods evaluated on healthy volunteers in randomised clinical trials (RCTs) conducted in compliance with good clinical practice (ICH-GCP).

In Paper I, we evaluated Electron paramagnetic resonance (EPR) spectroscopy for measuring reactive oxygen species (ROS) in blood and RNA sequencing (RNAseq) of monocytes in peripheral blood (PBMC), and compared HBOT and HIIT in ten healthy volunteers. We could measure ROS in blood in the same physiological range in both interventions. We also discovered pathways involved in adaption to hypoxia and inflammation that were similar in both interventions. In Papers II and III, we evaluated harms and explored RNAseq in PBMC in an open label RCT where 31 patients with severe COVID-19 were randomised to HBOT or best practice. We observed similar frequencies of adverse events (AEs) in the two groups and could not see any negative effect on vital signs or oxygenation. We discovered a unique transcriptomic signature in the subjects that had received HBOT. The differentially expressed genes were associated with the unfolded protein response, apoptosis, and immune response. In Paper IV, we evaluated harms and described health related quality of life (HRQoL)in an interim analysis of the first 20 subjects froma placebo controlled RCT where 80 patients with Long COIVD were randomised to HBOT or sham treatment. We reported more AEs than expected and severe physical and mental disabilities with a very poor HRQoL. Most AEs were mild, and all were transient.

We have shown that HBOT shares similarities in immune response with HIIT in healthy volunteers. HBOT has a favourable profile of harms and has a potent immunomodulatory effect that is associated with fast recovery for critical COVID-19 patients. HBOT has a favourable profile of harms for patients with post COVID-19 condition. The results provide a base for future clinical trials with HBOT.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals as indicated below:

I. **Comparing the blood response to hyperbaric oxygen with high intensity interval training - a cross-over study in healthy volunteers.** Kjellberg A, Lindholm ME, Zheng X, Liwenborg L, Rodriguez-Wallberg KA, Catrina S-B, Lindholm P. *Antioxidants* 2023; 12: 2043.

II. **COVID-19-Induced Acute Respiratory Distress Syndrome Treated with Hyperbaric Oxygen: Interim Safety Report from a Randomized Clinical Trial (COVID-19-HBO).** Kjellberg A, Douglas J, Hassler A, Al-Ezerjawi S, Bostrom E, Abdel-Halim L, Liwenborg L, Hetting E, Jonasdottir Njastad AD, Kowalski J, Catrina SB, Rodriguez-Wallberg KA, Lindholm P. *J Clin Med* 2023; 12.

III. **Fast recovery of COVID-19-induced acute respiratory distress syndrome after hyperbaric oxygen treatment and changes in endoplasmic reticulum (ER) stress response in peripheral monocytes – A randomized-controlled trial.** Kjellberg A, Zhao A, Lussier A, Hassler A, Al-Ezerjawi S, Boström E, Catrina S-B, Bergman P, Rodriguez-Wallberg KA, Lindholm P. *Manuscript Pre-print* doi.org/10.21203/rs.3.rs-3699049/v1

IV. **Hyperbaric Oxygen Therapy for Long COVID (HOT-LoCO), an interim safety report from a randomised controlled trial.** Kjellberg A, Hassler A, Bostrom E, El Gharbi S, Al-Ezerjawi S, Kowalski J, Rodriguez Wallberg KA, Bruchfeld J, Stahlberg M, Nygren-Bonnier M, Runold M, Lindholm P. *BMC Infect Dis* 2023; 23: 33.

Scientific papers not included in the thesis

Other publications on HBOT, listed by relevance:

I. Kjellberg A, De Maio A, Lindholm P. **Can hyperbaric oxygen safely serve as an anti-inflammatory treatment for COVID-19?** *Med Hypotheses*. 2020 Nov;144:110224. doi: 10.1016/j.mehy.2020.110224. Epub 2020 Aug 30.

II. Kjellberg, A., Douglas, J., Pawlik, M. T., Kraus, M., Oscarsson, N., Zheng, X., Bergman, P., Frånberg, O., Kowalski, J. H., Nyren, S. P., Silvanius, M., Skold, M., Catrina, S. B., Rodriguez-Wallberg, K. A., & Lindholm, P. (2021). **Randomised, controlled, open label, multicentre clinical trial to explore safety and efficacy of hyperbaric oxygen for preventing ICU**

admission, morbidity and mortality in adult patients with COVID-19. *BMJ open*, 11(7), e046738. doi.org/10.1136/bmjopen-2020-046738

III. Kjellberg, A., Abdel-Halim, L., Hassler, A., El Gharbi, S., Al-Ezerjawi, S., Boström, E., Sundberg, C. J., Pernow, J., Medson, K., Kowalski, J. H., Rodriguez-Wallberg, K. A., Zheng, X., Catrina, S., Runold, M., Ståhlberg, M., Bruchfeld, J., Nygren-Bonnier, M., & Lindholm, P. (2022). **Hyperbaric oxygen for treatment of long COVID-19 syndrome (HOT-LoCO): protocol for a randomised, placebo-controlled, double-blind, phase II clinical trial.** *BMJ open*, 12(11), e061870. doi.org/10.1136/bmjopen-2022-061870

IV. Oscarsson, N., Müller, B., Rosén, A., Lodding, P., Mölne, J., Giglio, D., Hjelle, K. M., Vaagbø, G., Hyldegaard, O., Vangedal, M., Salling, L., Kjellberg, A., Lind, F., Ettala, O., Arola, O., & Seeman-Lodding, H. (2019). **Radiation-induced cystitis treated with hyperbaric oxygen therapy (RICH-ART): a randomised, controlled, phase 2-3 trial.** *The Lancet. Oncology*, 20(11), 1602–1614. doi.org/10.1016/S1470-2045(19)30494-2

V. Kjellberg, A., Bjerin, O., Franzén-Röhl, E., Bartek, J., Jr, & Lindholm, P. (2021). **Lemierre's syndrome caused by Fusobacterium necrophorum complicated with multiple brain abscesses-A case report, literature review, and suggested management.** *Clinical case reports*, 9(12), e05142. doi.org/10.1002/ccr3.514

VI. Kjellberg, A., Nyström, H., Söderberg, M., Dlugosz, A., Jörnvall, H., & Steinberg, A. (2018). **Massive air embolism as a complication of upper gastrointestinal endoscopy: A case report illustrating a stroke mimic, literature review, and suggested management.** *Clinical case reports*, 6(9), 1862–1867. doi.org/10.1002/ccr3.1725

CONTENTS

List of abbreviations

AE	Adverse Event
ANOVA	Analysis of variance
AR	Adverse reaction
ATA	Absolute atmospheric pressure
BiP	ER-chaperone immunoglobulin heavy-chain-binding protein
CO	Carbon monoxide
CO_2	Carbon dioxide
COVID-19	Coronavirus disease 2019
DEGs	Differentially expressed genes
ECHM	European Committee of Hyperbaric Medicine
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum associated protein degradation
EQ-5D	EuroQuol questionnaire five dimensions
GPx	Glutathione peroxidase
GR	Glutathione reductase
GRP78	78 kDa glucose-regulated protein
H_2O_2	Hydrogen peroxide
HBO_2	Hyperbaric oxygen
HBOT	Hyperbaric oxygen treatment/therapy
HIF-1	Hypoxia inducible factor 1
HIIT	High intensity interval training
HHP	Hyperoxic-hypoxic paradox
HLoS	Hospital length of stay
HO	Heme oxygenase
HRQoL	Health Related Quality of Life
HSPA5	Heat shock 70 kDa protein 5
ICH-GCP	International Council for Harmonization, Good Clinical Practice

IH	Intermittent hypoxia
kPa	Kilo pascals
Long COVID	Post COVID-19 condition
NEWS	National Early Warning Score
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear factor erythroid 2-related factor 2
O_2	Molecular oxygen
$O_2^{\bullet -}$	Superoxide anion
OXPHOS	Oxidative phosphorylation
PaO_2/FiO_2	partial pressure of arterial oxygen/fraction of inspired oxygen
NO	Nitric oxide
PBMC	Peripheral blood mononuclear cells
QC	Quality control
RAND-36	RAND-36 questions questionnaire for HRQoL
RCT	Randomised controlled trial
RNAseq	Ribonucleic acid sequencing
ROS	Reactive oxygen species
RPE	Rating of perceived exertion
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SOD	Superoxide dismutase
UHMS	The Undersea and Hyperbaric Medical Society
UPR	Unfolded protein response
VO_2max	Maximal oxygen consumption
XBP1	X-box binding protein 1

1 INTRODUCTION

Hypoxic adaptation in COVID-19, can we find some clues in the early evolution?

Let us go back to where evolution began; how changes in levels of oxygen formed the adaptation to life with oxygen (O_2), adaptation of metabolism and the evolution of viruses and immune system. Even if we do not yet fully understand the complexity of the cellular redox pathways, how cells regulate the production and quality control of proteins and how they communicate abnormalities; if we follow the path of evolution it makes sense and follows the same patterns as we can see in nature. We have evolved by adding or deleting functions on a cellular level and many traces from the past can be found in the genome. Some functions from early evolution are merely silenced on behalf of more advanced functions, and many pathways share the same mechanisms. We have evolved through many parallel systems of adapting to oxygen and stress, and when the redox homeostasis is disturbed, this will induce changes to correct homeostasis in health and disease(1, 2).

Many doctors that managed cases of severe COVID-19 during the early pandemic were puzzled by how well adapted to hypoxemia patients were when they came to the hospital about a week after they were infected with SARS-CoV-2. They observed extremely low oxygen levels in blood (hypoxemia) but initially lacking signs of hypoxia in the tissue, normal respiratory rate ("happy hypoxemia"), normal or only moderately elevated lactate levels, normal or high carbon dioxide (3), and upregulated carbonic anhydrases that caused a metabolic alkalosis (4). The syndrome contradicted everything we learnt from J.S. Haldane and Niels Bohr about optimal oxygen delivery (5). It was as if patients evolved back in evolution, back to when the atmosphere was high on carbon dioxide and low on oxygen(6).

The syndrome resembled the way of fermentation, normally occurring in microorganisms like yeast, for energy production, creating a massive amount of carbon dioxide (CO_2) without the need for oxygen(7). There is very little evidence for ethanol fermentation in human cells. Instead, we have the ability to utilize lactate that does not create any CO_2 through a metabolic reprogramming towards glycolysis(8). Instead, we have the ability to utilize lactate that does not create any CO_2 through a metabolic reprogramming towards glycolysis(9). We see this phenomenon in cancer tumours, called the Warburg effect(10, 11). Humans can obviously survive in a hypoxemic state for quite some time when adapted, especially in intensive care, but some patients do not seem to be able to resolve inflammation.

Some people with a mild to moderate disease, without a documented hypoxemia, instead seem to adapt by going into a dormant or quiescent state to resolve inflammation and cannot switch back for unknown reasons. The dormant state may be an adaptation for

cell and organ survival, but it is not compatible with the expected lifestyle of a previously healthy and active person working or studying. The central question remains; what are the underlying mechanisms and what is the trigger for switching back? Can we use hyperbaric oxygen to trigger a switch back to a normal function in acute and post COVID-19 condition; and most importantly for this thesis: can we find evidence in clinical trials?

"Nothing in biology makes sense except in the light of evolution." –Theodosius Dobzhansky (1972)

1.1 Hyperbaric oxygen

1.1.1 Brief history

Hyperbaric medicine has evolved from diving medicine more than 300 years ago. In the 1930´s, when compressed oxygen became readily available, hyperbaric oxygen therapy (HBOT) became a treatment modality (12). Initially compressed air was used due to the fear of oxygen toxicity that was discovered more than a century ago (13). To overcome oxygen toxicity, air-breaks were introduced (14). It was realized empirically, from observation, that hyperbaric oxygen had a number of anti-bacterial and anti-inflammatory effects which caused a "boom" during the 1950´s and 1960´s led by surgeon Ite Boerema, attributed "the father of modern HBOT" (15). It should be noted that all modern protocols in hyperbaric medicine originate from the 1960´s and 1970´s, based on old dogmas that still prevail despite new insights in molecular mechanisms (16). The rationale behind the protocols is empirically established, limited by oxygen toxicity (17). Doctors prescribing HBOT reported a number of beneficial effects, but the underlying mechanisms were not understood and there was an almost complete absence of well-designed clinical trials. Medical adventurism and economical exploitation resulted in a somewhat ironical take on HBOT when evidence based medicine was introduced in the 1980´s (18). The article "A therapy in search of diseases", published in 1987 by Gabb and Robin lists 132 indications for the use of HBOT, present and in the past and highlights many conceptions and misconceptions that still apply today to HBOT.

1.1.2 General principles, protocols, and basic mechanisms

U.S. Navy (USN) Table 6 is the empirically based treatment for decompression sickness (DCS). For repeated treatments, Royal Navy (RN) Table 66 or its variants are normally used (19). The basic principle is breathing 100% oxygen at an increased pressure, Table 6 prescribes 280 kilo Pascals (kPa), and Table 66 prescribes 240 kPa which corresponds to 2.4-2.8 times absolute atmospheric pressure (ATA), equivalent to 14-18 meters of seawater (msw) (Fig. 1). The HBOT protocols are normally 60-90 minutes with two air-breaks of 5 minutes (12).

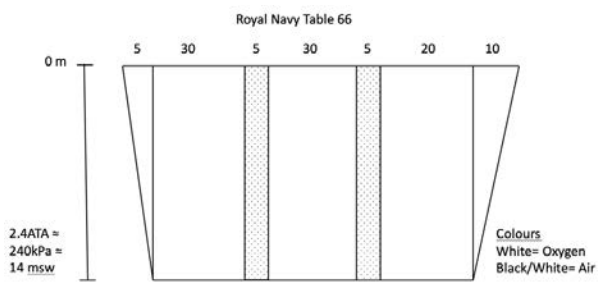

Figure 1. Shows RN Table 66 which is a typical treatment protocol for chronic inflammatory conditions such as diabetic foot ulcers (DFU) and late radiation tissue injury (LRTI).

The basic mechanisms according to current dogma are related to pressure, solubility of gases and diffusion of gases according to Boyle´s, Dalton´s and Henry´s laws (12). HBOT started as a treatment for DCS and gas embolism, but it was soon discovered that it was also beneficial in severe infections (20). It was first suggested to have bactericidal effects, but experimental discoveries during the 80´s suggest that the anti-bacterial properties are mostly related to innate immune cell function and potentiation of antimicrobial drugs (21, 22). Advances in our understanding of HBOT during the last two decades indicate that oxidative stress and/or changes in redox homeostasis are the central mechanism of action (23). Initially, most focus was on angiogenesis since Hypoxia inducible factor-1 (HIF-1) was one of the first transcription factors found to be involved and it was obvious that wounds healed faster when treated with HBO_2 (23, 24). With further insights into molecular mechanisms and redox-sensitive pathways, the anti-oxidative properties of HBOT were discovered, mediated by nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway (25, 26). Immunomodulating properties through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) have been suggested in both acute and chronic inflammatory diseases (27, 28). HIF-1 and NF-κB are intricately connected since hypoxia drives inflammation and vice versa (29, 30); together they transcribe more than a thousand genes that regulate key cellular processes including adaptation to oxidative stress, metabolic reprograming, inflammatory response, proliferation, differentiation, apoptosis and autophagy (29, 31). In order to treat acute inflammation, infections, and ischemia/reperfusion injury, 1-5 treatments with HBOT seem to be enough (32, 33), but for chronic inflammation and infections the empirical and/or evidence-based recommended dosage is 30-40 treatments (34, 35), and sometimes as many as 60 treatments(36).

1.1.3 Accepted indications

The Undersea Medical Society (UMS) was formed in 1967 and added Hyperbaric to its name in 1986 to become UHMS. A Committee on Hyperbaric Oxygen Therapy within UHMS systematically reviews scientific evidence for HBOT and suggests indications for HBOT (37). Most of the 14 indications recognised by UHMS are accepted by healthcare and insurance providers worldwide with some modifications. A similar committee in Europe, The European Committee of Hyperbaric Medicine (ECHM) recently updated their recommendations (38). Some indications have strong recommendations despite low GRADE evidence, such as decompression sickness and gas embolism where the evidence for its benefits is empirical, possibly even based on the wrong hypothesis (39). The GRADE system rates quality of evidence in four levels, from high to very low and the binary recommendations strong or weak (40). The use of "sham treatment" as control groups with associated logistics, costs and patient away time to produce high level of evidence and the use of strong recommendation despite low GRADE evidence highlights a problem

with clinical equipoise in clinical trials with HBOT (41). Below are the currently accepted indications for HBOT according to UHMS.

- Acute traumatic ischemia
- Air or gas embolism
- Arterial insufficiencies
    - Central retinal artery occlusion* (CRAO)
    - Selected problem wounds including diabetic ulcers (DFU)
- Carbon monoxide (CO) poisoning
- Clostridial myonecrosis (gas gangrene)
- Compromised grafts and flaps
- Decompression sickness (DCS)
- Delayed radiation injuries (Soft tissue and bony necrosis)
- Intracranial abscesses
- Necrotising soft tissue infections (NSTI)
- Refractory osteomyelitis
- Sudden sensorineural hearing loss* (SSHL)
- Severe anaemias*
- Thermal burns*

*Not an approved indication in Sweden

1.1.4 Risks and harms

Some of the criticism raised regarding HBOT during the last century concerned the lack of evidence, but also the lack of properly reported risks and harms (18, 42). Since the 90´s, UHMS regularly reports adverse reactions (ARs); but since the reporting is neither mandatory, nor structured according to international regulations for medical products, the reported ARs are likely underestimated (43).

On the other hand, HBOT has been widely used for half a century and it is generally regarded as safe when used for recognised indications (44, 45). HBOT has very few contraindications, and most of them are relative. These include pneumothorax, claustrophobia, and severe obstructive lung disease (46). Despite being regulated as a pharmaceutical intervention, very few clinical trials with HBOT are conducted in compliance with the International Council for Harmonization of technical requirements for registration of pharmaceuticals for human use, Good Clinical Practice (ICH-GCP) (47).

Most commonly reported ARs and frequencies:

- Transient myopia (10%), more common with more than 40 treatments
- Ear pain or sinus pain (2%)
- Confinement anxiety (8%)
- Hypoglycaemic event (0.05%)

- Seizures (0.02%)
- Pneumothorax (<0.01%)
- Transient pulmonary oxygen toxicity (0.02%)

In Sweden and most European countries, O_2 when used to treat an illness is classified as a pharmaceutical intervention and then sold as "Medical oxygen 100%". Regulation is overseen by the Swedish medical products agency (Läkemedelsverket) and the European Medicines Agency (EMA). One of the issues regarding regulatory bodies is that adverse reactions (AR) may arise both from the effects of pressure and of the drug (Medical oxygen 100%). Many regulatory bodies only regulate the drug. The Food and Drug Administration (FDA) in the U.S. is an exception, it also regulates the specific hyperbaric chambers for use of hyperbaric oxygen (HBO_2) (44).

It is well established that there is an inter-individual susceptibility to oxygen toxicity that also varies with different diseases (48). HBOT is delivered according to standardized protocols for a certain indication with minor variances between different centres depending on local routines, but the concept of dose is rarely discussed and there is no established surrogate marker except in the toxic range where central nervous toxicity with seizures indicates the maximum tolerated dose (49, 50). This highlights the importance of conducting clinical trials with HBOT in compliance with ICH-GCP and reporting all adverse events (AEs) when investigating HBOT for new indications.

1.1.5 Randomised clinical trials with HBOT

A few well-designed trials have been performed with HBOT, and yielded conflicting results (51-53). One example is diabetic ulcers, where a well-designed placebo-controlled trial shows better wound healing in selected patients (n= 94) (34) whereas the largest RCT to date (n=120) did not show a significant difference in limb salvage or wound healing in patients with diabetes and lower-limb ischemia (54). The latter was terminated early due to slow inclusion rate and poor subject compliance. Another example is late soft tissue radiation injury; two open label RCTs with crossover design suggested benefit in self-reported symptoms in proctitis and cystitis (35, 55), but a parallel-arm RCT could not show any evidence for benefit in proctitis (56). A well designed RCT that has gained surprisingly low attention has shown that pre-conditioning with only one HBO_2 session prior to on pump coronary artery bypass surgery improves left ventricular stroke volume, improves patient outcome, and shortens hospital length of stay (HLoS) with a positive health economic evaluation (32). However, most clinical trials with HBOT are small and neither designed nor conducted and/or reported in compliance with good clinical practice (GCP). Possible reasons for this are limited recourses, lack of adequate knowledge in trial design and conduct, but also likely a publication bias: 90% of all hyperbaric chambers in the world are private and there is little incentive for research as long as there are paying customers. There is thus no incentive to publish negative trials or

ways to profitably invest into large scale RCTs. The issue with clinical equipoise is also a problem as mentioned previously, and it will be discussed further in ethical considerations. In summary, HBOT is based on old dogmas and possibly wrong hypotheses, and has not adapted well enough into modern requirements of high-grade evidence for benefits and harms. Some major obstacles are lack of incentive and resources for unbiased clinical research, complex mechanisms, and the still poor understanding of the dose-response relationship and individual variance. This highlights the need of a paradigm shift in HBOT, adapting to next generation precision medicine (57).

1.2 Exercise

The benefit of exercise for general health is widely accepted, but the optimal dose is largely unknown (58, 59). There is a positive linear correlation between dose of exercise and health (60, 61). Vigorous exercise seems to be more beneficial for men than women, suggesting a gender difference (62). The sex differences may have developed during evolution with a more severe selection pressure that together with female sex hormones results in higher quality mitochondria and metabolism optimized for periods of starvation and low intensity stress, but not as suitable for glucose metabolism and high intensity stress (63). Since sex hormones seem important for redox homeostasis, the sex differences may vary depending on age and may partly explain why observed differences are larger during the reproductive part of life.

1.2.1 High intensity interval training

High intensity interval training (HIIT) has become popular over the last decade because it is time efficient and may be equally or more beneficial for health than moderate intensity training (64, 65). Its underlying principle is intermittent hypoxia (IH) induced by repeated intervals with >90% of maximal oxygen consumption (VO_2max) or >75% of maximal power followed by an active or passive recovery period, sessions normally lasting 30-60 min depending on intensity (66) (Fig. 2). It is known that HIIT has a greater effect on hypoxia and inflammatory pathways and induction of reactive oxygen species (ROS), which is not undisputedly beneficial (67). HIIT synergistically stimulates the immunosurveillance by regulating the function of various cells in both the innate and the adaptive immune system (68). One of the problems when comparing studies with HIIT is heterogeneity due to the use of different protocols and the lack of a clear definition of dose (69). Exercise, HIIT in particular, is very interesting as a comparator to HBOT since many of the shown mechanisms and proposed health benefits related to the immune system seem to be similar. Exercise also shares many of the shortcomings of HBOT in terms of generating scientific evidence: The effect on the body is holistic, hence very difficult to precisely attribute benefits and scientifically explain mechanisms or establish an effective dose (70). However, both methods are used as adjunctive therapies in conventional medicine and have shown comparable effects on pressure pain threshold, endurance, and

functional capacity, as well as physical performance in fibromyalgia, a syndrome that shares many common features with chronic fatigue syndrome and post COVID-19 condition (71).

Figure 2. A typical protocol for HIIT with four intervals and a total duration of 28 min, used in Paper I.

1.2.2 HIF and the Hyperoxic-Hypoxic Paradox (HHP)

HIF-1 was a ground-breaking discovery more than 30 years ago, named from its activation by hypoxia (72). Since then, many mechanisms have been explored which granted, Kaelin, Ratcliffe and Semenza the Nobel Prize in 2019. HIF-1 consists of a β-unit that is constitutively expressed and an α-unit that is hydroxylated by prolyl hydroxylase domain 2 (PHD2) protein on a proline residue. The α-unit can then be recognized by Von Hippel Lindau (VHL) protein that binds together with its cofactor p300 and recruits E3 Ligase that poly-ubiquitinates the HIF-1α, which is the tag for the complex to be sent to the 26S proteasome for recycling (73). Since its discovery, the understanding of complexity increases every year (74), and it is now known that there are three HIFs (HIF-1, HIF-2 and HIF-3) with overlapping and separate functions (75). HBOT and IH alter many similar pathways, including HIF-1/HIF-2, NF-κB and target genes such as Vascular Endothelial Growth Factor (VEGF), Insulin-like Growth Factor-1 (IGF-1): a phenomenon called "The normobaric oxygen paradox" since it was first discovered with normobaric oxygen (76) and later more accurately called the "The Hyperoxic-Hypoxic Paradox" (HHP) (77). The principle is based on hormesis and the upregulation of various transcription factors that respond to a change in redox homeostasis, induced by the relative change in reactive oxygen species (ROS) rather than the absolute oxygen level. However, many questions remain unanswered before an individualized and effective dosing can be defined (78). There is an ongoing debate if it is redox balance and ROS rather than oxygen that hydroxylates HIF-1α (79, 80) since other factors such as nitric oxide (NO)(81), hyperglycaemia (82, 83), specific microRNAs (miR) (84, 85), transcriptional and post-translational modification have been implicated as players in ROS mediated HIF-1α regulation (86, 87). The debate is semantic, and the importance of HIF-1 is indisputable,

but if we look at the environment from a cell's perspective and regard oxygen as one of many stressors that we have evolved to live with, we can better understand adaptive mechanisms, disease developments, and novel treatment options including HBOT.

1.3 The concept of dose-response

One central question to resolve is how to define and evaluate the right dosage for HBOT. Traditionally, for any type of stress the dose is defined as the area under the curve of exposure: Duration, Intensity (including specific protocols such as HIIT) (70). However, dosage is a much wider term including interval of administration and pharmacokinetics of a drug (88). There is an almost complete lack of evidence for interindividual dose-response and very little evidence for an optimal dose or dose-interval for HBOT (89). It is well understood that there are large interindividual differences related to heritage, sex, age, and disease for HIIT and IH (70, 90). No individual gene has been identified as a robust biomarker for dose-response and efforts focus rather on gene sets or pathways (91). For HIIT, cardio-pulmonary surrogate markers of intensity such as pulse, metabolic equivalents (METs) and metabolic biomarkers such as lactate, or subjective rating of perceived exertion (RPE) such as the Borg-scale are often used to individualize the dose (70, 92). However, there is no available dose-marker for HBOT in clincal practice with regards to transcriptomic or epigenomic effects, even though many recent attempts have been made to establish a better understanding of the effects on oxidative stress, inflammation, and angiogenesis biomarkers (93-95).

1.4 The concept of Hormesis

The ability to adapt to internal and external stressors is fundamental for all living organisms and any disturbance in the homeostasis (balance) will be counteracted to achieve a new homeostasis (Fig. 3). The basis of Hormesis is that the sub-lethal dose of a stressor will have strengthening effects on the exposed system; "that which does not kill us makes us stronger" and repeated exposures will have additive and/or prolonged effects (96). Hormesis is a reproducible and generalizable biological phenomenon in dose-response relationships (97), with a dose-response curve that is typically U-shaped (98) It has gained increasing interest in a wide range of biomedical applications, including drug discovery, benefits and harms assessment in clinical trials (99). A wide region of homeostasis enhances the ability to survive and this region will diminish with age, which also explains why older patients generally have lower ability to survive a severe infection or severe trauma (100). Hormesis is also referred to as "Arndt-Schulz Law", "biphasic dose-response", "U-shaped dose-response", "preconditioning/adaptive response", "overcompensation responses", "rebound effect", "repeat bout effect" and, "steeling effect" in the scientific literature (101). The molecular mechanisms responsible for hormesis are not fully understood, but they involve cells' ability to prevent, detect and repair molecular damage or induce programmed cell death if the damage is beyond repair

(102). It should be noted that there are three major hierarchical levels of hormetic effects in the body; 1. Cell, 2. Organ/organ system, and 3. Organism. Depending on timing, the response seen in the physiology of the patient may be an effect of adaptive responses that occur inside a cell or organ and the adequate treatment may be counterintuitive, which may explain contradictory mechanisms in cell cultures that do not translate into clinical practice (103). Pre-conditioning by exercise or adaptation to high altitude are two examples of hormesis that are easy to understand from an evolutionary perspective, but interestingly enough post-conditioning or "post-injury conditioning" has also been shown in many animal models with different interventions (101).

The concept of homeostasis and hormesis is fundamental for the understanding of oxidative stress, cellular adaptation and the benefits and harms of HBOT. Insights into the molecular mechanisms in the context of personalized medicine of HBOT would enable better design and interpretation of clinical trials.

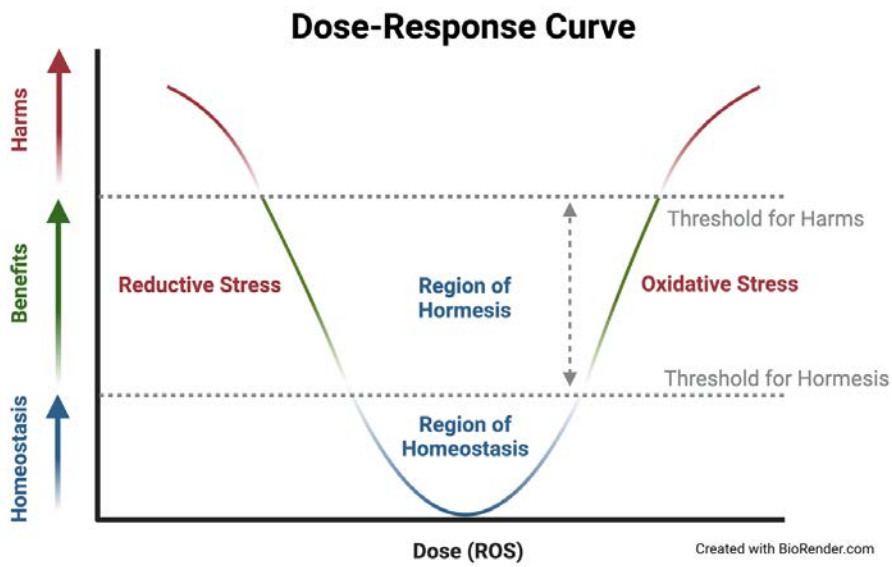

Figure 3. Shows a theoretical dose-response curve for reductive and oxidative stress translated to the possible benefits and harms of HBOT and HIIT. The dose must exceed the threshold for hormesis to induce an effect. If the dose is too high, it will instead cause harms. The regions and thresholds in the picture are individual and dynamic. With hormesis the region of homeostasis increases, and with age and chronic disease the region of homeostasis decreases, hence the threshold for harms will also likely change.

1.5 Redox homeostasis, reductive and oxidative stress

The body is divided into many different compartments that are actively or passively separated from each other, and these compartments need a common signalling system and many different buffering systems for redox homeostasis to prevail (104). To name a few, superoxide dismutase (SOD) is a critical enzyme that catalyses the dismutation of superoxide ($O_2^{\bullet -}$) into O_2 and hydrogen peroxide (H_2O_2)(105). Glutathione peroxidase (GPx) uses reduced glutathione (GSH) to reduce H_2O_2 to H_2O, while glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) back to GSH(106). Heme oxygenase (HO) catalyzes the degradation of heme into biliverdin, carbon monoxide, and free iron, hence it is important for redox signalling(107). Thioredoxin reductase (TrxR) is another important enzyme that together with Thioredoxin acts like a ROS scavenger, upholding the reductive state in proteins, including HIF-1α (108) (Fig.4). Cells and intracellular structures depend on active pumps to maintain homeostasis within each organelle, many pumps, receptors, enzymes and other proteins are redox-dependent (109). Emerging evidence indicates that ROS act as second messengers for a variety of growth factors and cytokines (110). Since we are adapted to atmospheric oxygen and mainly use oxidative phosphorylation (OXPHOS) for energy production, in health there is an overweight of anti-oxidizing systems. But to be able to adapt to infections and periods of stress, we also need to produce ROS by other means (111). The family of NADPH oxidases (NOX) whose sole purpose is to produce ROS, that are important for all cells to uphold/change redox-homeostasis, if the nicotinamide adenine dinucleotide (NADH/NAD+) and nicotinamide adenine dinucleotide phosphate (NADPH/NADP+) ratios are altered (112). NOXs are found in many different cells and many cells express different types of NOX, the vasculature express NOX1, 2, 4 and 5 whereas granulocytes mainly express NOX2 (113). ROS are, as the name suggests, highly reactive oxygen-containing molecules that have been considered harmful because they are known to react with and damage important biological molecules such as fatty acids and DNA, and there is a strong link between oxidative stress and chronic inflammatory diseases such as hypertension and diabetes (114, 115). However, it is now known that reductive stress is equally harmful if the redox balance is disrupted in a dose that exceeds the cells buffering capacity, since ROS are crucial for cell signalling (116). The balance between ROS, Reactive Nitrogen Species (RNS) and antioxidants, called redox homeostasis or redox balance, must be highly dynamic to start or shut down cellular processes (117). Some 90% of all oxygen consumed by humans is used for adenosine triphosphate (ATP) production in the mitochondria, inducing ROS when oxygen acts like the final electron acceptor in the electron transport chain. ROS also have important roles in many other cellular processes (118). A number of transcription factors are involved in maintaining the redox homeostasis including HIFs, peroxisome proliferator-activated receptor γ coactivator 1α (PGC1-α), Nrf2, and NF-κB (119, 120). HIFs are major regulators of the redox sensitivity in mitochondria (121), PGC1-α is a major regulator of energy metabolism and mitochondrial biogenesis (122), NRF

is the master regulator of cellular antioxidant systems and mitochondrial quality control (123), and NF-κB is a key regulator of inflammation and cell survival (124). Knowing the importance of redox balance, many attempts have been made to experimentally suppress inflammation with antioxidants such as vitamins and other antioxidant enzymes with paradoxical results; indicating that ROS vary with time and location (125). To regulate and signal redox homeostasis in each individual compartment, a transmitter is needed that can freely cross barriers. Substances that have that property are light gases. It is known that nitric oxide (NO), carbon dioxide (CO_2), carbon monoxide (CO), and hydrogen sulphide (H_2S) function as so-called gasotransmitters (126). $O2^{\bullet -}$ is extremely short-lived (~1μs) and due to its ionic properties does not readily cross membranes but appears to be intimately linked to the redox balance and a function of the gasotransmitters CO, NO and H_2S in blood (127-130). NO is an important signalling molecule that is even more short-lived than $O2^{\bullet -}$ and differs significantly because it readily passes biologic membranes, and hence may be intimately linked to superoxide in regulating the trans-membranous redox balance in inflammatory cells (131). NO also plays a central role in cellular respiration by binding to complexes in the mitochondrial respiratory chain, thereby regulating mitochondrial function (132). To date, there is no way of measuring NO availability directly. In fact, much of the published evidence for ROS is actually measuring products of NO and superoxide, such as peroxynitrite (ONOO-) or nitrotyrosine (133) (Fig. 4). A variety of methods have been developed for the measurement of ROS, but most of them are not specific for a particular oxygen radical but rather react with a set of radicals (134).

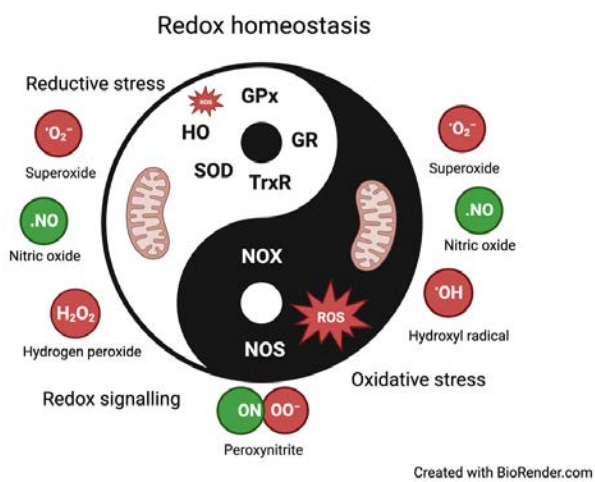

Figure 4. ROS and intermediates involved in redox homeostasis. Some of the most important ROS are $O_2^{\bullet -}$, H_2O_2, •OH and •NO. The balance between ROS and NO is critical for maintaining cellular homeostasis, and while they have distinct mechanisms of action, they can interact and influence each other. Together with various enzymes such as nitric oxide synthase (NOS) and NOX, and various antioxidants, homeostasis is maintained.

Electron paramagnetic resonance (EPR) is the gold standard for direct measurements of ROS (135). By using different spin probes and inhibitors of various redox enzymes, it is possible to measure site-specific ROS in various compartments (136). Cyclic hydroxyl amines such as CM-H rapidly react with ROS, producing stable nitroxide radicals, which can then be quantitatively measured by EPR (137).

Our understanding of redox homeostasis and signalling has grown significantly over the past few decades (138). However, due to the compartmentalized, context-sensitive, and short-lived nature of ROS, studies yield paradoxical results, leaving many questions unanswered still (116).

1.5.1 Redox sensitive immune response

The immune system has also evolved in a Darwinian way from the innate- to the addition of adaptive immune system that emerged some 500 million years ago and may have catalysed evolution as a positive survival factor (139). One of the theories on how viruses evolved is that single stranded RNA viruses, the ancestors of SARS-CoV-2, emerged even before the first eukaryotic cell (140). The innate and adaptive immune systems co-exist and have to be tightly regulated to fine-tune a timely defence upon invasion, not to be activated by endogenous receptors or non-pathogenic organisms that co-exist in our bodies (141). The differentiation map of the immune system is slowly being re-drawn since Waddington developed the concept of "the epigenetic landscape" (142). Advances in stem cell research have rocked the rigid landscape and suggest that the differentiation and maturation in the Waddington landscape can not only be partly reversed but also cross-tracked, called trans-differentiation or polarisation when it comes to immune cells (143). Innate immune cells such as monocytes respond to external and intrinsic stimuli and change their phenotype "on demand" (144). The phenotype was traditionally based on the expression of co-receptors for toll-like receptor 4 (TLR4). The introduction of next generation sequencing (NGS) and multi-omics has increased our understanding of monocyte function, and thus increased the awareness of additional complexity, which leaves many questions unanswered (145, 146). PBMC can choose path to inflammatory or anti-inflammatory phenotypes depending on redox homeostasis in blood; in a similar fashion B-cells, T-cells, NK-cells, macrophages and dendritic cells can also revert its phenotype when in tissue depending on redox balance, a way to locally turn on or off inflammation depending och supply and demand (147, 148). Chronic hypoxia alters the functions and fate of the immune cells depending on redox homeostasis, and leads to an exhaustion of T-cells with an increase in senescent cells that are dysfunctional (148-150). Imbalance in the different phenotypes of T-helper cells (CD4+) is associated with immune deficiency and auto-immunity (151, 152). T-killer cells (CD8+) are highly dependent on OXPHOS for optimal function, hence hypoxia and dysregulation of redox homeostasis will impair their killing capacity (153, 154). Viruses can exploit this phenomenon to evade the immune system; typical examples are chronic viral infections such as Hepatitis B and C,

Epstein-Barr, and other Herpes viruses (155, 156). Redox homeostasis is crucial for an adequate immune response. Interestingly, HIF-1α protein activation during hypoxia is NF-κB dependent in macrophages, a relationship not seen with HIF-2 that is associated with chronic hypoxia (29) Many questions remain to be answered regarding the host-pathogen response and the interplay between HIFs and NF-κB in viral infections, but there is compelling evidence for the importance of redox regulation of multiple pathways (157).

1.6 Micro RNA

Micro-RNA (miR) is a group of small RNA, 19-25 nucleotides long, that do not encode for proteins but have other functions including regulation of post-transcriptional gene expression (158). More than 1800 miRs have been identified in the human genome (159). Some miRs have been identified as important regulators of specific biological processes, such as ER stress and the UPR (160, 161). Many of the miRs with known functions seem to be involved in negative feedback mechanisms and act as adaptive switches (162-164), MiR-210 is "the master hypoxamiR", a direct regulator of HIF-1(165) and miR-34a is intricately involved in NFκB signalling (166). Due to their involvement in adaptive mechanisms, miRs are interesting as biomarkers in many settings, including exercise, due to their stability and biological diversity (167). Circulating miR have been evaluated as biomarkers for different types of exercise in humans (168) and a recent attempt to evaluate some miR as dose markers for HBO_2 has been made (94). One of the biggest hurdles of translating miR to become reliable and reproducible biomarkers in clinical practice is the problem to find suitable internal reference genes, especially for circulating miR (c-miR) in plasma or serum. The search for suitable reference miRs is time consuming and expensive, therefore U6 is commonly used in tissue and cells, but this is not suitable for c-miR(169).

1.7 The endoplasmic reticulum

The Endoplasmic reticulum (ER) is a large dynamic organelle that is situated adjacent to the nucleus, lodges ribosomes for protein synthesis on the cytosolic side but also functions as calcium storage and is involved in lipid metabolism (170). The ER is the major protein factory in the cell, responsible for processes of translation of mRNA and posttranslational modification with chaperones and folding factors that implement an extensive quality control (QC), endorsing perfect products before they are transported to their intended destination (171). The logistics of protein production is complex. A number of different pathways and transcription factors are involved, including HIF-1, NF-κB, and Nrf2, to fine-tune the speed and optimize the quality of finished proteins. The importance of the system is evident since many developmental, acute, and chronic diseases are associated with, if not caused by a dysregulation of protein quality control (172).

1.7.1 ER stress and the Unfolded protein response

ER stress is basically an overload of the protein production that can be caused by a plethora of stressors that change the redox balance within the cell; this can be toxins, infections with virus, bacteria or other pathogens that trigger an inflammatory response, but also environmental stress such as heat (173). ER stress, loss of homeostasis, leads to an accumulation of misfolded or unfolded proteins and vice versa, and evidently causes various diseases in humans if not adequately controlled (174).

The unfolded protein response (UPR) is a central part of the DNA-RNA-protein flow. One of the primary responses is a global inhibition of protein synthesis to reduce the load on the ER (175). The UPR is mediated by one master control protein, the ER chaperone immunoglobulin heavy-chain-binding protein (BiP) also known as Glucose-Regulated Protein 78 (GRP78), and the three sensors: PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and the ER transmembrane protein kinase/endoribonuclease (IRE1) (176) (Fig. 5). The UPR is triggered when the cell cannot maintain homeostasis, which leads to a build-up of inadequately processed or unfolded proteins, hence the name UPR. The UPR will activate adaptive programs across the protein assembly line to restore the homeostasis; through feedback mechanisms, it senses the result and triggers autophagy or apoptosis if homeostasis cannot be restored despite actions taken (177). An important part of the regulation involves energy production, therefore the UPR is intimately connected to mitochondria that are the main producer of ATP and ROS in most cells (178). Mitochondria have their own UPR (UPR_{mt}) that is induced in a very similar fashion by unfolded proteins in the mitochondrial matrix (179). ER and mitochondria are connected in a synapse-like way where the signalling substances are Calcium and ROS (180). There is no doubt that the mitochondria and the ER are intertwined and crucial parts of the cells adaption to stress, but to fully understand the complex mechanisms involved in the integrated stress response (ISR) we may need to trace it back to the beginning of evolution (181). Mitochondria are far from being only the power plants of the cells; they are highly dynamic and mobile organelles that regulate the internal environment in the cells by redirecting metabolism to maintain redox homeostasis (182). Interestingly, the same adaptive mechanism of UPR and mitochondria maintaining cellular redox homeostasis has been shown over the whole kingdom of life, from yeast *(Saccharomyces cerevisiae),* and animals (*Caenorhabditis elegans*) to plants (*Arabidopsis thaliana) (178).* Most of the current mechanistic insights into mitochondrial regulation in health and disease are derived from studies on yeast, and many acute and chronic inflammatory diseases in humans suggest a direct or indirect involvement of mitochondrial dysfunction (183).

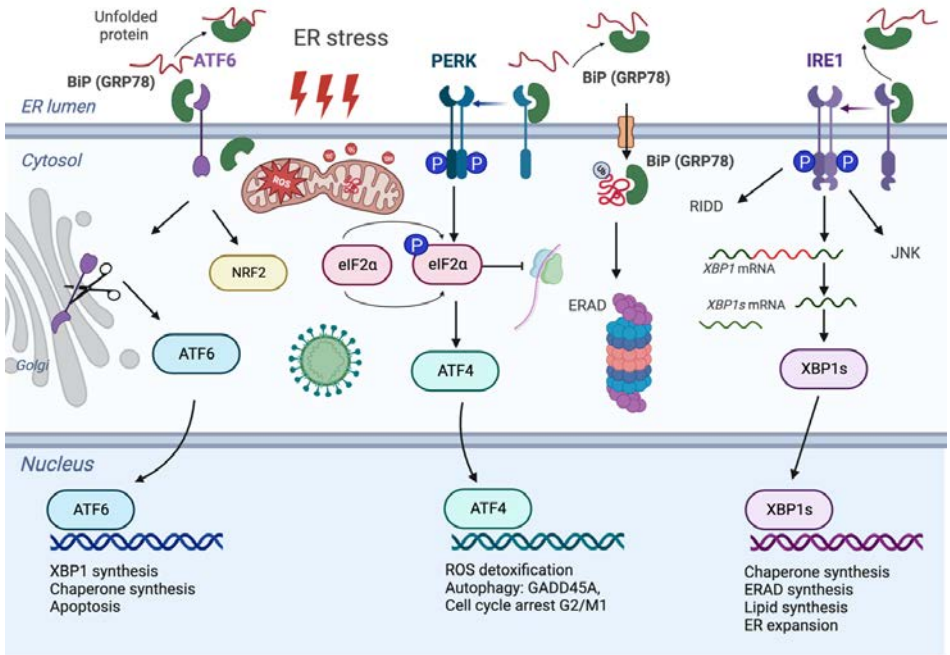

Figure 5. Basic mechanisms and signalling involved in the UPR. In homeostasis, the ER chaperone BiP (GRP78) associates with three UPR sensors: IRE1, PERK, and ATF6, inhibiting their activity. Upon ER stress or misfolded protein sensing, BiP dissociates from the UPR transducers, allowing the activation of downstream signalling. Each UPR pathway is activated by a distinct signal transduction system. IRE1, undergoes homodimerization and autophosphorylation which activates its RNase domain that splices XBP1 mRNA to its active form. XBP1s translocate to the nucleus, promoting the expression of UPR target genes, including chaperones and ERAD. IRE1 also participates in the RIDD pathway, where it degrades mRNAs, reducing the protein load in the ER lumen. IRE1 also enhances the c-Jun N-terminal kinase (JNK) pathway, leading to apoptosis. PERK increases eIF2 alpha subunit phosphorylation, dampening protein translation to alleviate ER protein overload. Paradoxically, this process upregulates ATF4 mRNA, triggering the activation of proapoptotic C/EBP homologous protein (CHOP) and other UPR target genes. ATF6 translocate to the Golgi apparatus and undergoes cleavage by proteases. The cleaved fragments then move to the nucleus, activating ATF6's target genes, including chaperones and XBP1. If the adaptive response fails to resolve ER stress, UPR signalling may be upregulated, ultimately leading to apoptosis. (Created with BioRender.com)

1.7.2 Molecular Chaperones

Molecular chaperones are proteins that are essential as facilitators in protein folding by preventing misfolding and aggregation, assisting in the transport of proteins across cellular compartments, and aiding in the degradation of misfolded or damaged proteins (184). These functions are vital for maintaining cellular homeostasis and preventing the accumulation of non-functional or harmful protein aggregates (185). Molecular chaperones were first identified to be induced by heat, hence the name heat-shock proteins (hsp), but this is now known to be a universal defence mechanism against ER stress, conserved among species, from procaryotes, yeast, plants to mammals (184, 186, 187). The production of proteins is "core business" for all cells except erythrocytes. Many of the proteins need to be folded by chaperones before they are finished, hence it's not surprising that cells have an intricate network of more than 200 chaperones and co-chaperones (188). The 70-kDa heat shock proteins (hsp70) seem to be one of the most important families of chaperones, since they are involved in maintaining homeostasis at all levels of protein production from transcription to degradation (189). The hsp70 family member GRP78/BiP is of specific interest, since not only is it a key player in the UPR but is also known to translocate to the plasma cell membrane where it acts like a receptor for many important ligands, including major histocompatibility complex 1 (MHC-1) involved in T-cell immediate response (190) (Fig. 6).

1.7.3 UPR and viral infections

Viruses cannot replicate on their own and therefore they need to exploit their hosts protein factories. SARS CoV is known to induce ER stress and activate the UPR because of high translational turnover and accumulation of unfolded or small post-translational polypeptides in the cytoplasm as virus assembles (191). If the UPR is activated but the cell despite this cannot restore redox homeostasis, the cell is triggered to go into apoptosis (177). Many viruses have adapted to UPR and use this to their advantage (192). By halting the cell cycle in G2/M, the cells will not go into apoptosis and instead go into senescence which gives the virus time to continue to replicate even though the cells reduce the production rate of proteins (193). SARS-CoV-2 S protein induces all three key pathways in the UPR (194) (Fig. 6).

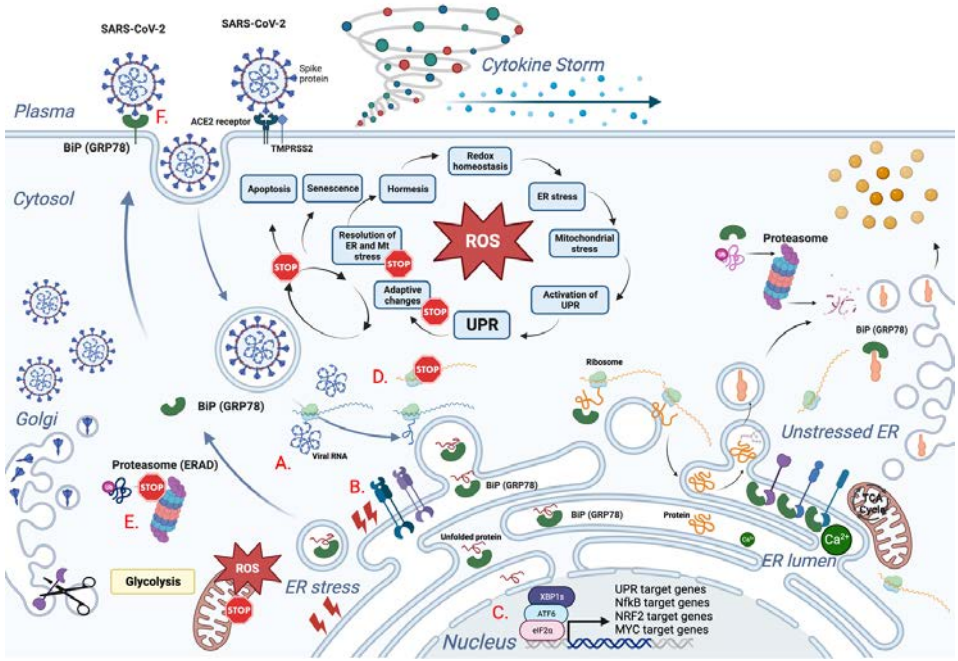

Figure 6. Summary of alterations involving the UPR when SARS-CoV-2 invades a cell. SARS-CoV strategically manipulates the UPR to create an environment supporting its replication and survival. After entry, SARS-CoV-2 releases its RNA and by overwhelming the ER with the synthesis of viral proteins the accumulation of unfolded or misfolded proteins, induces ER stress and is activated by BiP (A). IRE1, when activated, can promote cell survival or apoptosis. The virus may modulate this pathway to favour its replication and survival (B). The spliced XBP1 regulates the expression of UPR target genes. SARS-CoV-2 may exploit this to enhance the expression of factors that benefit viral replication (C). ATF6 is activated, translocates to the Golgi apparatus, and is cleaved to an active transcription factor that regulates the expression of UPR-related genes. PERK activation and phosphorylation of eIF2α attenuates global protein synthesis but SARS-CoV-2 may downregulate host cell translation, redirecting cellular resources to favour viral protein synthesis (D). SARS-CoV may interfere with ERAD, contributing to the virus ability to evade host cell defence mechanisms (E). During prolonged ER stress, BiP translocate to the plasma surface, acting like a receptor that may be used by SARS-CoV-2 to entry and evade the immune response (F). (Created with BioRender.com).

1.8 COVID-19 and dysregulated immune response

The SARS-CoV-2 pandemic has put an unprecedented burden on society and healthcare with 771,820,937 confirmed cases, including 6,978,175 deaths reported on the WHO dashboard per November 20th 2023 (195). Despite 400 000 articles including >40 000 review articles and >4000 clinical trials with published results, no silver bullet is available

for treatment of severe and critical COVID-19 and quite ironically, some of the oldest tools in the box seem to be the most effective; Low molecular weight heparin (LMWH) and corticosteroids, as learnt from the adaptive pragmatic REMAP-CAP and RECOVERY trials (192, 193). In COVID-19 some patients, for unknown reasons, the innate immune system seems to over-react and cause an uncontrolled inflammation, popularly called a cytokine storm (194). Recent evidence suggests that the activation of innate immune cells in severe COVID-19 causes a disseminated vascular inflammation that leads to a cascade of vascular damage and coagulopathy (196). Endothelial cells seem to be key players in the disease, and emerging evidence suggests that the endothelitis and pathways involved in the disseminated inflammation are potential treatment targets (197, 198). Obesity, diabetes, cardiovascular disease, and male gender are associated with more severe COVID-19; interestingly, these risk factors are also associated with inflammatory profile or classically activated (M1) macrophages (199). In the broncho alveolar fluid of patients with mild COVID-19, the M1 infiltration was minimal and clones of CD8+ T cells with a tissue-resident memory T-cell gene signature were observed (200). The homeostasis between M1 and the anti-inflammatory type or alterative activated macrophages (M2) is context sensitive and complex and during chronic ER stress the resolution is rather associated with M1 phenotype (201). Different immunological signatures are associated with disease severity of COVID-19 and development of post COVID condition (202, 203). We have deepened our understanding of immunological mechanisms known from the SARS-CoV and MERS epidemics, suggesting to target the innate immune system, but it has become evident that precision medicine is required to tailor individual treatment (204). There are interesting similarities in how cancer-cells interact with healthy cells and their respective impact on the immune response that can help us understand adaptation to hypoxia in COVID-19 (205). The communication between monocytes/macrophages and T-cells is complex and dependent on the intra- and extra cellular environment. It seems that we should look beyond infection in severe COVID-19 to better understand the immune response.

1.8.1 HBOT for severe COVID-19

Endothelitis with impaired redox-balance of endothelial cells may explain many of the complications seen in COVID-19 (206, 207). Inflammation drives hypoxia and vice versa, hence it is not surprising that major risk factors for severity of COVID-19 are associated with chronic inflammation and a dysregulation of HIF-1 (208). HIF-1 stabilization is one well described mechanism of HBOT (209). HBOT has also been shown to polarize M1 macrophages to M2 in animal models (210) and shifted macrophages from M1 to M2 in an experimental model of acute lung injury (211). The known anti-inflammatory and immune-modulating effects of HBOT with evidence from the SARS-CoV-1 and MERS epidemics formed our hypothesis in the dawn of the pandemic (212). This hypothesis is yet to be proven or rejected, but clinical data from four randomised controlled trials including 227

patients support that HBOT improves oxygenation and reduces the inflammatory response(213-217).

1.9 Post COVID-19 condition (Long COVID)

The WHO definition of the post COVID-19 condition, also called Long COVID is: the continuation or development of new symptoms three months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least two months with no other explanation(218) But there is still not a universal consensus on the definition of the syndrome (219, 220). COVID-19 causes lingering physical and mental symptoms in 45% of infected patients regardless of disease severity (221). In a recent longitudinal follow up after COVID-19 in unvaccinated patients, 23% had not recovered after six months. The disease put a big burden on society, healthcare, and individuals since most of the patients with symptoms after 12 months showed little or no improvement and up to 18% had ongoing symptoms at two years after infection (222). Most patients with low HRQoL in Long COVID have not been hospitalized, and female sex is consistently reported as a risk factor for Long COVID (223, 224). More than 200 symptoms are described, with the most common symptoms being fatigue, post exertional-malaise and brain fog (225). The initial hypoxia may have triggered cellular adaptations, but for unknown reasons the immune response becomes dysregulated (226). Long COVID patients with brain fog have elevated IFN-γ responses to internal SARS-CoV-2 proteins, enhanced activation of Th-cells, but impaired $CD8^+$ T cell memory compared with patients that recovered from COVID-19 (227). Endothelial dysfunction is associated with chronic inflammatory disease and a risk factor for cardio-vascular events (228). Endothelial dysfunction is associated with COVID-19 (229, 230). Endothelial dysfunction seems to be equally associated with Long COVID that share many similar features with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) (231, 232). The chance of improvement is particularly low for patients that fulfil the Canadian consensus criteria for ME/CFS (233).

Long COVID with its many different diffuse symptoms appears as a heterogeneous disease but almost all symptoms could be explained by a dysregulated redox homeostasis with secondary mitochondrial dysfunction and cell senescence (207). Metformin started within three days of COVID-19 onset in an outpatient population reduced the risk for Long COVID by 41.3%, absolute risk reduction with 4% (224). Interestingly, Metformin is known to activate the UPR by downregulation of GRP78 and inhibition of mTOR (234) and switch cancer cells from senescence to apoptosis in experimental settings(235, 236).

1.9.1 HBOT for post COVID condition

HBOT has previously been shown to increase cell proliferation and oxidative stress resistance in endothelial cells (237). HBOT is widely used as an "off label" treatment despite lack of evidence, due to a plausible pathophysiological mechanism (238, 239).

Recently, the first randomised controlled, double blinded placebo-controlled trial with HBOT for Long COVID showed that 40 sessions of HBOT improve cognitive function and symptoms, also associated with changes in MRI of the brain, in patients with neurocognitive symptoms (240). Two major shortcomings with the otherwise well designed RCT with HBOT for Long-COVID are that it is not conducted in compliance with ICH-GCP and that it lacks a long-term follow-up. There are still many unanswered questions regarding mechanisms in Long COVID, the potential long-term benefits and cost-efficiency of HBOT (241).

1.10 Rationale for the thesis

Hyperbaric therapy has been used in alternative medicine for more than 300 years as an alternative therapy and in conventional medicine for more than half a century. With the inclusion of HBOT into conventional medicine, the requirements for evidence and cost-benefit analyses have increased. Extensive research has been conducted in the field over the past 40 years, yet many questions are unanswered, some of the mechanisms are over-simplified, and some are based on old dogmas, possibly based on the wrong hypotheses. Most importantly, there is a huge knowledge gap regarding dose and timing and how to individualize the treatment to maximize benefits and reduce harms. The initial plan for this thesis was to try to find a biomarker for dose-response with preclinical research and healthy volunteers, but COVID-19 came in the way during early 2020 so we changed course into clinical trials. With modern technology such as transcriptomics it would be possible to gain a deeper understanding of the complex mechanisms induced by HBOT. Understanding the mechanisms involved in virus-host response has important clinical implications for the management of severe COVID-19 patients, and it may also add knowledge to the conundrum of inflammatory diseases in the ICU such as sepsis and ARDS, as well as chronic inflammatory diseases and complex syndromes such as post COVID-19 condition. Cumpstey et al. have summarized their holistic perspective on COVID-19 as a "redox disease" that aligns well with our understanding and overall hypothesis (242). Conducting RCTs in compliance with ICH-GCP guidelines ensures scientific rigor, patient safety, and data integrity. These strengths contribute to the credibility of the trial results, promote ethical conduct, safeguard participant welfare, and provide reliable evidence to support medical and regulatory decisions.

1.11 ER stress explained from a lean management perspective

The human body has used lean management some million years before Toyota made it famous in our generation. Every part of each cell practices the philosophy to reduce waste. The lean assembly line (ER and Golgi apparatus) with the workstations (ribosomes) assembles proteins on demand according to the blueprint (mRNA), printed from the template type (DNA). The assembly line is overseen by the QC manager (BiP/GRP78) from the QC department, division of protein assembly (UPR) that regulate the production speed on demand to minimize waste and do constant quality checks at all parts of the production line. If manufactured proteins do not pass QC, they cannot continue through packing and logistics (Golgi) to leave the factory, but are instead discarded in a recycling container (proteasome). The QC managers are key positions for lean production and just in time delivery to optimize cost efficiency. The QC managers (BiP/GRP78) seem to be very important for lean training since they are also involved in logistics and communication (plasma membrane receptors) and in power source allocation (ER-mitochondria interface) in times of stress and crisis. The board of directors (transcription factors such as NF-κB, HIF-1, NRF2 and MYC) oversees the daily business operations, changing blueprints, setting production goals, while also providing strategic oversight with decision-making involving sustainability (redox regulation), expanding or closing down assembly lines (membrane expansion, mitophagy, autophagy), whole factories (cell apoptosis) or sometimes the whole company (organ or organ system shutdown). The latter with inevitable consequences in terms of human disease. (Fig. 6).

The working environment is an integrated part of lean production. All protein factories in the cells (with the exception of red blood cells) are mainly powered by powerplants (mitochondria) that run on fossil fuel (glucose) which consumes plenty of oxygen and leaves a significant environmental footprint in terms of CO_2 and other intermediates for greenhouse gases (ROS). During lean production, we have enough catalysators and filters (antioxidant enzymes and ROS scavengers) to cope with this, but if these systems break down, we are faced with an environmental crisis. In conditions of crisis or stress we can use less economical, but more environmentally friendly power sources (glycolysis and lactate fermentation). We use alternative sources of energy all the time, but the fraction depends on the type of factory, sustainability goals and environmental awareness of the board of directors' and more than anything depends on supply and demand for protein production and energy (lifestyle factors, acute and chronic diseases).

It's generally known that support functions are needed in larger companies, and this is where it becomes complicated! There are many different departments such as IT, human resources, legal, economy, communication, marketing, maintenance, and repair etc.

In my view, this is where conventional and integrative medicine have different approaches with their respective pros and cons. Conventional medicine focuses on fixing the supply and demand problem by changing the company's marketing or sales strategies (cytokines and other substances that can be measured in plasma) by receptor blockers. This is effective for symptoms since cutting down marketing and sales will result in a lower production rate, but is not sustainable for lean production and environmental health. A toxic workplace culture is rarely fixed by changing external communication or replacing a manager; the workers need to be involved in major changes. Integrative medicine tries to analyse the structural problems and to change the company culture which may be effective for chronic disease but probably most beneficial for preventing disease. Unfortunately, the complexity of the problems is not fully understood and trying different managing consultant strategies (alternative and holistic treatments, including HBOT) are often expensive, time consuming, rarely evidence based and only effective if it changes the working climate in the factory, and not sustainable unless the company culture is actually changed.

"Research is to see what everybody else has seen, and to think what nobody else has thought."–Albert Szent-Györgyi

2 RESEARCH AIMS

The overall aim of this thesis was to evaluate harms of hyperbaric oxygen therapy (HBOT) for novel indications and explore biomarkers in experimental and clinical trials to enable future precision medicine. Specific aims are listed for each study.

- To evaluate methods and protocols for oxidative stress and transcriptomic changes in response to HBO_2 for future studies of dose-response. (Study I)

- To evaluate harms of HBOT for patients with severe COVID-19 with moderate to severe ARDS and explore oxygen toxicity in a randomised clinical trial in compliance with ICH-GCP. (Study II)

- To explore transcriptomic changes related to hypoxic adaptation and inflammation in a randomised clinical trial in compliance with ICH-GCP. (Study III)

- To evaluate harms of HBOT for post COVID condition and describe self-reported quality of life, symptoms, and objective findings in a randomised clinical trial in compliance with ICH-GCP (Study IV)

""The aim of science is not to open the door to infinite wisdom, but to set a limit to infinite error." -Bertolt Brecht

3 MATERIALS AND METHODS

The following section provides a brief description and discussion of materials and methods used in this thesis. Detailed information on all materials and methods is presented in the respective papers I-IV and in the protocols previously published.

3.1 ETHICAL CONSIDERATIONS AND APPROVALS

3.1.1 Paper I: Ethical approval

EPM dnr: 2019-01864.

HIITO$_2$, the experimental study with ten healthy volunteers, was conducted according to the principles of the Declaration of Helsinki (2013). There were very small risks involved for healthy individuals, but a thorough risk-benefit-assessment was conducted and presented in the ethical application. We used a dose of HBO$_2$, much lower than that used for medical treatment, that is the dose used by many diving special forces to predict the risk of seizures when diving with oxygen. The main ethical consideration was regarding blood samples and biobanking; we analysed gene-expression and there was detailed information in the subject information that was also approved by the responsible biobank (KI biobank). Since we detected a significant effect on gene expression, it could be argued that we induced a pharmacological effect of oxygen despite a much lower dose than that used in clinical practice. If repeated, the study should be conducted in compliance with ICH-GCP, including approval from Läkemedelsverket since even a lower dose may have pharmacological effects.

3.1.2 Papers II and III: Ethical approval

EPM dnr: 2020-01705, and amendments 2020-06279 and 2021-01215.
Läkemedelsverket approval: Dnr 5.1-2020-36673.

The trial was registered in the European Medicines Agency (EMA) database, EudraCT number: 2020-001349-37 and on ClinicalTrials.gov, Identifier: NCT04327505.

COVID-19-HBO was conducted in compliance with ICH-GCP. There was a thorough risk-benefit evaluation in the protocol that was approved by EPM and Läkemedelsverket in May 2020. There is an ongoing debate in the medical community regarding clinical equipoise, some still argue that HBOT may be harmful for these patients. The trial was stopped due to futility, with only 34 of the planned 200 subjects included. The trial could not start at Karolinska University Hospital until December 2020 and not until November 2021 in Germany, because the management at Karolinska and the ethical committee in Regensburg did not agree with the clinical equipoise and suspected harms, despite the approval of EPM and Läkemedelsverket in Sweden. Hence it was also important to publish the protocol early to help other researchers to plan trials elsewhere. The potential benefit

would have been the greatest early during the pandemic when resources were limited. In retrospect it could be argued if it was the right thing to do to initiate the trial with such a long delay, but at that time we did not know how the pandemic and the virus would evolve. The interim analysis was reviewed by an independent DSMB. The data provided to the DSMB-members raised no safety or ethical issues, and the recommendation was to continue the trial as planned based on safety and ethical concerns. This highlights the importance of conducting future trials in compliance with ICH-GCP, but also informing local ethical committees and the medical community in general about the principles of ICH-GCP.

3.1.3 Paper IV: Ethical approval

EPM dnr: 2021-02634, amendment 2021-04572.
Läkemedelsverket approval: Dnr: 5.1-2021-43347.

The trial was registered in the European Medicines Agency (EMA) database, EudraCT number: 2021-000764-30 and on ClinicalTrials.gov, Identifier: NCT04842448.

HOT-LoCO was conducted in compliance with ICH-GCP. In the protocol there was a thorough risk-benefit evaluation, and the protocol was approved by EPM and Läkemedelsverket in September 2021. In the HOT-LoCO trial there was an ethical consideration regarding "sham treatment". Many subjects had severe fatigue and especially post exertional malaise. Some subjects were not able to complete the ten treatments. Two interim analyses have been reviewed by an independent DSMB. The data provided to the DSMB-members raised no safety or ethical issues and the recommendation was to continue the trial as planned based on safety and ethical concerns. There are still many unanswered questions related to Long COVID and no convincingly effective treatment exists, hence it is important to adhere to the protocol.

"Ethics is not about the way things are, it is about the way things ought to be." -Michael Josephson

3.2 STUDY DESIGN AND SUBJECTS

3.2.1 HIITO2 (Paper I)

Ten healthy physically active volunteers, aged 20–55, were included in this crossover-study and performed two interventions: a 28 min HIIT session and 28 min HBO2 in a crossover design. Blood gases, peripheral venous oxygen saturation (SpvO2), and ROS levels were measured in peripheral venous blood (Fig. 7). We evaluated bulk RNA sequencing data from PBMCs, with a separate analysis of mRNA and microRNA.

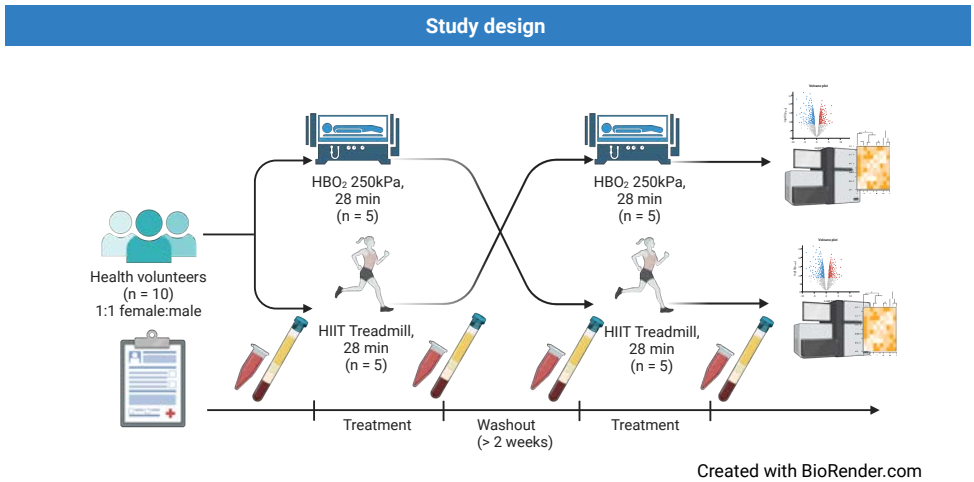

Figure 7. Study design for the HIITO2 study

3.2.2 COVID-19-HBO (Paper II and III)

34 patients with severe COVID-19 were included in Sweden and Germany, 31 in Sweden of which 23 at Karolinska University Hospital in this open-label, parallel-arms randomised controlled trial comparing HBOT plus best practice (Intervention) with best practice (Control). The patients were recruited directly on hospital wards. The inclusion criteria were: aged 18–90 years, moderate-to-severe ARDS induced by COVID-19, a ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) of <26.7 kPa (200 mmHg), and at least two defined risk factors for ICU admission and/or mortality. Exclusion criteria were severe COPD, significant pulmonary fibrosis, or other contraindications for HBOT. HBOT was delivered at 2.4 ATA for 60 min with 10 min compression/decompression time and one air-break, total time 80 min. The first HBOT was given within 24 h of randomization to subjects allocated to HBOT. The subjects received a maximum of five treatments within seven days of randomization. The subjects were followed up on nine visits, daily for the first seven days, at 14 and 30 days (Fig. 8).

The national early warning score (NEWS), PaO2/FiO2 (PFI), adverse events and daily oxygen requirement were recorded, and blood samples were collected. A pre-defined sub-study of 20 subjects at Karolinska included extended immunological profiling, including RNAseq to explore mechanisms. The protocol adheres to ICH-GCP(243) and was written according to SPIRIT guidelines(244) the published summary of the protocol is available as appendix VI.

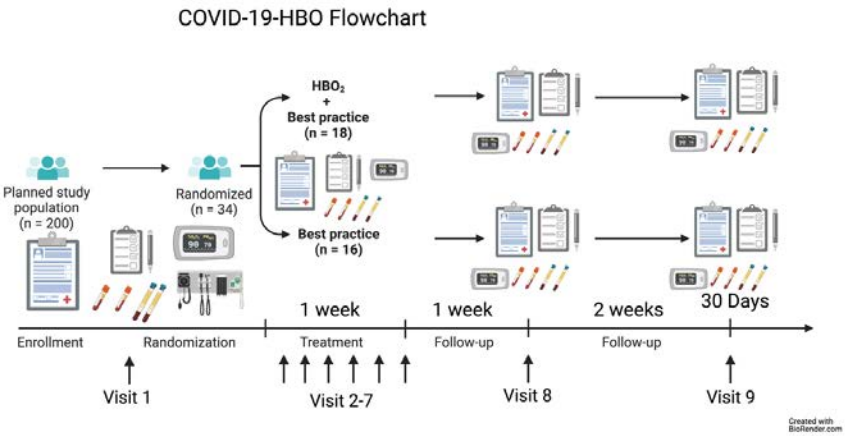

Figure 8. Study design for the COVID-19-HBO trial

3.2.3 HOT-LoCO (Paper IV)

80 previously healthy patients aged 18-60, diagnosed with post COVID-19 condition (U09.9) and substantial self-reported physical disability were included in this parallel-arms, randomised, placebo-controlled, double blind, clinical trial. Exclusion criteria included major systemic diseases, diabetes, and hypertension prior to COVID-19. HBOT was delivered at 2.4 ATA, 90 min with two air-breaks. Placebo was delivered as a 'sham treatment' with air breathing at 1.34 ATA to equate the sensation of HBOT, and air-breaks were simulated. Both groups received a maximum of 10 treatments within 6 weeks of randomisation. Subjects were followed up on five visits, and evaluation was done with questionnaires, physical tests, and objective measurements. Primary endpoint was the physical domains in RAND-36 at 13 weeks, the subjects will be followed-up for 52 weeks (Fig. 9).The protocol adhere to ICH-GCP (243) and was written according to SPIRIT-PRO guidelines (245) the published summary of the protocol is available as appendix VII.

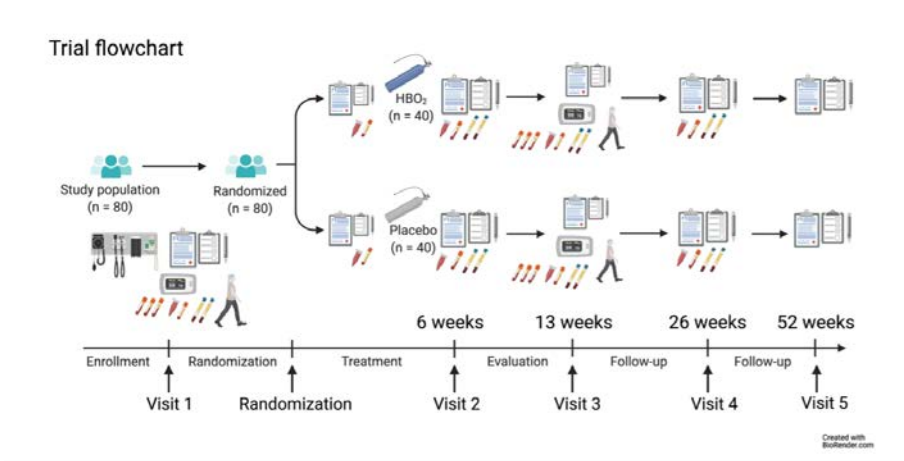

Figure 9. Study design for the HOT-LoCO trial

3.3 METHODS

3.3.1 HBOT and placebo protocols

The different protocols are described in the respective paper, but in summary we used 2.5 ATA, 28 min 100% oxygen without air break for healthy volunteers, 2.4 ATA, 80 minutes 100% oxygen with one air break for severe COVID-19. For Long COVID we used 2.4 ATA, 87 minutes 100% oxygen with two air breaks as active treatment, and 1.34 ATA-1.2 ATA, 87 minutes air with two fake air breaks as sham treatment. The placebo has been previously used in double blinded RCTs (246).

3.3.2 Trial conduct and reporting

ICH-GCP is the international standard since 1997 for design and conduct of clinical trials evaluating pharmaceutical interventions. Hence trials with HBO_2 should comply with these guidelines (47), and today this is a requirement for all clinical research at least in Europe. ICH-GCP consists of 13 principles including the Declaration of Helsinki. Conducting the RCTs in compliance with ICH-GCP guidelines ensures a high level of scientific rigor and credibility. ICH-GCP places a strong emphasis on protecting the rights, safety, and well-being of trial participants, which is absolutely crucial when dealing which critically ill and vulnerable groups of patients. The commitment to ethical conduct in accordance with ICH-GCP enhances the overall safety profile of the trial, promoting the welfare of participants and maintaining public trust in the research process. ICH-GCP guidelines provide a framework for the collection, documentation, and verification of clinical trial data. Strict record-keeping, source data verification, and data management practices are employed to ensure the accuracy and reliability of the collected data. This commitment to data quality enhances the overall robustness of the trial results, facilitating the

interpretation of outcomes and supporting evidence-based decision-making in both clinical practice and regulatory assessments (47).

Protocols for clinical trials should be written according to the SPIRIT guidelines (244) with adequate extensions such as SPIRIT-PRO for patient reported outcome measures (PROM) (245).

Randomised clinical trials should be reported according to the CONSORT guidelines with the recently recommended Harms extension, which is a prerequisite for publication in many high impact medical journals (42, 247).

3.3.3 Electron Paramagnetic Resonance (EPR) spectroscopy (Paper I)

In paper I, we used EPR for measuring ROS. To stabilize ROS that are very short-lived in blood a cyclic hydroxylamine (CMH) spin probe and iron chelators were used (136). The EPR technique is based on paramagnetic resonance, which utilizes the magnetic properties of ROS with its unpaired electrons to exhibit a magnetic signal. The energy required to flip the spin of the unpaired electrons corresponds to a microwave frequency (135). When the microwave frequency is swept, energy is absorbed and creates a signal that is recorded (Fig. 10). By comparing the amplitude of the signal made from a known concentration of the probe, the concertation in the sample can be calculated from a CP radical standard curve (135).

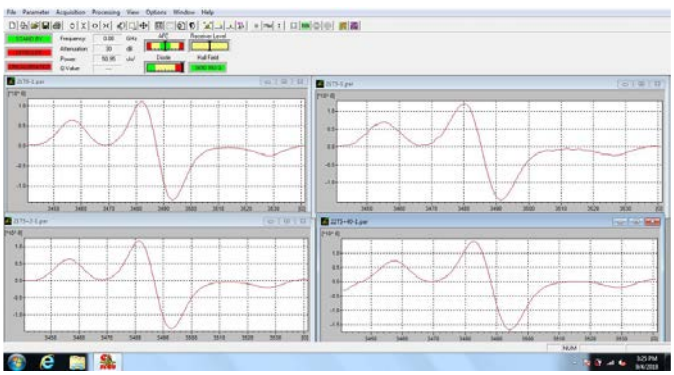

Figure 10. Shows a typical resonance spectrum from EPR

3.3.4 PBMC isolation, RNA extraction and QC (Paper I and III)

PBMCs were isolated by collection in Ficoll-Hypaque tubes, centrifuged and washed. After isolation of PBMCs, half were cryopreserved and the rest were treated with RNAlater, a universal preservative suitable for multi-omics that fixes the cells and preserves RNA and proteins to prevent degradation during storage and thaw-freezing (248, 249). The quality and integrity of the extracted RNA was evaluated with Nanodrop and TapeStation respectively, in order to assure pure RNA without degradation.

3.3.5 RNA sequencing (Paper I and III)

The RNA sequencing was outsourced to the core facility at Novum, Bioinformatics and Expression Analysis, but in summary the first part of RNAseq was the same as for polymerase chain reaction (PCR). The isolated RNA is fragmented into smaller pieces, the fragmented RNA was then reverse-transcribed into complementary DNA (cDNA) by using reverse transcriptase. This step converts the RNA into double-stranded cDNA. Then adapters, short DNA sequences necessary for sequencing library preparation, were ligated to the ends of the cDNA fragments which were then amplified through PCR and the resulting cDNA library was assessed for quality and quantity (QC) (250). The prepared library was loaded onto a high-throughput sequencing Illumina platform. During sequencing, the cDNA fragments generated millions or billions of short DNA sequences that was presented to us as raw data. The raw data was processed through a series of bioinformatics analyses, including alignment of the short sequences to a reference genome (Human PBMCs) for quantification of gene expression levels (251). The human PBMCs contain some 20-25 thousand genes, hence, in order to understand what these genes do, the data is also referenced against known pathways or functions, such as "hallmarks" or gene otology (GO) (252). This information provided insights into the functional elements of the transcriptome and helped identify differentially expressed genes in a pathway of interest under different conditions, that can be further validated with protein expression for a more detailed understanding of the functions.

3.3.6 PROM questionnaires (Paper IV)

3.3.6.1 RAND 36-item Health Survey 1.0 (RAND 36)

RAND 36 is a self-reporting questionnaire that contains 36 items that measure eight concepts of health in general terms, at present and past four weeks: physical functioning (ten items), role limitations due to physical health (four items), role limitations due to emotional problems (three items), energy/fatigue (four items), emotional well-being (five items), social functioning (two items), pain (two items) and general health (five items). It also includes a single item that provides an indication of perceived change in health over the last year. Scoring RAND 36 is a two-step process. First, numeric values from the survey are coded so that all items are scored from 0 (lowest score) to 100 (highest possible score). Scores then represent the percentage of total possible score achieved. In step two, items in the same scale are averaged together to create the eight-scale scores. Items that are left blank (missing data) are not considered when calculating the scale scores. Hence, scale scores represent the average for all items in the scale that the respondent answered. RAND 36 is well documented in terms of reliability and variability also for Swedish translation (253). National gender and age normative data are available for comparison (253) The questionnaire was sent out digitally to the subjects on the day of the visit, and when filled out it was uploaded to

the medical records. The dimensions in RAND-36 are presented separately. The rationale for choosing RAND-36 was that it is well validated and used in previous studies with similar methodology to enable power calculations. We chose the physical domains RP and PF as primary endpoint for two reasons:

The physical domains seem to be severely affected in conditions associated with chronic fatigue and POTS (254, 255). We expected the physical domains to be least affected by placebo.

3.3.6.2 EuroQol-5 Dimensions questionnaire (EQ-5D)

EQ-5D is a widely used patient-reported questionnaire aimed at measuring five different dimensions of present health with three or five levels of severity: no problems, some/moderate problems, and severe/extreme problems. The five different dimensions are mobility, self-care, usual activities, pain/discomfort, anxiety/depression. It also uses a visual analogue scale (VAS) 0-100 for quantifying measures of overall health. EQ-5D is a well-validated tool and the index that is calculated from the dimensions gives an estimate of Quality Adjusted Life Years (QALY), with a low index indicating a low HRQoL (256). We used five levels of severity (EQ-5D-5L) in our trial. One of the strengths of EQ-5D is that gender and age normative data for the Swedish population is available for use in health economic evaluation (257), and the index can be used to predict ability to work or study. The questionnaire was sent out digitally to the subjects on the day of the visit and when filled out, uploaded to the medical records

EQ-5D was chosen to provide an evaluation of HRQoL in a shorter perspective, as it is easier to fill in and may therefore be a better option for long term follow-up, to enable a simple health economic evaluation.

3.3.7 Objective evaluation (Paper IV)

3.3.7.1 EndoPAT 2000

Endothelial function was evaluated in fasting state using an *EndoPAT 2000* device (Itamar Medical, Caesarea, Israel). The subjects were connected to the pulse amplitude tonometry (PAT) device for non-invasive determination of digital endothelial function. The PAT device comprises a pneumatic plethysmograph that allows measurements of pulse amplitude at baseline and during hyperaemia following a five minutes arterial occlusion of the forearm (258). The change in the PAT signal was used for calculating the reactive hyperaemia index (RHI), which has been shown to reflect microvascular endothelial dysfunction and reduced NO bioavailability, and to predict cardiovascular events (259).

3.3.7.2 Non-invasive cardiac output monitoring with Nexfin technology

The *Nexfin* monitor was connected to a fasting subject. This was a non-invasive measurement of cardiovascular indices, with a beat-to-beat pulse wave analyser. The

Nexfin device (Edwards Lifesciences, Malmö, Sweden) was placed on the middle phalanx of the middle finger on the right hand. The Nexfin device comprises a pneumatic plethysmograph that provides advanced hemodynamic parameters and continuous non-invasive blood pressure (BP) from a finger cuff, with a redesigned self-coiling mechanism that reconstructs the clinical standard brachial arterial waveform from the finger arterial pressure waveform; it has been validated towards invasive measurements in several clinical trials (260).

3.3.8 Physical evaluation (Paper IV)

3.3.8.1 6-minute walk test

The 6 minute (min) walk test (American Thoracic Society), The 6MWT (American Thoracic Society) was performed in a corridor with a measured distance of 30 metres (m), with markings for every metres. The subject carried a pulse oximeter with a probe attached to their forehead. The test was monitored by an experienced instructor recording parameters every minute, the total number of metres walked in six minutes, the subject's graded and subjective feeling of leg-fatigue and dyspnoea according to the Borg CR-10-scale, as well as the feeling of general exertion according to the Borg-RPE-scale, both at baseline and at the end of the tests (261).

3.3.8.2 30/60 seconds Chair stand test (CST)

The subject would stand up straight and sit down completely as many times as possible for 30/60 seconds (s). An instructor recorded the number of times the subject managed to perform the movement, as well as the subject's graded and subjective feeling of general exertion according to the Borg-RPE-Scale, and dyspnoea and leg fatigue according to the Borg CR-10-scale at baseline and the end of the test(262). The rationale for recording both 30 and 60 s was that some subjects may not be able to perform the full 60 s test.

3.3.9 Randomisation (Paper II, III and IV)

Two different web-based randomisation tools were used. For COVID-19-HBO we used (randomize.net) and for HOT-LoCO we used (randomizer.au). The randomisation sequence with blocks was generated by an independent clinical research associate and statistician. Randomisation was stratified for sex and centre in the COVID-19-HBO trial (Paper II and III) and for sex and disease severity in the HOT-LoCO trial (Paper IV).

3.3.10 Blinding (Paper IV)

Subjects as well as all personnel participating in assessments of symptoms and any objective findings were blinded to the treatment. The placebo 'Sham treatment' protocol is well established, and even experienced divers cannot differ between Sham treatment and HBOT (246). Designated personnel, experienced in HBOT and trained in GCP and the specific protocols administered the assigned treatments. All subjects will furthermore be

asked during the first week of treatment whether they believe they received the placebo treatment or HBOT, to validate the blinding process.

3.3.11 Statistical methods

The statistical methods used are summarized in each paper. For the two clinical trials, a Statistical analysis plan (SAP) was written together with the external statistician, and the statistical methods are also outlined in the published protocols. Sample size calculation was done in nQuery version 7. The statistics for primary and main secondary endpoints were performed using Analysis of Covariance (ANCOVA) with IBM SPSS Statistics version 29 (Paper II and IV). For exploratory endpoints, the repeated measures two-way Analysis of Variance (ANOVA) with Fisher's Least Significant Difference (LSD) tests for multiple comparisons were performed using GraphPad Prism version 10. (Paper I and III). For RNAseq the R/Bioconductor package DESeq2 (263) was used to call differential gene expression based on the gene counts generated by featureCounts. Correction for multiple testing was performed using the Benjamini–Hochberg false discovery rate (FDR). The significance level was set to FDR < 0.05 and a log2 fold change (Log2FC) of at least ±0.5 (Paper I and III).

"There is no shortcut to truth, no way to gain a knowledge of the universe except through the gateway of scientific method." -Karl Pearson

4 SUMMARY OF RESULTS

4.1 PAPER I

In paper I, we evaluated methods and protocols on healthy volunteers for future clinical trials. We measured ROS in blood with EPR. On group level there were no significant changes in ROS levels in response to HBO_2, whereas in response to HIIT the ROS levels increased from baseline at 30 min (p = 0.04) and stayed elevated at 60 min (p = 0.02). We observed a large inter-individual variation in the ROS levels for both interventions (Fig. 11).

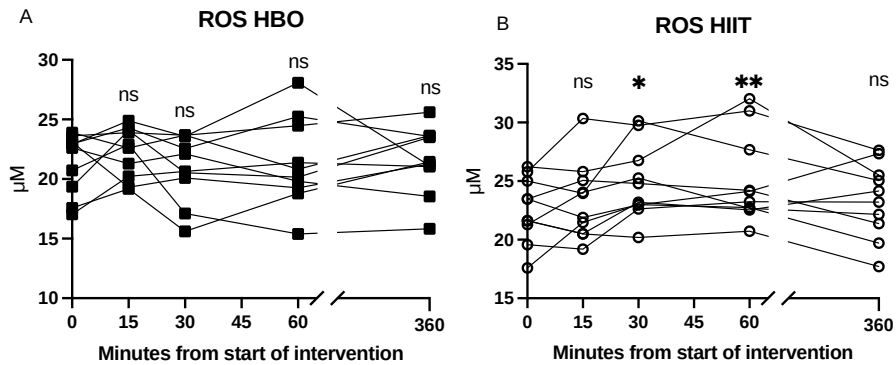

Figure 11. Panels (**A** and **B**) show individual values of ROS levels in venous blood measured by EPR. The zero, 15 and 30 min timepoints correspond to baseline, mid and end of intervention. Significance level of the mean at each timepoint compared to baseline is indicated by * $p < 0.05$, ** $p < 0.01$; ns = not significant

The $SpvO_2$ increased during the HBO_2 session ($p < 0.05$). There was a trend towards lower $SpvO_2$ immediately after HBO_2 (p = 0.20), and the level returned to baseline at 60 min. $SpvO_2$ decreased during the HIIT session (p = 0.02), but increased immediately after HIIT ($p < 0.001$), remained elevated at 60 min (p = 0.03), and returned to baseline at 6 hours (Fig. 12).

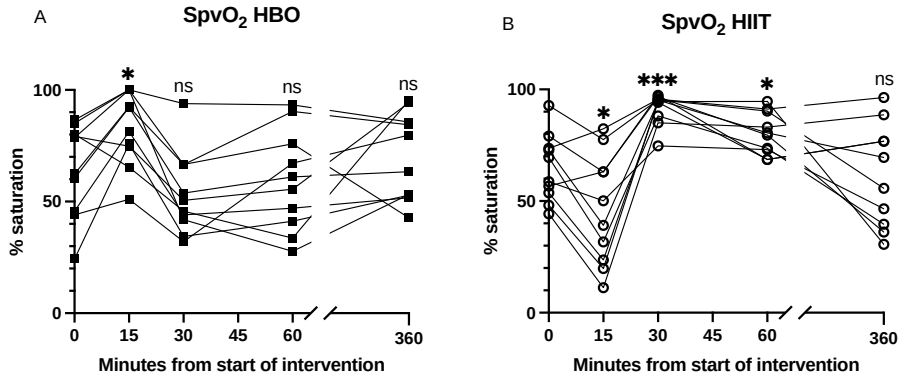

Figure 12. Panels (**A** and **B**) show individual values of $SpvO_2$ in venous blood. The zero, 15 and 30 min timepoints correspond to baseline, mid and end of intervention. Significance level of the mean at each timepoint compared to baseline is indicated by * $p < 0.05$, *** $p < 0.001$; ns = not significant.

There was a significant overlap between the genes altered in response to both HBO_2 and HIIT (n=166, Fig. 13C). To further compare the responses between the two interventions, we correlated the log2 fold changes in the common DEGs between HIIT and HBO_2. There was a highly significant correlation (Spearman's rho of 0.81, $p < 2.2 \times 10^{-16}$) of the PBMC expression changes 6h after HIIT and HBO_2 (Fig. 13D). The top enriched pathways in response to both HBO_2 and HIIT are shown in (Fig. 13E). We observed downregulation of several immune response pathways and mitochondrial oxidative respiration in response to HBO_2, a positive enrichment of calcium regulation in response to both interventions, and an upregulation of the adaptive immune response in HIIT. Of particular interest, we observed downregulation of several NF-κB signalling genes in response to both interventions (Fig. 13F). The NF-κB inhibitors *NFKBIA* and *TNFAIP3* were two of the most downregulated genes in response to HBO_2. In contrast, several interferon α/γ signalling genes were upregulated in response to both HBO_2 and HIIT.

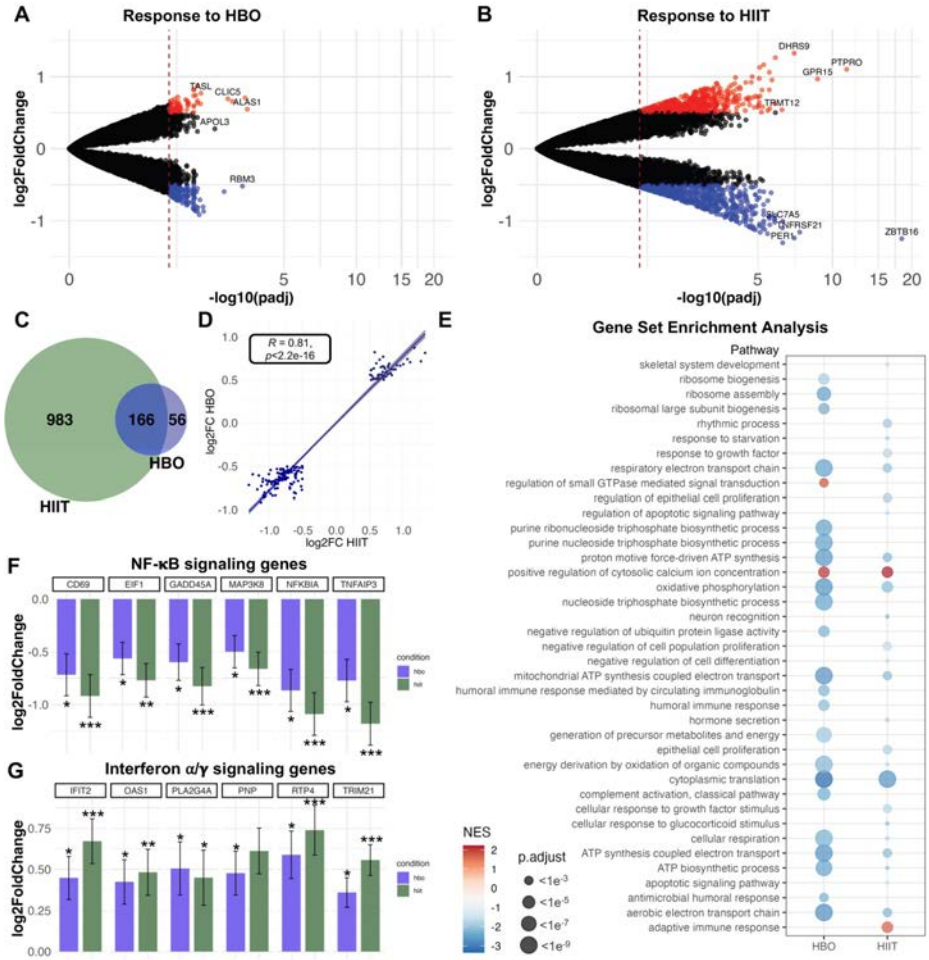

Figure 13. RNA sequencing results. Volcano plot for all expressed genes in response to HBO_2 (**A**) and HIIT (**B**). The coloured dots indicate significant DEGs, red for upregulated genes, and blue for downregulated genes (log2FC<−0.5; FDR<0.05) (**A,B**). Venn diagram of the overlap of DEGs between HIIT in green and HBO in purple (**C**). Correlation between the log2FCs of DEGs in response to HBO_2 and HIIT (**D**). Top enriched pathways in the two interventions from the GSEA (**E**). Gene expression changes for selected NF-κB-associated genes (**F**), and selected interferon α/γ-associated genes (**G**). Bars correspond to the mean log2FC, and error bars show the standard error. Significance is indicated by * for FDR < 0.05, ** for FDR < 0.01, and *** for FDR < 0.001 (**F**,**G**).

4.2 PAPER II

Paper II, was an early planned interim analysis to evaluated harms. we showed that it was feasible to treat critical COVID-19 patients with HBOT in monoplace chambers. The subjects were critically ill, most were admitted to intermediate care, which by international standards was ICU (Table 1). Of the 31 randomised subjects, 29 subjects were analysed, one subject in the HBOT group withdrew consent before treatment and one subject in the Control group was negative for SARS-CoV-2.

Baseline variable	HBOT, n =14	Control, n=15
Age	67.4 (10.8)	63.3 (8.2)
Male Sex	8 (57.1 %)	8 (53.3%)
Caucasian Ethnicity	13 (92.8%)	15 (100%)
BMI	29.4 (4.5)	29.2 (5.0)
Number of Risk factors	2.93 (0.96)	3.13 (1.06)
Smoker (Every day)	1 (7.1%)	0 (0%)
Former smoker	5 (35.7%)	5 (33.3%)
Never smoker	8 (57.1%)	10 (66.7%)
Time since initial symptoms	9.93 (3.58)	11.67 (3.62)
NEWS at randomisation	5.3 (2.0)	5.4 (1.7)
PaO_2/FiO_2 at randomisation	14.0 (3.5)	17.3 (6.4)

Table 1. Baseline variables for subjects in paper II

Adverse events were common. A total of 95 AEs were registered; of the 23 SAEs, 9 (in six subjects) were in the HBOT group and 14 (in six subjects) in the control group. Hypoxia was the most commonly reported AE. Two SAE were possbibly related to HBOT. One SAE (hypoxia) coincided with HBOT and was assessed as a Serious adverse drug reaction (SADR). Three subjects died in the trial, at day two and 25 in the HBOT group and day 13 in the Control group.

Other safety endpoints were NEWS and PaO_2/FiO_2. There was no significant difference in the safety endpoints. In the HBOT group the PaO_2/FiO_2 was significantly higher at Day 14, but this was not a pre-defined endpoint (Fig. 14).

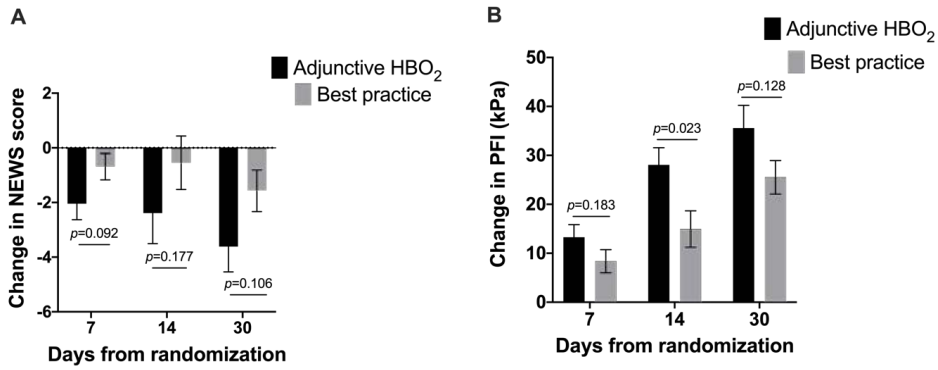

Figure 14. Changes from baseline in NEWS (**A**) and PaO_2/FiO_2 (**B**) Day 7, Day 14, and Day 30 (Mean and SD).

4.3 PAPER III

This was a pre-defined sub study of the parental trial to explore mechanisms, 23 patients were randomised, and the 17 subjects that had RNAseq data for baseline and Day 7 were analysed (Fig. 15). The groups were balanced at baseline (Fig. 15A). 791 DEGs in the HBOT group compared to only 46 in the control group were discovered in the RNAseq analysis at Day 7 vs. baseline (Fig. 15B, C and D). GSEA revealed a unique transcriptomic signature in response to HBOT that included downregulation of key genes in the UPR, other pathways regulating the UPR and inflammatory response (Fig. E).

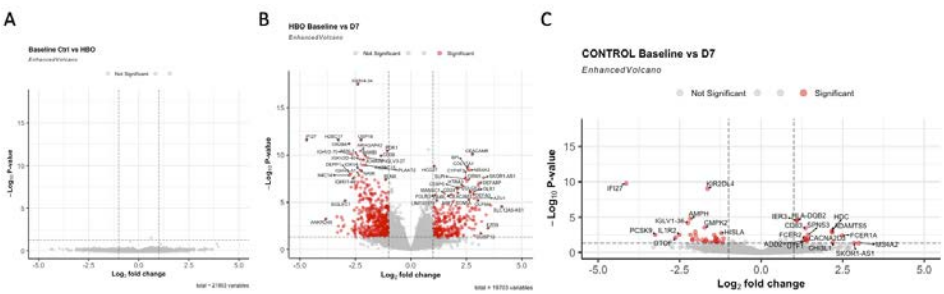

Figure 15. RNA sequencing results. Volcano plots for all expressed genes. Unpaired-t test comparing baseline for HBOT and Control (A), in response to HBOT (B) and Control (C). The coloured dots indicate significant DEGs (FDR<0.05, to the left for upregulated genes log2FC>1, and to the right for downregulated genes log2FC<-1) (A–C).

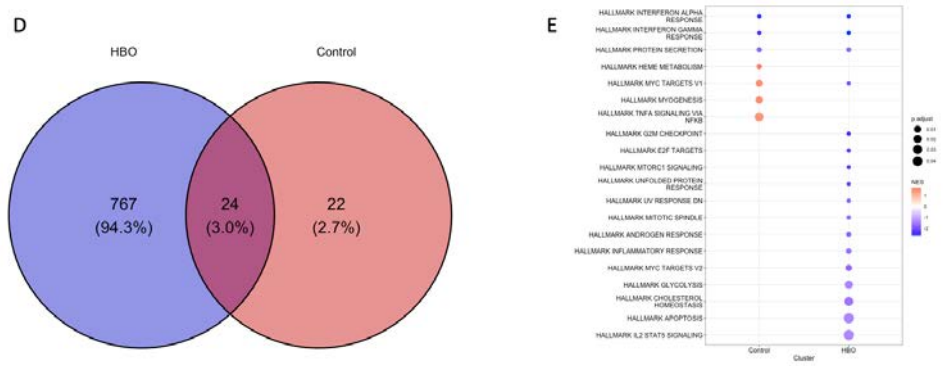

Figure 16. Venn diagram of the unique and overlapping DEGs in HBOT in and Control (D). Top pathway enrichment results from the GSEA (E).

Since we were specifically interested in the UPR, we performed a non-hierarchical clustering. We could see a clear pattern where baseline and D7 clustered together in the HBOT group but not in the Control group (Fig. 17).

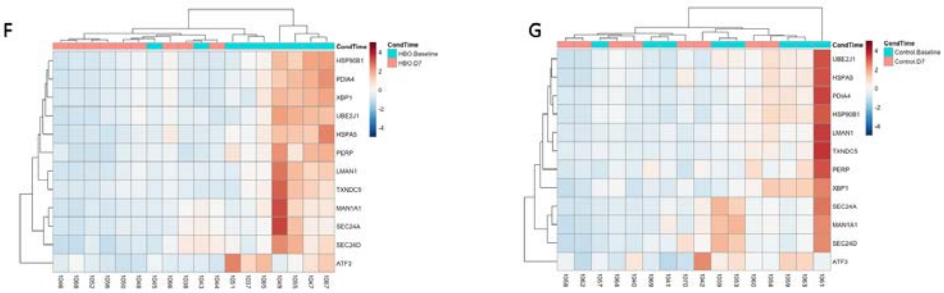

Figure 17. Unsupervised hierarchical clustering of genes in Hallmark UPR. HBOT cluster with upregulated genes at baseline and downregulated genes day 7 (F), but no distinct clustering in the control group (G)

Gene abbreviations: *HSP90B1*- heat shock protein 90kDa beta member 1; *XBP1*- X-box binding protein 1; *UBE2J1*- Ubiquitin Conjugating Enzyme E2 J1; HSPA5- Heat Shock Protein Family A (Hsp70) Member 5 (GRP78/BiP); *PERP*- P53 Apoptosis Effector Related To PMP22; ATF3- Activating Transcription Factor 3

HLoS was statistically significant only for survivors, but was clinically significant with almost 40% shorter time in hospital. Only one of eight surviving patients in the HBOT group stayed more than 17 days, compared to five out of seven patients in the Control group. One patient in each group died; in the HBOT group the patient died on Day 25 (withdrawal of care), while in the BP group the patient died on Day 13 (septic shock) (Fig. 18).

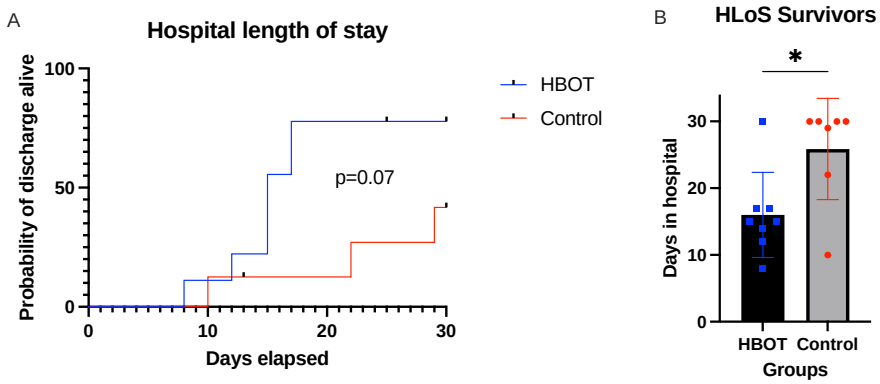

Figure 18. Kaplan-Meier plot describing day of discharge for the two groups, deaths and patients censored at 30 days are marked with a square on respective line, Log rank HR 2.98 [95% CI 0.9-10.4], p= 0.07 (A). Mean (SD) days in hospital for survivors in the two groups, HBOT =16 days vs Control =26 days, Mann-Whitney U= 11, [95.99% CI -16.0 to 0.0], p= 0.045. * Indicates p<0.05 (B).

NEWS and PaO_2/FiO_2 were safety endpoints in the original trial. There was no indication of harms in these endpoints, on the contrary the HBOT group recovered faster. NEWS was lower in the HBOT group (ANOVA, $F(8, 120) = 3.817$, $p < 0.001$), and after post hoc analysis with Fisher's LSD test for individual timepoints, NEWS was lower in the HBOT group at Day 7 (mean difference -2,7 [95% CI -4.8 to -0.5], p = 0.02) and at Day 14, (mean difference -4,1 [95% CI -7.4 to -0.8], p = 0.02. PaO_2/FiO_2 was higher in the HBOT group (Mixed effects model (time-by-treatment interaction), $F(8, 94) = 2.900$, $p < 0.01$), and at individual timepoints lower in the HBOT group at Day 14 (mean difference 20,1 [6.0 to 34.2], p = 0,01 (Fig. 19).

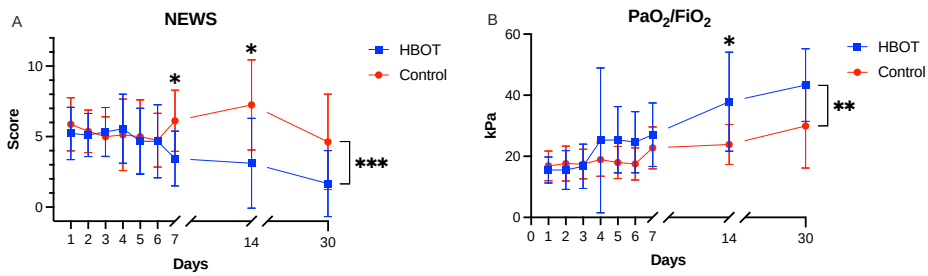

Figure 19. Mean (SD) NEWS score over the course of the trial for the two groups; a reduced NEWS score indicates that vital signs were less affected (A). Mean (SD) PaO_2/FiO_2 over the course of the trial for the two groups expressed as PaO_2 in kPa; a higher PaO_2/FiO_2 indicated better lung function and gas exchange (B). The significant differences in the ANOVA/mixed effects model (time by treatment interaction) and Fisher's LSD test for

individual timepoints are marked. * Indicates p<0.05, ** indicates p<0.01 and *** indicates p<0.001

4.4 PAPER IV

Paper IV was an early planned interim analysis to evaluate harms. Thirty-one AEs were recorded, at least one in 60% of subjects. No SAE was reported. Most AEs were mild, six were moderate. In 20 AEs, there was at least a possible relationship with the study drug. The most common AE was cough and chest pain/discomfort. All AE were transient (Paper IV).

The HRQoL was very low in our cohort compared to age and sex matched Swedish norm data(253, 264) (Fig. 20).

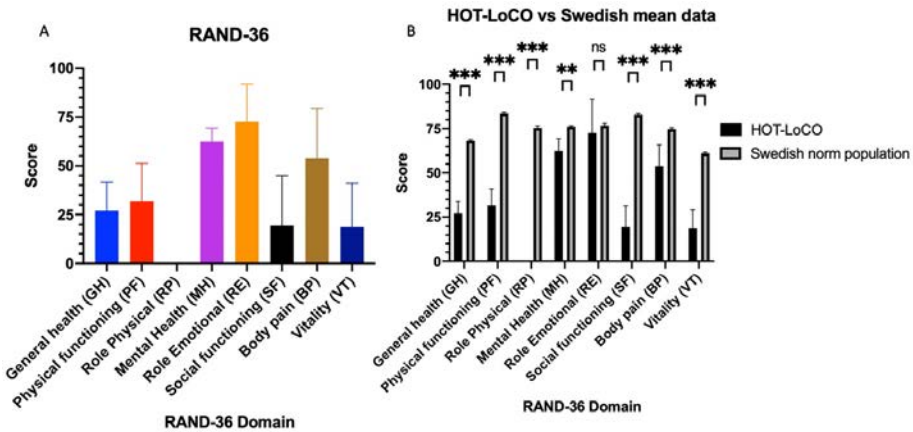

Figure 20. The eight domains of RAND-36 expressed as Mean (SD). A low value represents low HRQoL in that domain. A) shows mean in our cohort (n=20) and B) our cohort compared to a Swedish norm population (n=3432). ns indicates not significant, ** indicates p<0.01 and *** indicates p<0.001

The physical performance was very low in the two tests at baseline compared to international norm data; 6MWT 442 (180) [95 % CI; 357,7-525,8] vs 662(18) m and CST 13(5.1) [95 % CI; 10,5-15,3] vs 25 (1.2) [95 % CI; 22,9-27,6] stands in 30s (Fig. 21).

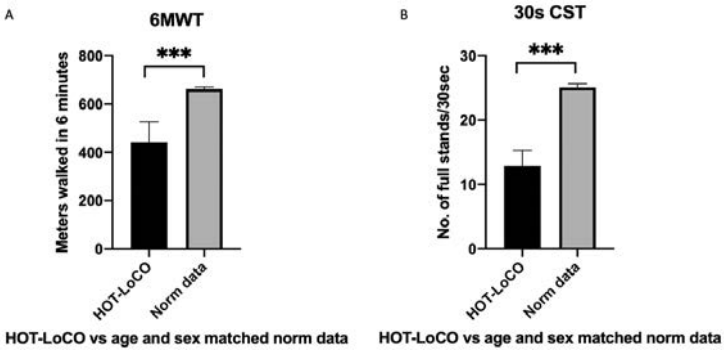

Figure 21. Measurements of 6MWT (A) and 30s CST (B), both compared to age and sex matched norm data. Expressed as Mean (SD). *** indicates p<0.001

5 DISCUSSION

In this thesis, we evaluated methods for future studies of dose response, explored transcriptomic changes and described harms in clinical trials on the novel indications severe COVID-19 and post COVID-19 condition. This is the first time that HBOT has been evaluated in compliance with ICH-GCP for these novel indications, which strengthen the validity and integrity of our results. We registered harms in compliance with ICH-GCP, which not surprisingly disclosed more adverse events than previously reported in HBOT trials. With RNAseq we uncovered new and much more complex mechanisms of HBOT than previously described when we evaluated gene expression in patients with severe COVID-19. These findings provide a base for a more fundamental understanding of the limitations and possibilities of HBOT.

5.1 Trial conduct

Protocols and standard operations procedures (SOPs) are only as good as the people who execute them, while education on GCP guidelines, data collection and follow-up are particularly challenging when conducting international multi centre trials (265). ICH-GCP have been criticized for not focusing enough on the ethical aspects of research but rather focusing on procedures (266). We spent much time on producing manuals and SOPs, and on educating staff in the principles of ICH-GCP in Paper II, III and IV). We had the advantage of being a small group that collected data and had external monitoring organizations that helped us finding weaknesses and possible points of improvement. Every trial has its specific challenges related to clinical setting and procedures, but ICH-GCP guidelines are very helpful as a framework for planning and conducting of any clinical trial.

5.2 Oxidative stress

The human body that consists of trillions of cells with specialized functions is extremely compartmentalized. However, all organelles and cells have some way of communication with each other, and there is a basic code and pattern that have evolved from a single-cell organism even if all divisions have their unique features. A metaphor for the human body can be an organization or an ecosystem.

If one could directly measure the gasotransmitters or redox balance in human compartments, we would better understand the specific hormetic mechanisms that differ for different tissues and find possible therapies in humans (138, 267, 268). Our first approach was to look for the common signal substance all the way from organelle to organ system. We therefore wanted to measure ROS in blood with the aim of finding a clinically available mirror of intracellular ROS. We reviewed the literature and found many different ways of measuring ROS, but EPR seemed to be the gold standard and available in Pernow and Catrina's lab (135, 269-272). In Paper I, we could measure changes in ROS in the same physiological range as exercise, but the individual variance was greater than expected in

healthy individuals (273) possible reason for this is that we included both men and women, and there are most likely cultural and national differences in the definition of "normally active". We realize that blood is a mix of cells with individual redox capacity, and a single type of cells such as erythrocytes or monocytes may be more suitable for a small sample size. Monocyte iNOS activity and eNOS uncoupling in endothelial cells are identified as key factors for endothelial dysfunction and vascular inflammation in ATII-induced arterial hypertension, while depletion of inflammatory monocytes was able to restore iNOS-derived nitro-oxidative stress and to recouple eNOS (274). Erythrocytes represent 45-50% of the blood volume and may also be clinically relevant in endothelial dysfunction and COVID-19 (275). On the other hand, mature erythrocytes are very different from all other cells in the body because they lack a nucleus and most organelles, including mitochondria, and instead depend on low molecular chaperones and scavengers for their redox homeostasis(276). Redox balance in blood may still be interesting to mirror redox balance and offers an easy and minimally invasive route to evaluate transient effects of HBOT. A partially unanswered question is whether ROS in blood purely correlates with oxygen availability in a U-shaped manner, or if the ROS measured in blood actually reflects intracellular oxidative stress. It is possible that pO_2 or SvO_2 are much easier and cheaper surrogates for oxidative stress. The ROS-results and inverse relationship between $SpvO_2$ in HIIT and HBOT from paper I, even though not statistically significant, were hypothesis generating and warrant further investigation. The long treatment periods with associated costs and effort for patients are some of the major shortcomings of HBOT. A biomarker that could predict outcome and possibly reduce the number of treatments on an individual basis would make HBOT more cost efficient and reduce harms. More experimental research, as well as a readily availible biomarker for dose-response are needed in order to be able to tailor individual treatment regimens to comply with modern requirements of evidence based and precision medicine.

5.3 RNA sequencing results

We decided to use PBMCs for RNAseq since they are easily accessible cells including both innate and adaptive immune cells. One of the limitations of our method is that we do not include neutrophils that are an important part of the innate immune system; they may be relevant in the effects of HIIT and HBOT, and may also be relevant for the cytokine storm that is thought to be the driving mechanism for severe and critical COVID-19 (277). The aim with paper I was to evaluate methods and protocols, and therefore we used HIIT as a reference. Interestingly, we observed changes in the same physiological range as HIIT with a very small dose of HBO_2, one session with 17-25% of the dose normally used in clinical HBOT. We used the protocols tested in Paper I for the subsequent clinical trials.

The results from Paper I should be interpreted with caution due to the small sample size and exploratory nature of the study design. However, we observed some interesting

similarities in genes associated with hypoxia and inflammation between the two interventions that were hypothesis generating for the two clinical trials . ROS downregulate NF-κB in a mutual and bidirectional fashion, where the UPR seems to be an integrated pathway (124, 278). Of particular interest in our results was that we observed a downregulation in *GADD45A* that is involved in both DDR and UPR (279) and *EIF* that is involved in the UPR (280) when we evaluated methods in healthy volunteers with a very low dose (Paper I). *GADD45A* is a regulator of transitional switch from survival to apoptosis during ER stress (281). The top downregulated genes in the NF-κB pathway were *NFKBIA* and *TNFAIP3* (Paper I). *CD69* was also downregulated. CD69 is an early activation marker on lymphocytes that has been shown to be upregulated in COVID-19, and a higher expression is associated with more severe disease (282). One to two sessions of HBOT at 2.8 ATA, 90 minutes, have recently been shown to upregulate genes involved in T-helper cell differentiation and downregulation of the NF-κB pathway. Interestingly, *GADD45A* and *TNFAIP3* were the top downregulated genes in a prospective cohort of NSTI patients (283). The observation of large differences in the response between healthy men and women suggests that sex differences also should be considered, at least in young and healthy individuals (Paper I). In summary, the results form paper I suggest that even a very low dose of HBO_2 may have anti-inflammatory and immunomodulating effects, which warrants further investigation with a larger sample size.

In Paper III we observed a distinct change in transcriptomic signature from baseline to day seven in severe and critical COVID-19 patients treated with HBOT, that was not seen in the control group. The change was associated with faster recovery and reduced HLoS. Many pathways related to ER stress, quality control, cell fate and modulation of the immune response seemed to be involved. One of the most interesting findings was the transcriptional downregulation of the UPR since it is intricately involved in virus-host response and progression of COVID-19 (193). One of the central players in the UPR is the pleiotropic molecular chaperone BiP/GRP78, that is coded by the gene *heat shock protein family. A (Hsp70) member 5, (HSPA5)* that has also been suggested as a therapeutic target for viral disease *(191, 284-287)*. BiP/GRP78 has been shown to be more abundantly expressed on the plasma interface of human airway epithelial cells than the ACE2 receptor in severe COVID-19 patients other than in patients with HIV and Tuberculosis, consistent with a large publicly available single cell sequencing database FANTOM5, and as such it may be an important alternative/co-receptor for SARS-CoV-2 (288). In normal cells BiP/GRP78 is adapted to support protein synthesis, folding and export, but during ER stress, alterations in the cytoplasmic environment seem to induce changes in its structural topography, converting GRP78 into a receptor with additional functions not observed in the original ER chaperone (285). BiP/GRP78 was one of the most significantly DEG in our analysis, suggesting that this may be an important factor involved in the clinical outcome. In line with previous findings (288), there were no major sex differences in expression of the UPR genes in our trial (Paper III). This is an interesting observations since

we saw big sex differences in healthy individuals (Paper I). Possible explanations may be that females in Paper III were all post-menopausal, the dose was higher, and concomitant diseases or the critical illness may have relatively reduced the sex-related difference. We also observed downregulation of mTORC1, which is interesting since activated B-cells seem to depend on mTORC1 to switch on and off the UPR in response to demand of antibody production (289). There is compelling evidence suggesting that the UPR is involved in macrophage polarization and acts as a switch for turning on and off inflammation (290, 291). However, evaluation of RNA is only a part of the puzzle and our evaluation of differentially expressed genes that make UPR guilty by association, needs to be validated on protein level (292). Opposite patterns are seen on protein level in non-HBOT treated subjects, but it is difficult to draw conclusions from the direction of the change since a downregulation on mRNA level may not directly correspond to an upregulation on protein level (293, 294). We further observed a downregulation of genes in metabolic pathways such as MYC, glycolysis and cholesterol homeostasis that suggests a metabolic reprogramming of mitochondrial OXPHOS, known to be an integrated part of T-cell exhaustion and macrophage polarisation in hypoxia (153, 290). There is strong circumstantial evidence that the effects seen are related to the clinical outcome, but it should be noted that the design of the sub study is exploratory and not confirmatory, and to fully understand the complex interplay and the potential role of HBOT more clinical and pre-clinical research are needed. Understanding how viruses interact with the UPR is not only important for understanding the effects of HBOT; it may also have an impact for elucidating host-virus interactions, and provide insights for the development of other antiviral strategies.

5.4 HBOT Harms

In paper II we described AEs and safety endpoints observed from the first 29 subjects (all Swedish subjects). In the intention to treat population we did not see any statistically significant difference in the safety endpoints NEWS and PaO2/FiO2 and there was a trend towards benefit; PaO2/FiO2 was significantly better in the HBOT group at Day 14, but this was not a predefined endpoint. This trend that is also supported by a previous RCTs on HBOT for COVID-19 that used our protocol (215), suggests that HBOT does not cause harm even for critical COVID-19. The AEs or SAEs were similar in the two groups and only two SAEs were assessed as at least possibly related to the treatment. We reported more AEs than any other RCT on HBOT for COVID-19 (215) This could be because our cohort was more critically ill than those in the other studies, but also because we reported in compliance with ICH-GCP. Since oxygen is generally regarded as harmful, and toxic for the lungs above FiO_2 0,5 (295), we also recorded the daily average inspired oxygen for each subject throughout the trial. Despite the much higher dose during the HBOT treatment, there was no difference in $CPTD_{ICU}$ and we observed a trend towards lower need for oxygen in the HBOT group throughout the trial (Paper II, Fig. 3). There were

numerically more barotrauma events in the Control group, and also more secondary infections. There were numerically more deaths in the HBOT group; this could be explained by the fact that these subjects were too sick and beyond salvation, but it can also be argued that they would have benefitted from continuing the HBOT after intubation, or that the dose was already too high and caused harm (Paper II, Table 2). Taken together, the profile of harms seems favourable for HBOT in severe and critical COVID-19 but the number of patients is too small to draw definite conclusions regarding harms.

In paper IV we described the observed AEs and baseline characteristics from the first 20 subjects. AEs were more frequent that previously reported. One reason could be that we reported in compliance with ICH-GCP, but it is also possible that the dose is too high for this condition. The previous RCT that reported beneficial effects on cognitive function, HRQoL and objective findings on MRI brain and global longitudinal strain (GLS) on echocardiography with 40 treatments, used 2.0 ATA, 90 minutes with five minutes air-breaks every 20 minutes, five days a week (240, 296, 297). The low HRQoL and poor physical function observed in our cohort are important information for health care personnel and decision makers, because at first glance you cannot see post COVID-19 condition when only observing the patient for a short time (298, 299). One of the most interesting findings was the very low score in RAND-36 RP. This finding can be interpreted as that the cohort has very high expectations that are not met by their function and this dissonance is an important part of the poor HRQoL (300). The low HRQoL scores may also suggest that our cohort had a worse disease severity than in previously reported trials on Long COVID (240). The dose in our trial differed from previously reported RCT; we designed the dosage 2.4 ATA/87 min, ten sessions over up to six weeks. Really, most subjects received the treatments two to three times a week over four weeks to accommodate patient preferences well within the protocol of the trial. Previous trial used 2.0 ATA/90min, 40 sessions, five days a week over eight weeks. It is also possible that the number of treatments (10 vs 40) is not enough to see a clinically relevant difference.

5.5 Potential benefits with HBOT

Results from Paper I suggest that HIIT and HBOT induce similar transcriptomic effects on the immune system, despite a trend towards opposite effects on ROS and $SpvO_2$ in blood. The observed similarities in anti-inflammatory and immune modulating effects correspond well with the somewhat over-simplified HHP hypothesis published by Hadanny et al. shortly after our study was conducted (77), with the difference that the HHP only includes HIF-1 and the molecular chaperones Sirtuins that are primarily involved in the DNA damage response (DDR) to explain the mechanisms. Balestra, et al. generated a similar hypothesis already ten years ago named "the normobaric oxygen paradox", where they discovered a dose-response relationship suggesting a negative effect in the hyperoxic range on inducing Erythropoietin (76, 301), highlighting the need for

individualized dose in different settings. Many other researchers have similar hypotheses. Numerous experimental and clinical studies have been published in the past few years, but there are still more questions than answers regarding how to define and evaluate dose and response(78, 90, 95, 302-305). Taken together, they all point towards that it is rather a "relative pO_2" or the intermittent difference of partial pressure (ΔpO_2) and not the absolute pO_2 that is the main determinant of the effect. Whether ROS and $SpvO_2$ after HBOT can be used as a biomarker for dose-response, warrants further investigation. HIIT seems like the obvious choice for healthy individuals that can perform heavy exercise (64). However, the critical difference and advantage of HBOT is that almost all mechanisms involved in the effect are energy dependent and it would be impossible to induce these effects in elderly, disabled or hypoxic patients with exercise (306). Further, the targeted effects are associated with ER-mitochondria dissociation and mitophagy (307, 308), suggesting that a ROS-source other than mitochondria is needed to overcome the reductive state(302).

The effects of HBOT on immune modulation have been studied in animal models. It has been suggested that HBOT may suppress atopic dermatitis (AD) by improving ROS levels in the skin (309). It has also been suggested that HBOT can alleviate psoriasis by improving T-regulator cell (Treg) function (310). HBOT does not appear to have the same risk-profile for bacterial superinfections as corticosteroids and other immunomodulating drugs. On the contrary, HBOT is associated with a number of benefits as an effective adjuvant intervention for severe infections (311-314), and our results in Paper III suggest that HBOT may be beneficial for viral infections. HBOT has been shown to affect many different chaperones in cell models suggesting a potential positive effect for endothelitis (237). Acute and chronic inflammatory diseases are associated with ER stress and an imbalance in the redox homeostasis that triggers the UPR (315). In most cases, the body has the ability to reset the redox homeostasis and resolve inflammation, but in severe COVID-19 endothelitis and damaged lungs causing immune cell infiltration and ARDS creates a catch-22 with hypoxia (316). The sustained inflammation in COVID-19 induced ARDS seems to depend on pathological interactions between macrophages and T-cells that get exhausted (317-319). There has been an ongoing discussion over the past decade whether senescence and exhaustion of immune cells are two intertwined or distinct unrelated processes (320, 321). A similar phenomenon is seen in tumours where cancer cells create a hypoxic micro-environment to evade the immune system (9, 322). I have chosen to refer to these processes with the general term of senescence since my understanding is that both processes can be induced by a sustained dysregulation of UPR and redox homeostasis that are seen in severe COVID-19 but with the features of autophagy and mitochondrial dysfunction some may argue that it is exhaustion (323, 324), or even anergy (325). Our results suggest that HBOT reduces inflammation, possibly by resetting the UPR and other pathways by changing the redox homeostasis in immune

cells, and at a transcriptional level we find features from both senescence and exhaustion (Paper III).

The interim analysis (Paper II) was reviewed by an independent DSMB, and the interim report was important to describe the profile on harms. It was also important to submit the results of the predefined sub-study at Karolinska (Paper III), since we discovered important mechanisms in the virus-host stress response that may explain possible benefits with HBOT that may pave the way for future trials on critically ill patients.

In HOT-LoCO (Paper IV) all subjects have completed follow-up for primary and secondary endpoints at three months. The DSMB reviewed the data 27 September 2022, they found no ethical, or safety reasons to discontinue the trial. This suggests that no difference was seen on the primary endpoint. We will also analyse gene expression in PBMCs for post COVID-19 condition to further explore the HBOT mechanisms. Regardless of benefits or harms, this may give us useful clues regarding mechanisms for the syndrome and future interventions.

6 CONCLUSIONS

Based on the results in this thesis, we conclude that HBOT acts like a potent immunomodulatory drug regulating cell fate with a favourable profile of harms. HBOT is beneficial for selected patients with critical COVID-19, associated with faster recovery and shorter HLoS compared to best practice. However, the mechanisms of action seem to be more complex than previously described, and the optimal dose and timing warrant further investigation. In detail we conclude:

- Oxidative stress can be measured in blood in the same physiological range as HIIT (Paper I)
- HBOT shares important transcriptomic mechanisms with HIIT in healthy subjects, but the required dose is individual and sex differences should be considered (Paper I).
- HBOT has a favourable profile of harms for both severe COVID-19 and post COVID condition, but the optimal timing and individual dosing are still unknown (Paper II, IV).
- HBOT improves gas exchange and vital signs and shortens HLoS in selected patients with severe and critical COVID-19 compared to best practice per 2021.
- The transcriptomic response in severe COVID-19 suggests that HBOT has a far more complex effect on many pathways involved in cellular quality control and cell fate than previously described (Paper III).
- HBOT alters the unfolded protein response and related pathways involved in senescence/exhaustion in immune cells (Paper I, III).

"It is paradoxical, yet true, to say, that the more we know, the more ignorant we become in the absolute sense, for it is only through enlightenment that we become conscious of our limitations. Precisely one of the most gratifying results of intellectual evolution is the continuous opening up of new and greater prospects."

Nikola Tesla

7 POINTS OF PERSPECTIVE

In this thesis we have generated many more new questions than answers, but the immunomodulatory effect seen from different doses of HBOT in healthy volunteers and in critical COVID-19 patients provides food for thought. The mechanisms of HBOT seem to be more complex than previously shown. With transcriptomics we have shown a link between regulation of the UPR, suggesting resolution of ER stress, and clinically relevant outcomes in critical COVID-19 patients treated with HBOT. We have shown that it is feasible to treat patients with severely injured lungs, affecting mechanisms involved in cell senescence that are not specific for COVID-19, which opens up new avenues for trials with HBOT in the ICU setting.

The common denominator of the somewhat ironically listed potential 132 conditions by Gabb and Robin in the publication "A therapy in search of diseases" (1987) is that they are all acute or chronic inflammatory diseases associated with ER stress and an imbalance in the redox homeostasis (18, 184, 188, 326). 37 years later, an extensive effort has been made to explore mechanisms, but they are still far from fully understood (46). Attempts have been made to conduct well designed clinical trials with HBOT on a few of these conditions, but even the accepted indications need more high-grade evidence to adhere to modern requirements for evidence-based medicine. Despite controversies in clinical trials, HBOT is a readily availible option with a favourable profile of harms for accepted conditions (44).

HBOT has a huge potential as a unique and short acting regulator of immune cell senescence/exhaustion. HBO_2 has the outstanding theoretical advantage over all conventional pharmacological drugs that it seems to be holistic and naturally selective. This means that cells that are dysfunctional with acute or chronic ER stress seem to be affected with the commonly used doses of HBOT, but healthy cells without ER stress will at most have some temporary ER stress from which they are equipped to quickly recover. In this aspect, the downregulation of GRP78/BiP is by far the most interesting finding for the use of HBOT as an adjuvant therapy for overcoming T-cell exhaustion in infection and cancer (327). The cell surface GRP78 (csGRP78) has been extensively studied and autoantibodies to GRP78 have been found in various cancers and autoimmune pathologies (190). Antibodies towards GRP78 have been shown to successfully inhibit tumour growth and metastases in cell and animal models of malignant melanoma (328), prostate cancer (329), and colon cancer (330). Anti-GRP78 antibodies also enhanced the effect of radiotherapy for lung cancer and glioblastoma (331). HBOT has been studied as an adjuvant therapy for malignancies including glioblastoma since the 1950´s, it may be time to revise the hypotheses with modern multi-omics methods and conduct clinical trials in compliance with ICH-GCP (332, 333). HBOT has been used for NSTI since the 1950´s, a recent observational study including transcriptomics showed a downregulation of genes involved in T-cell signalling and programmed cell death protein-1 (PD-1) /pro-

grammed death ligand-1 (PD-L1) immune checkpoint (283). Vinkel et al also found a downregulation of GADD45A and TNFAIP3 that were top downregulated genes in Paper I. We are currently planning an RCT in compliance with ICH-GCP on HBOT for NSTI.

We speculate that HBOT resolves ER stress either by selecting cells for apoptosis, or by autophagy/mitophagy separating the sheep from the goats, and/or metabolically reprograms the cells by polarisation (148). The holistic mechanism is the blessing and the curse; since we currently do not have a reliable plasma biomarker for ER stress or redox balance (334-336), the only way to really prove that HBOT has a place in conventional medicine is to prove its clinical benefits and harms in randomised trials. In parallel, we should include multi-omics in HBOT trials to be able to connect the potential clinical outcomes with molecular effects. Despite its complexity, the UPR seem to be a great place to start looking for biomarkers for personalized HBOT dose and effect.

Preclinical research is needed to get a more detailed understanding of how the UPR, and redox regulation is responsible for the positive effects. Molecular chaperones, in particular the hsp70 family including GRP78/BiP, have the great advantage of being conserved among species, and *S. cerevisiae* (yeast) (337), *C. elegans* (animal) (338) and *Arabidopsis Thaliana* (plants) (339)are potential 3R (Replace, Reduce, Refine) models for this (340). The ER-mitochondria interface seems to be another important and plausible site of action since it is the hub for Ca2+ regulation, autophagy including mitophagy (selective degradation of mitochondria) and inflammation; involved in cancer and many chronic inflammatory-, metabolic- and neurodegenerative diseases (341, 342). We need to continue to search for reliable clinical biomarkers for ER stress and individual dose-response for better patient selection and trial designs.

Our results together with previous research suggest that ΔpO_2 or rather the transient change in redox balance could be the target mechanism (76, 78, 95, 304, 343). It may be feasible to have a similar effect with much lower dose both regarding time and pressure, which may reduce harms and make HBOT more time and cost efficient if it is used in a HIIT fashion (344). It is possible that the hyperoxic periods can be limited to 10-15 minutes and repeated 3-4 times. It may also be possible to achieve selected effects with normobaric oxygen 100% (103kPa) and a hypoxic gas mixture with 9% oxygen (10kPa) during breaks to maximize the ΔpO_2 in the normobaric range, but this is something that first needs to be evaluated in a preclinical setting.

Further understanding of timing, dose and frequency would make HBOT cost-efficient, potentially minimizing harms and improving outcomes. Precision medicine is the future for HBOT.

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9 REFERENCES

1. Dyall SD, Brown MT, Johnson PJ. Ancient invasions: from endosymbionts to organelles. Science. 2004;304(5668):253-7.
2. Zimorski V, Mentel M, Tielens AGM, Martin WF. Energy metabolism in anaerobic eukaryotes and Earth's late oxygenation. Free radical biology & medicine. 2019;140:279-94.
3. Tobin MJ, Laghi F, Jubran A. Why COVID-19 Silent Hypoxemia Is Baffling to Physicians. American journal of respiratory and critical care medicine. 2020;202(3):356-60.
4. Deniz S, Uysal TK, Capasso C, Supuran CT, Ozensoy Guler O. Is carbonic anhydrase inhibition useful as a complementary therapy of Covid-19 infection? J Enzyme Inhib Med Chem. 2021;36(1):1230-5.
5. Haldane J. The Relation of the Action of Carbonic Oxide to Oxygen Tension. The Journal of physiology. 1895;18(3):201-17.
6. De Marais DJ. Evolution. When did photosynthesis emerge on Earth? Science. 2000;289(5485):1703-5.
7. Bennett CF, Kaeberlein M. The mitochondrial unfolded protein response and increased longevity: cause, consequence, or correlation? Exp Gerontol. 2014;56:142-6.
8. . !!! INVALID CITATION !!! (6).
9. Icard P, Lincet H, Wu Z, Coquerel A, Forgez P, Alifano M, et al. The key role of Warburg effect in SARS-CoV-2 replication and associated inflammatory response. Biochimie. 2021;180:169-77.
10. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029-33.
11. Xu Y, Hao X, Ren Y, Xu Q, Liu X, Song S, et al. Research progress of abnormal lactate metabolism and lactate modification in immunotherapy of hepatocellular carcinoma. Front Oncol. 2022;12:1063423.
12. Gill AL, Bell CN. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. QJM. 2004;97(7):385-95.
13. Lorrain Smith J. The pathological effects due to increase of oxygen tension in the air breathed. . 1899(24): 19–35.
14. Chavko M, Mahon RT, McCarron RM. Mechanisms of protection against pulmonary hyperbaric O(2) toxicity by intermittent air breaks. European journal of applied physiology. 2008;102(5):525-32.
15. Leopardi LN, Metcalfe MS, Forde A, Maddern GJ. Ite Boerema--surgeon and engineer with a double-Dutch legacy to medical technology. Surgery. 2004;135(1):99-103.
16. McCallum RI. Decompression sickness: a review. Br J Ind Med. 1968;25(1):4-21.
17. Chawla A, Lavania AK. Oxygen Toxicity. Med J Armed Forces India. 2001;57(2):131-3.
18. Gabb G, Robin ED. Hyperbaric oxygen. A therapy in search of diseases. Chest. 1987;92(6):1074-82.
19. Försvarsmakten. Regler för militär sjöfart RMS-Dyk 2013 2013 [updated 02/04/2019. Regler för militär sjöfart (RMS) ska tillämpas på örlogsfartyg och dykerisystem. Regler för det militära sjösäkerhetssystemet (RMS-S) ska tillämpas för all verksamhet inom Försvarsmakten med örlogsfartyg och dykare samt de

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20. Brummelkamp WH, Boerema I, Hoogendyk L. Treatment of clostridial infections with hyperbaric oxygen drenching. A report on 26 cases. Lancet. 1963;1(7275):235-8.
21. Thom SR, Lauermann MW, Hart GB. Intermittent hyperbaric oxygen therapy for reduction of mortality in experimental polymicrobial sepsis. J Infect Dis. 1986;154(3):504-10.
22. Park MK, Myers RA, Marzella L. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents, and immunologic responses. Clin Infect Dis. 1992;14(3):720-40.
23. Thom SR. Oxidative stress is fundamental to hyperbaric oxygen therapy. J Appl Physiol (1985). 2009;106(3):988-95.
24. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell. 2012;148(3):399-408.
25. Meng XE, Zhang Y, Li N, Fan DF, Yang C, Li H, et al. Effects of hyperbaric oxygen on the Nrf2 signaling pathway in secondary injury following traumatic brain injury. Genet Mol Res. 2016;15(1).
26. Ma Q. Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol. 2013;53:401-26.
27. Yu X, Li YG, He XW, Li XR, Din BN, Gan Y, et al. Hyperbaric oxygen reduces inflammatory response in acute pancreatitis by inhibiting NF-kappaB activation. Eur Surg Res. 2009;42(2):130-5.
28. Anguiano-Hernandez YM, Contreras-Mendez L, de Los Angeles Hernandez-Cueto M, Muand Oz-Medina JE, Santillan-Verde MA, Barbosa-Cabrera RE, et al. Modification of HIF-1alpha, NF-akappaB, IGFBP-3, VEGF and adiponectin in diabetic foot ulcers treated with hyperbaric oxygen. Undersea Hyperb Med. 2019;46(1):35-44.
29. Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, et al. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature. 2008;453(7196):807-11.
30. Taylor CT, Colgan SP. Regulation of immunity and inflammation by hypoxia in immunological niches. Nat Rev Immunol. 2017;17(12):774-85.
31. D'Ignazio L, Bandarra D, Rocha S. NF-kappaB and HIF crosstalk in immune responses. FEBS J. 2016;283(3):413-24.
32. Yogaratnam JZ, Laden G, Guvendik L, Cowen M, Cale A, Griffin S. Hyperbaric oxygen preconditioning improves myocardial function, reduces length of intensive care stay, and limits complications post coronary artery bypass graft surgery. Cardiovasc Revasc Med. 2010;11(1):8-19.
33. Dulai PS, Raffals LE, Hudesman D, Chiorean M, Cross R, Ahmed T, et al. A phase 2B randomised trial of hyperbaric oxygen therapy for ulcerative colitis patients hospitalised for moderate to severe flares. Aliment Pharmacol Ther. 2020.
34. Londahl M, Katzman P, Nilsson A, Hammarlund C. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. Diabetes care. 2010;33(5):998-1003.
35. Oscarsson N, Muller B, Rosen A, Lodding P, Molne J, Giglio D, et al. Radiation-induced cystitis treated with hyperbaric oxygen therapy (RICH-ART): a randomised, controlled, phase 2-3 trial. The lancet oncology. 2019;20(11):1602-14.

36. Ablin JN, Lang E, Catalogna M, Aloush V, Hadanny A, Doenyas-Barak K, et al. Hyperbaric oxygen therapy compared to pharmacological intervention in fibromyalgia patients following traumatic brain injury: A randomized, controlled trial. PloS one. 2023;18(3):e0282406.
37. Moon RE, editor. Hyperbaric Oxygen Therapy Indications. 14th ed: Undersea and Hyperbaric Medical Society; 2019.
38. Mathieu D, Marroni A, Kot J. Tenth European Consensus Conference on Hyperbaric Medicine: recommendations for accepted and non-accepted clinical indications and practice of hyperbaric oxygen treatment. Diving and hyperbaric medicine : the journal of the South Pacific Underwater Medicine Society. 2017;47(1):24-32.
39. Kjellberg A, Nystrom H, Soderberg M, Dlugosz A, Jornvall H, Steinberg A. Massive air embolism as a complication of upper gastrointestinal endoscopy: A case report illustrating a stroke mimic, literature review, and suggested management. Clin Case Rep. 2018;6(9):1862-7.
40. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. Bmj. 2008;336(7650):924-6.
41. Sherlock S. ECHM Consensus Conference and levels of evidence. Diving and hyperbaric medicine : the journal of the South Pacific Underwater Medicine Society. 2017;47(2):133.
42. Junqueira DR, Zorzela L, Golder S, Loke Y, Gagnier JJ, Julious SA, et al. CONSORT Harms 2022 statement, explanation, and elaboration: updated guideline for the reporting of harms in randomised trials. Bmj. 2023;381:e073725.
43. Heyboer M, 3rd, Sharma D, Santiago W, McCulloch N. Hyperbaric Oxygen Therapy: Side Effects Defined and Quantified. Adv Wound Care (New Rochelle). 2017;6(6):210-24.
44. FDA. Hyperbaric Oxygen Therapy: Get the Facts: U.S. Food and Drug administration; 2021 [updated 07/26/2021. Available from: https://www.fda.gov/consumers/consumer-updates/hyperbaric-oxygen-therapy-get-facts.
45. Jokinen-Gordon H, Barry RC, Watson B, Covington DS. A Retrospective Analysis of Adverse Events in Hyperbaric Oxygen Therapy (2012-2015): Lessons Learned From 1.5 Million Treatments. Advances in skin & wound care. 2017;30(3):125-9.
46. Ortega MA, Fraile-Martinez O, Garcia-Montero C, Callejon-Pelaez E, Saez MA, Alvarez-Mon MA, et al. A General Overview on the Hyperbaric Oxygen Therapy: Applications, Mechanisms and Translational Opportunities. Medicina (Kaunas). 2021;57(9).
47. EMA. ICH E6 (R2) Good clinical practice: European Medicines Agency; 2022 [updated 15/12/2016. Available from: https://www.ema.europa.eu/en/ich-e6-r2-good-clinical-practice#current-version---revision-2-section.
48. Winter PM, Smith G. The toxicity of oxygen. Anesthesiology. 1972;37(2):210-41.
49. Camporesi EM. Side effects of hyperbaric oxygen therapy. Undersea Hyperb Med. 2014;41(3):253-7.
50. Arieli R, Yalov A, Goldenshluger A. Modeling pulmonary and CNS O(2) toxicity and estimation of parameters for humans. J Appl Physiol (1985). 2002;92(1):248-56.
51. Kranke P, Bennett MH, Martyn-St James M, Schnabel A, Debus SE, Weibel S. Hyperbaric oxygen therapy for chronic wounds. The Cochrane database of systematic reviews. 2015(6):CD004123.

52. Bennett MH, Feldmeier J, Hampson NB, Smee R, Milross C. Hyperbaric oxygen therapy for late radiation tissue injury. The Cochrane database of systematic reviews. 2016;4:CD005005.
53. Villeirs L, Tailly T, Ost P, Waterloos M, Decaestecker K, Fonteyne V, et al. Hyperbaric oxygen therapy for radiation cystitis after pelvic radiotherapy: Systematic review of the recent literature. Int J Urol. 2019.
54. Santema KTB, Stoekenbroek RM, Koelemay MJW, Reekers JA, van Dortmont LMC, Oomen A, et al. Hyperbaric Oxygen Therapy in the Treatment of Ischemic Lower-Extremity Ulcers in Patients With Diabetes: Results of the DAMO2CLES Multicenter Randomized Clinical Trial. Diabetes care. 2018;41(1):112-9.
55. Clarke RE, Tenorio LMC, Hussey JR, Toklu AS, Cone DL, Hinojosa JG, et al. Hyperbaric oxygen treatment of chronic refractory radiation proctitis: a randomized and controlled double-blind crossover trial with long-term follow-up. International journal of radiation oncology, biology, physics. 2008;72(1):134-43.
56. Glover M, Smerdon GR, Andreyev HJ, Benton BE, Bothma P, Firth O, et al. Hyperbaric oxygen for patients with chronic bowel dysfunction after pelvic radiotherapy (HOT2): a randomised, double-blind, sham-controlled phase 3 trial. The lancet oncology. 2016;17(2):224-33.
57. Tsakiroglou M, Evans A, Pirmohamed M. Leveraging transcriptomics for precision diagnosis: Lessons learned from cancer and sepsis. Front Genet. 2023;14:1100352.
58. Shephard RJ. How much physical activity is needed for good health? Int J Sports Med. 1999;20(1):23-7.
59. Martinez-Gomez D, Esteban-Cornejo I, Lopez-Garcia E, Garcia-Esquinas E, Sadarangani KP, Veiga OL, et al. Physical activity less than the recommended amount may prevent the onset of major biological risk factors for cardiovascular disease: a cohort study of 198 919 adults. Br J Sports Med. 2020;54(4):238-44.
60. Haennel RG, Lemire F. Physical activity to prevent cardiovascular disease. How much is enough? Can Fam Physician. 2002;48:65-71.
61. Lee IM, Hsieh CC, Paffenbarger RS, Jr. Exercise intensity and longevity in men. The Harvard Alumni Health Study. JAMA : the journal of the American Medical Association. 1995;273(15):1179-84.
62. Shiroma EJ, Sesso HD, Moorthy MV, Buring JE, Lee IM. Do moderate-intensity and vigorous-intensity physical activities reduce mortality rates to the same extent? J Am Heart Assoc. 2014;3(5):e000802.
63. Mauvais-Jarvis F. Sex differences in energy metabolism: natural selection, mechanisms and consequences. Nat Rev Nephrol. 2023.
64. Khalafi M, Symonds ME. The impact of high-intensity interval training on inflammatory markers in metabolic disorders: A meta-analysis. Scand J Med Sci Sports. 2020;30(11):2020-36.
65. Weston KS, Wisloff U, Coombes JS. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. Br J Sports Med. 2014;48(16):1227-34.
66. Atakan MM, Li Y, Kosar SN, Turnagol HH, Yan X. Evidence-Based Effects of High-Intensity Interval Training on Exercise Capacity and Health: A Review with Historical Perspective. International journal of environmental research and public health. 2021;18(13).
67. Powers SK, Radak Z, Ji LL. Exercise-induced oxidative stress: past, present and future. The Journal of physiology. 2016;594(18):5081-92.
68. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. J Sport Health Sci. 2019;8(3):201-17.

69. Andreato LV, Coimbra DR, de Carvalho T. High-intensity interval training vs. moderate interval training: the lack of equalisation, an ongoing problem. Obes Rev. 2017;18(10):1223-4.
70. Herold F, Muller P, Gronwald T, Muller NG. Dose-Response Matters! - A Perspective on the Exercise Prescription in Exercise-Cognition Research. Front Psychol. 2019;10:2338.
71. Izquierdo-Alventosa R, Ingles M, Cortes-Amador S, Gimeno-Mallench L, Sempere-Rubio N, Chirivella J, et al. Comparative study of the effectiveness of a low-pressure hyperbaric oxygen treatment and physical exercise in women with fibromyalgia: randomized clinical trial. Ther Adv Musculoskelet Dis. 2020;12:1759720X20930493.
72. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Molecular and cellular biology. 1992;12(12):5447-54.
73. Semenza GL. Oxygen sensing, homeostasis, and disease. The New England journal of medicine. 2011;365(6):537-47.
74. Semenza GL. A compendium of proteins that interact with HIF-1alpha. Exp Cell Res. 2017;356(2):128-35.
75. Duan C. Hypoxia-inducible factor 3 biology: complexities and emerging themes. Am J Physiol Cell Physiol. 2016;310(4):C260-9.
76. Balestra C, Germonpre P. Hypoxia, a multifaceted phenomenon: the example of the "normobaric oxygen paradox". European journal of applied physiology. 2012;112(12):4173-5.
77. Hadanny A, Efrati S. The Hyperoxic-Hypoxic Paradox. Biomolecules. 2020;10(6).
78. Balestra C, Kot J. Oxygen: A Stimulus, Not "Only" a Drug. Medicina (Kaunas). 2021;57(11).
79. Hwang AB, Lee SJ. Regulation of life span by mitochondrial respiration: the HIF-1 and ROS connection. Aging (Albany NY). 2011;3(3):304-10.
80. Hagen T. Oxygen versus Reactive Oxygen in the Regulation of HIF-1alpha: The Balance Tips. Biochem Res Int. 2012;2012:436981.
81. Hagen T, Taylor CT, Lam F, Moncada S. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha. Science. 2003;302(5652):1975-8.
82. Xiao H, Gu Z, Wang G, Zhao T. The possible mechanisms underlying the impairment of HIF-1alpha pathway signaling in hyperglycemia and the beneficial effects of certain therapies. Int J Med Sci. 2013;10(10):1412-21.
83. Zheng X, Narayanan S, Xu C, Eliasson Angelstig S, Grunler J, Zhao A, et al. Repression of hypoxia-inducible factor-1 contributes to increased mitochondrial reactive oxygen species production in diabetes. Elife. 2022;11.
84. Wan DY, Zhang Z, Yang HH. Cardioprotective effect of miR-214 in myocardial ischemic postconditioning by down-regulation of hypoxia inducible factor 1, alpha subunit inhibitor. Cell Mol Biol (Noisy-le-grand). 2015;61(2):1-6.
85. Bartoszewska S, Kochan K, Piotrowski A, Kamysz W, Ochocka RJ, Collawn JF, et al. The hypoxia-inducible miR-429 regulates hypoxia-inducible factor-1alpha expression in human endothelial cells through a negative feedback loop. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2015;29(4):1467-79.
86. Movafagh S, Crook S, Vo K. Regulation of hypoxia-inducible factor-1a by reactive oxygen species: new developments in an old debate. J Cell Biochem. 2015;116(5):696-703.

87. Fong GH, Takeda K. Role and regulation of prolyl hydroxylase domain proteins. Cell Death Differ. 2008;15(4):635-41.
88. Medem AV, Seidling HM, Eichler HG, Kaltschmidt J, Metzner M, Hubert CM, et al. Definition of variables required for comprehensive description of drug dosage and clinical pharmacokinetics. Eur J Clin Pharmacol. 2017;73(5):633-41.
89. Hayes W. Hyperbaric Oxygen Therapy (HBOT) for Tissue Damage, Including Wound Care and Treatment of Central Nervous System (CNS) Conditions- A Health Technology Assessment HTA analysis. Online: Washington State Health Care Authority; 2013 Feb 15 2013.
90. Camacho-Cardenosa A, Camacho-Cardenosa M, Tomas-Carus P, Timon R, Olcina G, Burtscher M. Acute physiological response to a normobaric hypoxic exposure: sex differences. Int J Biometeorol. 2022;66(7):1495-504.
91. Sarzynski MA, Ghosh S, Bouchard C. Genomic and transcriptomic predictors of response levels to endurance exercise training. The Journal of physiology. 2017;595(9):2931-9.
92. Noble BJ, Borg GA, Jacobs I, Ceci R, Kaiser P. A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. Med Sci Sports Exerc. 1983;15(6):523-8.
93. De Wolde SD, Hulskes RH, Weenink RP, Hollmann MW, Van Hulst RA. The Effects of Hyperbaric Oxygenation on Oxidative Stress, Inflammation and Angiogenesis. Biomolecules. 2021;11(8).
94. Bosco G, Paganini M, Giacon TA, Oppio A, Vezzoli A, Dellanoce C, et al. Oxidative Stress and Inflammation, MicroRNA, and Hemoglobin Variations after Administration of Oxygen at Different Pressures and Concentrations: A Randomized Trial. International journal of environmental research and public health. 2021;18(18).
95. Balestra C, Lambrechts K, Mrakic-Sposta S, Vezzoli A, Levenez M, Germonpre P, et al. Hypoxic and Hyperoxic Breathing as a Complement to Low-Intensity Physical Exercise Programs: A Proof-of-Principle Study. Int J Mol Sci. 2021;22(17).
96. Gems D, Partridge L. Stress-response hormesis and aging: "that which does not kill us makes us stronger". Cell Metab. 2008;7(3):200-3.
97. Calabrese EJ, Baldwin LA, Holland CD. Hormesis: a highly generalizable and reproducible phenomenon with important implications for risk assessment. Risk Anal. 1999;19(2):261-81.
98. Hayes DP. Adverse effects of nutritional inadequacy and excess: a hormetic model. Am J Clin Nutr. 2008;88(2):578S-81S.
99. Calabrese EJ. Hormetic mechanisms. Crit Rev Toxicol. 2013;43(7):580-606.
100. Demirovic D, Rattan SI. Establishing cellular stress response profiles as biomarkers of homeodynamics, health and hormesis. Exp Gerontol. 2013;48(1):94-8.
101. Calabrese EJ, Mattson MP. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech Dis. 2017;3:13.
102. Wiegant FA, de Poot SA, Boers-Trilles VE, Schreij AM. Hormesis and Cellular Quality Control: A Possible Explanation for the Molecular Mechanisms that Underlie the Benefits of Mild Stress. Dose Response. 2012;11(3):413-30.
103. Li X, Yang T, Sun Z. Hormesis in Health and Chronic Diseases. Trends Endocrinol Metab. 2019;30(12):944-58.
104. Kietzmann T. Cellular Redox Compartments. Antioxid Redox Signal. 2019;30(1):1-4.
105. Fridovich I. Oxygen: how do we stand it? Med Princ Pract. 2013;22(2):131-7.

106. Gansemer ER, McCommis KS, Martino M, King-McAlpin AQ, Potthoff MJ, Finck BN, et al. NADPH and Glutathione Redox Link TCA Cycle Activity to Endoplasmic Reticulum Homeostasis. iScience. 2020;23(5):101116.
107. Duarte TL, Talbot NP, Drakesmith H. NRF2 and Hypoxia-Inducible Factors: Key Players in the Redox Control of Systemic Iron Homeostasis. Antioxid Redox Signal. 2021;35(6):433-52.
108. Milovanova TN, Bhopale VM, Sorokina EM, Moore JS, Hunt TK, Hauer-Jensen M, et al. Lactate stimulates vasculogenic stem cells via the thioredoxin system and engages an autocrine activation loop involving hypoxia-inducible factor 1. Molecular and cellular biology. 2008;28(20):6248-61.
109. Fisher AB. Redox signaling across cell membranes. Antioxid Redox Signal. 2009;11(6):1349-56.
110. Finkel T. Redox-dependent signal transduction. FEBS Lett. 2000;476(1-2):52-4.
111. Mennerich D, Kellokumpu S, Kietzmann T. Hypoxia and Reactive Oxygen Species as Modulators of Endoplasmic Reticulum and Golgi Homeostasis. Antioxid Redox Signal. 2019;30(1):113-37.
112. Petry A, Gorlach A. Regulation of NADPH Oxidases by G Protein-Coupled Receptors. Antioxid Redox Signal. 2019;30(1):74-94.
113. Lassègue B, Griendling KK. NADPH oxidases: functions and pathologies in the vasculature. Arterioscler Thromb Vasc Biol. 2010;30(4):653-61.
114. Montezano AC, Touyz RM. Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies. Ann Med. 2012;44 Suppl 1:S2-16.
115. Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PI, Jr. Molecular mechanisms of ROS production and oxidative stress in diabetes. The Biochemical journal. 2016;473(24):4527-50.
116. Kuczynska M, Jakubek P, Bartoszek A. More than Just Antioxidants: Redox-Active Components and Mechanisms Shaping Redox Signalling Network. Antioxidants (Basel). 2022;11(12).
117. Muraille E, Leo O, Moser M. TH1/TH2 paradigm extended: macrophage polarization as an unappreciated pathogen-driven escape mechanism? Front Immunol. 2014;5:603.
118. Lennicke C, Cocheme HM. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. Mol Cell. 2021;81(18):3691-707.
119. Samanta D, Semenza GL. Maintenance of redox homeostasis by hypoxia-inducible factors. Redox Biol. 2017;13:331-5.
120. Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Nikolaidis MG. Antioxidants in Personalized Nutrition and Exercise. Adv Nutr. 2018.
121. Dehne N, Brune B. Sensors, transmitters, and targets in mitochondrial oxygen shortage-a hypoxia-inducible factor relay story. Antioxid Redox Signal. 2014;20(2):339-52.
122. Wu J, Ruas JL, Estall JL, Rasbach KA, Choi JH, Ye L, et al. The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1alpha/ATF6alpha complex. Cell Metab. 2011;13(2):160-9.
123. Shilovsky GA, Ashapkin VV. Transcription Factor Nrf2 and Mitochondria - Friends or Foes in the Regulation of Aging Rate. Biochemistry (Mosc). 2022;87(12):1477-86.
124. Bubici C, Papa S, Dean K, Franzoso G. Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: molecular basis and biological significance. Oncogene. 2006;25(51):6731-48.

125. Gelderman KA, Hultqvist M, Olsson LM, Bauer K, Pizzolla A, Olofsson P, et al. Rheumatoid arthritis: the role of reactive oxygen species in disease development and therapeutic strategies. Antioxidants & Redox Signaling. 2007;9(10):1541-67.
126. Hartmann C, Nussbaum B, Calzia E, Radermacher P, Wepler M. Gaseous Mediators and Mitochondrial Function: The Future of Pharmacologically Induced Suspended Animation? Frontiers in physiology. 2017;8:691.
127. Mistry RK, Brewer AC. Redox regulation of gasotransmission in the vascular system: A focus on angiogenesis. Free radical biology & medicine. 2017;108:500-16.
128. Roderique JD, Josef CS, Feldman MJ, Spiess BD. A modern literature review of carbon monoxide poisoning theories, therapies, and potential targets for therapy advancement. Toxicology. 2015;334:45-58.
129. Stasko A, Brezova V, Zalibera M, Biskupic S, Ondrias K. Electron transfer: a primary step in the reactions of sodium hydrosulphide, an H2S/HS(-) donor. Free Radic Res. 2009;43(6):581-93.
130. Wang R. The gasotransmitter role of hydrogen sulfide. Antioxid Redox Signal. 2003;5(4):493-501.
131. Laroux FS, Pavlick KP, Hines IN, Kawachi S, Harada H, Bharwani S, et al. Role of nitric oxide in inflammation. Acta physiologica Scandinavica. 2001;173(1):113-8.
132. Larsen FJ, Schiffer TA, Weitzberg E, Lundberg JO. Regulation of mitochondrial function and energetics by reactive nitrogen oxides. Free radical biology & medicine. 2012;53(10):1919-28.
133. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. The American journal of physiology. 1996;271(5 Pt 1):C1424-37.
134. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. Nat Chem Biol. 2008;4(5):278-86.
135. Dikalov SI, Polienko YF, Kirilyuk I. Electron Paramagnetic Resonance Measurements of Reactive Oxygen Species by Cyclic Hydroxylamine Spin Probes. Antioxid Redox Signal. 2018;28(15):1433-43.
136. Dikalov SI, Kirilyuk IA, Voinov M, Grigor'ev IA. EPR detection of cellular and mitochondrial superoxide using cyclic hydroxylamines. Free Radic Res. 2011;45(4):417-30.
137. Dikalov SI, Polienko YF, Kirilyuk IA. EPR measurements of reactive oxygen species by cyclic hydroxylamine spin probes. Antioxid Redox Signal. 2017.
138. Umemura A. Editorial: Impacts of drug-induced oxidative stress. Front Med (Lausanne). 2023;10:1191864.
139. Cooper MD, Alder MN. The evolution of adaptive immune systems. Cell. 2006;124(4):815-22.
140. Forterre P. The origin of viruses and their possible roles in major evolutionary transitions. Virus Res. 2006;117(1):5-16.
141. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. Science. 2010;327(5963):291-5.
142. Waddington CH. The strategy of the genes; a discussion of some aspects of theoretical biology. London,: Allen & Unwin; 1957. ix, 262 p. p.
143. Takahashi K, Yamanaka S. A decade of transcription factor-mediated reprogramming to pluripotency. Nat Rev Mol Cell Biol. 2016;17(3):183-93.
144. Orekhov AN. Monocyte differentiation and macrophage polarization. Vessel Plus. 2019;Online.
145. Sampath P, Moideen K, Ranganathan UD, Bethunaickan R. Monocyte Subsets: Phenotypes and Function in Tuberculosis Infection. Front Immunol. 2018;9:1726.

146. Kapellos TS, Bonaguro L, Gemund I, Reusch N, Saglam A, Hinkley ER, et al. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol. 2019;10:2035.
147. Brune B, Dehne N, Grossmann N, Jung M, Namgaladze D, Schmid T, et al. Redox control of inflammation in macrophages. Antioxid Redox Signal. 2013;19(6):595-637.
148. Morris G, Gevezova M, Sarafian V, Maes M. Redox regulation of the immune response. Cell Mol Immunol. 2022;19(10):1079-101.
149. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12(6):492-9.
150. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15(8):486-99.
151. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol. 2010;28:445-89.
152. Knosp CA, Johnston JA. Regulation of CD4+ T-cell polarization by suppressor of cytokine signalling proteins. Immunology. 2012;135(2):101-11.
153. Liu YN, Yang JF, Huang DJ, Ni HH, Zhang CX, Zhang L, et al. Hypoxia Induces Mitochondrial Defect That Promotes T Cell Exhaustion in Tumor Microenvironment Through MYC-Regulated Pathways. Front Immunol. 2020;11:1906.
154. Davoodzadeh Gholami M, Kardar GA, Saeedi Y, Heydari S, Garssen J, Falak R. Exhaustion of T lymphocytes in the tumor microenvironment: Significance and effective mechanisms. Cell Immunol. 2017;322:1-14.
155. Vallejo AN, Weyand CM, Goronzy JJ. T-cell senescence: a culprit of immune abnormalities in chronic inflammation and persistent infection. Trends Mol Med. 2004;10(3):119-24.
156. Jackson SR, Berrien-Elliott MM, Meyer JM, Wherry EJ, Teague RM. CD8+ T cell exhaustion during persistent viral infection is regulated independently of the virus-specific T cell receptor. Immunol Invest. 2013;42(3):204-20.
157. Riffelmacher T, Richter FC, Simon AK. Autophagy dictates metabolism and differentiation of inflammatory immune cells. Autophagy. 2018;14(2):199-206.
158. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-97.
159. Kim K, Baek SC, Lee YY, Bastiaanssen C, Kim J, Kim H, et al. A quantitative map of human primary microRNA processing sites. Mol Cell. 2021;81(16):3422-39 e11.
160. Malhi H. MICRORNAs IN ER STRESS: DIVERGENT ROLES IN CELL FATE DECISIONS. Curr Pathobiol Rep. 2014;2(3):117-22.
161. Bartoszewska S, Kochan K, Madanecki P, Piotrowski A, Ochocka R, Collawn JF, et al. Regulation of the unfolded protein response by microRNAs. Cell Mol Biol Lett. 2013;18(4):555-78.
162. Serocki M, Bartoszewska S, Janaszak-Jasiecka A, Ochocka RJ, Collawn JF, Bartoszewski R. miRNAs regulate the HIF switch during hypoxia: a novel therapeutic target. Angiogenesis. 2018;21(2):183-202.
163. Janaszak-Jasiecka A, Bartoszewska S, Kochan K, Piotrowski A, Kalinowski L, Kamysz W, et al. miR-429 regulates the transition between Hypoxia-Inducible Factor (HIF)1A and HIF3A expression in human endothelial cells. Scientific reports. 2016;6:22775.
164. Kalinowski L, Janaszak-Jasiecka A, Siekierzycka A, Bartoszewska S, Wozniak M, Lejnowski D, et al. Posttranscriptional and transcriptional regulation of endothelial nitric-oxide synthase during hypoxia: the role of microRNAs. Cell Mol Biol Lett. 2016;21:16.

165. Chan YC, Banerjee J, Choi SY, Sen CK. miR-210: the master hypoxamir. Microcirculation. 2012;19(3):215-23.
166. Hart M, Walch-Ruckheim B, Friedmann KS, Rheinheimer S, Tanzer T, Glombitza B, et al. miR-34a: a new player in the regulation of T cell function by modulation of NF-kappaB signaling. Cell Death Dis. 2019;10(2):46.
167. Sapp RM, Shill DD, Roth SM, Hagberg JM. Circulating microRNAs in acute and chronic exercise: more than mere biomarkers. J Appl Physiol (1985). 2017;122(3):702-17.
168. Cui S, Sun B, Yin X, Guo X, Chao D, Zhang C, et al. Time-course responses of circulating microRNAs to three resistance training protocols in healthy young men. Scientific reports. 2017;7(1):2203.
169. Xiang M, Zeng Y, Yang R, Xu H, Chen Z, Zhong J, et al. U6 is not a suitable endogenous control for the quantification of circulating microRNAs. Biochem Biophys Res Commun. 2014;454(1):210-4.
170. Schwarz DS, Blower MD. The endoplasmic reticulum: structure, function and response to cellular signaling. Cell Mol Life Sci. 2016;73(1):79-94.
171. Hebert DN, Molinari M. In and out of the ER: protein folding, quality control, degradation, and related human diseases. Physiological reviews. 2007;87(4):1377-408.
172. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2016;529(7586):326-35.
173. Wang Y, Boerma M, Zhou D. Ionizing Radiation-Induced Endothelial Cell Senescence and Cardiovascular Diseases. Radiat Res. 2016;186(2):153-61.
174. Cao SS, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. Antioxid Redox Signal. 2014;21(3):396-413.
175. Carreras-Sureda A, Hetz C. RNA metabolism: putting the brake on the UPR. EMBO Rep. 2015;16(5):545-6.
176. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007;8(7):519-29.
177. Hetz C, Papa FR. The Unfolded Protein Response and Cell Fate Control. Mol Cell. 2018;69(2):169-81.
178. Lima T, Li TY, Mottis A, Auwerx J. Pleiotropic effects of mitochondria in aging. Nat Aging. 2022;2(3):199-213.
179. Nguyen TT, Wei S, Nguyen TH, Jo Y, Zhang Y, Park W, et al. Mitochondria-associated programmed cell death as a therapeutic target for age-related disease. Exp Mol Med. 2023;55(8):1595-619.
180. Carreras-Sureda A, Kroemer G, Cardenas JC, Hetz C. Balancing energy and protein homeostasis at ER-mitochondria contact sites. Sci Signal. 2022;15(741):eabm7524.
181. Magalhaes-Novais S, Blecha J, Naraine R, Mikesova J, Abaffy P, Pecinova A, et al. Mitochondrial respiration supports autophagy to provide stress resistance during quiescence. Autophagy. 2022;18(10):2409-26.
182. Detmer SA, Chan DC. Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol. 2007;8(11):870-9.
183. Pfanner N, Warscheid B, Wiedemann N. Mitochondrial proteins: from biogenesis to functional networks. Nat Rev Mol Cell Biol. 2019;20(5):267-84.
184. Hartl FU. Molecular chaperones in cellular protein folding. Nature. 1996;381(6583):571-9.
185. Horowitz M, Robinson SD. Heat shock proteins and the heat shock response during hyperthermia and its modulation by altered physiological conditions. Progress in brain research. 2007;162:433-46.

186. Chang HC, Tang YC, Hayer-Hartl M, Hartl FU. SnapShot: molecular chaperones, Part I. Cell. 2007;128(1):212.
187. Tang YC, Chang HC, Hayer-Hartl M, Hartl FU. SnapShot: molecular chaperones, Part II. Cell. 2007;128(2):412.
188. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. Nature. 2011;475(7356):324-32.
189. Rosenzweig R, Nillegoda NB, Mayer MP, Bukau B. The Hsp70 chaperone network. Nat Rev Mol Cell Biol. 2019;20(11):665-80.
190. Gonzalez-Gronow M, Gopal U, Austin RC, Pizzo SV. Glucose-regulated protein (GRP78) is an important cell surface receptor for viral invasion, cancers, and neurological disorders. IUBMB Life. 2021;73(6):843-54.
191. Chan CP, Siu KL, Chin KT, Yuen KY, Zheng B, Jin DY. Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. J Virol. 2006;80(18):9279-87.
192. Ravindran MS, Bagchi P, Cunningham CN, Tsai B. Opportunistic intruders: how viruses orchestrate ER functions to infect cells. Nature reviews Microbiology. 2016;14(7):407-20.
193. Prasad V, Greber UF. The endoplasmic reticulum unfolded protein response - homeostasis, cell death and evolution in virus infections. FEMS microbiology reviews. 2021;45(5).
194. Echavarria-Consuegra L, Cook GM, Busnadiego I, Lefevre C, Keep S, Brown K, et al. Manipulation of the unfolded protein response: A pharmacological strategy against coronavirus infection. PLoS Pathog. 2021;17(6):e1009644.
195. (WHO) WHO. WHO Coronavirus Disease (COVID-19) Dashboard [web page]. 2023 [updated 2023/11/08. Available from: https://covid19.who.int/.
196. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol. 2020.
197. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395(10234):1417-8.
198. Huertas A, Montani D, Savale L, Pichon J, Tu L, Parent F, et al. Endothelial cell dysfunction: a major player in SARS-CoV-2 infection (COVID-19)? The European respiratory journal. 2020;56(1).
199. Kraakman MJ, Murphy AJ, Jandeleit-Dahm K, Kammoun HL. Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? Front Immunol. 2014;5:470.
200. Merad M, Blish CA, Sallusto F, Iwasaki A. The immunology and immunopathology of COVID-19. Science. 2022;375(6585):1122-7.
201. Oh J, Riek AE, Weng S, Petty M, Kim D, Colonna M, et al. Endoplasmic reticulum stress controls M2 macrophage differentiation and foam cell formation. The Journal of biological chemistry. 2012;287(15):11629-41.
202. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell. 2020;181(7):1489-501 e15.
203. Wiech M, Chroscicki P, Swatler J, Stepnik D, De Biasi S, Hampel M, et al. Remodeling of T Cell Dynamics During Long COVID Is Dependent on Severity of SARS-CoV-2 Infection. Front Immunol. 2022;13:886431.
204. Diamond MS, Kanneganti TD. Innate immunity: the first line of defense against SARS-CoV-2. Nat Immunol. 2022;23(2):165-76.

205. Ferraro E, Germano M, Mollace R, Mollace V, Malara N. HIF-1, the Warburg Effect, and Macrophage/Microglia Polarization Potential Role in COVID-19 Pathogenesis. Oxid Med Cell Longev. 2021;2021:8841911.
206. Libby P, Luscher T. COVID-19 is, in the end, an endothelial disease. Eur Heart J. 2020;41(32):3038-44.
207. Chang R, Mamun A, Dominic A, Le NT. SARS-CoV-2 Mediated Endothelial Dysfunction: The Potential Role of Chronic Oxidative Stress. Frontiers in physiology. 2020;11:605908.
208. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. The New England journal of medicine. 2011;364(7):656-65.
209. Thom SR. Hyperbaric oxygen: its mechanisms and efficacy. Plast Reconstr Surg. 2011;127 Suppl 1:131S-41S.
210. Geng CK, Cao HH, Ying X, Zhang HT, Yu HL. The effects of hyperbaric oxygen on macrophage polarization after rat spinal cord injury. Brain research. 2015;1606:68-76.
211. Han CH, Zhang PX, Xu WG, Li RP, Xu JJ, Liu WW. Polarization of macrophages in the blood after decompression in mice. Med Gas Res. 2017;7(4):236-40.
212. Kjellberg A, De Maio A, Lindholm P. Can hyperbaric oxygen safely serve as an anti-inflammatory treatment for COVID-19? Medical Hypotheses. 2020;144.
213. Cannellotto M, Duarte M, Keller G, Larrea R, Cunto E, Chediack V, et al. Hyperbaric oxygen as an adjuvant treatment for patients with COVID-19 severe hypoxaemia: a randomised controlled trial. Emergency medicine journal : EMJ. 2021.
214. Toledo-Orozco S, et al. Hyperbaric Oxygen Therapy Efficacy as an Adjuvant for the Systemic Inflammation Reduction in Patients with SARS-CoV-2 Infection. J Clin Resp. 2022;4(1).
215. Siewiera J, Brodaczewska K, Jermakow N, Lubas A, Kłos K, Majewska A, et al. Effectiveness of Hyperbaric Oxygen Therapy in SARS-CoV-2 Pneumonia: The Primary Results of a Randomised Clinical Trial. Journal of Clinical Medicine. 2023;12(1):8.
216. Kjellberg A, Douglas J, Hassler A, Al-Ezerjawi S, Bostrom E, Abdel-Halim L, et al. COVID-19-Induced Acute Respiratory Distress Syndrome Treated with Hyperbaric Oxygen: Interim Safety Report from a Randomized Clinical Trial (COVID-19-HBO). J Clin Med. 2023;12(14).
217. Keller GA, Colaianni I, Coria J, Di Girolamo G, Miranda S. Clinical and biochemical short-term effects of hyperbaric oxygen therapy on SARS-Cov-2+ hospitalized patients with hypoxemic respiratory failure. Respir Med. 2023;209:107155.
218. Soriano JB, Murthy S, Marshall JC, Relan P, Diaz JV, Condition WHOCCDWGoP-C-. A clinical case definition of post-COVID-19 condition by a Delphi consensus. Lancet Infect Dis. 2021.
219. Villar JC, Gumisiriza N, Abreu LG, Maude RJ, Colebunders R. Defining post-COVID condition. Lancet Infect Dis. 2022;22(3):316-7.
220. Pan D, Pareek M. Toward a Universal Definition of Post-COVID-19 Condition-How Do We Proceed? JAMA Netw Open. 2023;6(4):e235779.
221. O'Mahoney LL, Routen A, Gillies C, Ekezie W, Welford A, Zhang A, et al. The prevalence and long-term health effects of Long Covid among hospitalised and non-hospitalised populations: A systematic review and meta-analysis. EClinicalMedicine. 2023;55:101762.
222. Ballouz T, Menges D, Anagnostopoulos A, Domenghino A, Aschmann HE, Frei A, et al. Recovery and symptom trajectories up to two years after SARS-CoV-2 infection: population based, longitudinal cohort study. Bmj. 2023;381:e074425.

223. Venkatesan P. NICE guideline on long COVID. The lancet Respiratory medicine. 2021.
224. Bramante CT, Buse JB, Liebovitz DM, Nicklas JM, Puskarich MA, Cohen K, et al. Outpatient treatment of COVID-19 and incidence of post-COVID-19 condition over 10 months (COVID-OUT): a multicentre, randomised, quadruple-blind, parallel-group, phase 3 trial. Lancet Infect Dis. 2023.
225. Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y, et al. Characterizing Long COVID in an International Cohort: 7 Months of Symptoms and Their Impact. medRxiv. 2020:2020.12.24.20248802.
226. Brodin P, Casari G, Townsend L, O'Farrelly C, Tancevski I, Loffler-Ragg J, et al. Studying severe long COVID to understand post-infectious disorders beyond COVID-19. Nat Med. 2022.
227. Visvabharathy L, Hanson B, Orban Z, Lim PH, Palacio NM, Jain R, et al. Neuro-COVID long-haulers exhibit broad dysfunction in T cell memory generation and responses to vaccination. medRxiv. 2021.
228. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol. 2003;23(2):168-75.
229. Marchetti M. COVID-19-driven endothelial damage: complement, HIF-1, and ABL2 are potential pathways of damage and targets for cure. Ann Hematol. 2020;99(8):1701-7.
230. Evans PC, Rainger GE, Mason JC, Guzik TJ, Osto E, Stamataki Z, et al. Endothelial dysfunction in COVID-19: a position paper of the ESC Working Group for Atherosclerosis and Vascular Biology, and the ESC Council of Basic Cardiovascular Science. Cardiovasc Res. 2020;116(14):2177-84.
231. Scherbakov N, Szklarski M, Hartwig J, Sotzny F, Lorenz S, Meyer A, et al. Peripheral endothelial dysfunction in myalgic encephalomyelitis/chronic fatigue syndrome. ESC Heart Fail. 2020;7(3):1064-71.
232. Newton DJ, Kennedy G, Chan KK, Lang CC, Belch JJ, Khan F. Large and small artery endothelial dysfunction in chronic fatigue syndrome. Int J Cardiol. 2012;154(3):335-6.
233. Legler F, Meyer-Arndt L, Modl L, Kedor C, Freitag H, Stein E, et al. Long-term symptom severity and clinical biomarkers in post-COVID-19/chronic fatigue syndrome: results from a prospective observational cohort. EClinicalMedicine. 2023;63:102146.
234. Conza D, Mirra P, Cali G, Insabato L, Fiory F, Beguinot F, et al. Metformin Dysregulates the Unfolded Protein Response and the WNT/beta-Catenin Pathway in Endometrial Cancer Cells through an AMPK-Independent Mechanism. Cells. 2021;10(5).
235. Tran LNK, Kichenadasse G, Butler LM, Centenera MM, Morel KL, Ormsby RJ, et al. The Combination of Metformin and Valproic Acid Induces Synergistic Apoptosis in the Presence of p53 and Androgen Signaling in Prostate Cancer. Mol Cancer Ther. 2017;16(12):2689-700.
236. Lee DE, Lee GY, Lee HM, Choi SY, Lee SJ, Kwon OS. Synergistic apoptosis by combination of metformin and an O-GlcNAcylation inhibitor in colon cancer cells. Cancer Cell Int. 2023;23(1):108.
237. Godman CA, Chheda KP, Hightower LE, Perdrizet G, Shin DG, Giardina C. Hyperbaric oxygen induces a cytoprotective and angiogenic response in human microvascular endothelial cells. Cell Stress Chaperones. 2010;15(4):431-42.

238. Robbins T, Gonevski M, Clark C, Baitule S, Sharma K, Magar A, et al. Hyperbaric oxygen therapy for the treatment of long COVID: early evaluation of a highly promising intervention. Clin Med (Lond). 2021;21(6):e629-e32.
239. Bhaiyat AM, Sasson E, Wang Z, Khairy S, Ginzarly M, Qureshi U, et al. Hyperbaric oxygen treatment for long coronavirus disease-19: a case report. J Med Case Rep. 2022;16(1):80.
240. Zilberman-Itskovich S, Catalogna M, Sasson E, Elman-Shina K, Hadanny A, Lang E, et al. Hyperbaric oxygen therapy improves neurocognitive functions and symptoms of post-COVID condition: randomized controlled trial. Scientific reports. 2022;12(1):11252.
241. Kjellberg A. Hyperbar syrgas kan förbättra kognitiv funktion vid postcovid. Läkartidningen. 2022;40/2022.
242. Cumpstey AF, Clark AD, Santolini J, Jackson AA, Feelisch M. COVID-19: A Redox Disease-What a Stress Pandemic Can Teach Us About Resilience and What We May Learn from the Reactive Species Interactome About Its Treatment. Antioxid Redox Signal. 2021.
243. Agency EM. ICH guideline for good clinical practice E6(R2) 2016 [ICH-GCP Guidelines]. Available from: https://www.ema.europa.eu/en/ich-e6-r2-good-clinical-practice-scientific-guideline#current-version--section.
244. Chan AW, Tetzlaff JM, Altman DG, Dickersin K, Moher D. SPIRIT 2013: new guidance for content of clinical trial protocols. Lancet. 2013;381(9861):91-2.
245. Calvert M, Kyte D, Mercieca-Bebber R, Slade A, Chan AW, King MT, et al. Guidelines for Inclusion of Patient-Reported Outcomes in Clinical Trial Protocols: The SPIRIT-PRO Extension. JAMA : the journal of the American Medical Association. 2018;319(5):483-94.
246. Lansdorp CA, van Hulst RA. Double-blind trials in hyperbaric medicine: A narrative review on past experiences and considerations in designing sham hyperbaric treatment. Clin Trials. 2018;15(5):462-76.
247. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMC medicine. 2010;8:18.
248. Alyethodi RR, Karthik S, Muniswamy K, Ravi SK, Perumal P, Bhattacharya D, et al. Assessment of Protein Profiles of RNAlater Stored and Fresh PBMC Cells Using Different Protein Extraction Buffers. Protein J. 2020;39(3):291-300.
249. van Eijsden RG, Stassen C, Daenen L, Van Mulders SE, Bapat PM, Siewers V, et al. A universal fixation method based on quaternary ammonium salts (RNAlater) for omics-technologies: Saccharomyces cerevisiae as a case study. Biotechnol Lett. 2013;35(6):891-900.
250. Ura H, Togi S, Niida Y. A comparison of mRNA sequencing (RNA-Seq) library preparation methods for transcriptome analysis. BMC Genomics. 2022;23(1):303.
251. Hrdlickova R, Toloue M, Tian B. RNA-Seq methods for transcriptome analysis. Wiley Interdiscip Rev RNA. 2017;8(1).
252. Garcia-Campos MA, Espinal-Enriquez J, Hernandez-Lemus E. Pathway Analysis: State of the Art. Frontiers in physiology. 2015;6:383.
253. Orwelius L, Nilsson M, Nilsson E, Wenemark M, Walfridsson U, Lundstrom M, et al. The Swedish RAND-36 Health Survey - reliability and responsiveness assessed in patient populations using Svensson's method for paired ordinal data. J Patient Rep Outcomes. 2017;2(1):4.

254. Hardt J, Buchwald D, Wilks D, Sharpe M, Nix WA, Egle UT. Health-related quality of life in patients with chronic fatigue syndrome: an international study. J Psychosom Res. 2001;51(2):431-4.
255. Bagai K, Song Y, Ling JF, Malow B, Black BK, Biaggioni I, et al. Sleep disturbances and diminished quality of life in postural tachycardia syndrome. J Clin Sleep Med. 2011;7(2):204-10.
256. Dolan P. Modeling valuations for EuroQol health states. Med Care. 1997;35(11):1095-108.
257. Burstrom K, Sun S, Gerdtham UG, Henriksson M, Johannesson M, Levin LA, et al. Swedish experience-based value sets for EQ-5D health states. Qual Life Res. 2014;23(2):431-42.
258. Hamburg NM, Benjamin EJ. Assessment of endothelial function using digital pulse amplitude tonometry. Trends Cardiovasc Med. 2009;19(1):6-11.
259. Alexander Y, Osto E, Schmidt-Trucksass A, Shechter M, Trifunovic D, Duncker DJ, et al. Endothelial function in cardiovascular medicine: a consensus paper of the European Society of Cardiology Working Groups on Atherosclerosis and Vascular Biology, Aorta and Peripheral Vascular Diseases, Coronary Pathophysiology and Microcirculation, and Thrombosis. Cardiovasc Res. 2021;117(1):29-42.
260. Ameloot K, Van De Vijver K, Broch O, Van Regenmortel N, De Laet I, Schoonheydt K, et al. Nexfin noninvasive continuous hemodynamic monitoring: validation against continuous pulse contour and intermittent transpulmonary thermodilution derived cardiac output in critically ill patients. ScientificWorldJournal. 2013;2013:519080.
261. Enright PL. The six-minute walk test. Respiratory care. 2003;48(8):783-5.
262. Munblit D, Nicholson T, Akrami A, Apfelbacher C, Chen J, De Groote W, et al. A core outcome set for post-COVID-19 condition in adults for use in clinical practice and research: an international Delphi consensus study. The lancet Respiratory medicine. 2022;10(7):715-24.
263. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
264. Ohlsson-Nevo E, Hiyoshi A, Noren P, Moller M, Karlsson J. The Swedish RAND-36: psychometric characteristics and reference data from the Mid-Swed Health Survey. J Patient Rep Outcomes. 2021;5(1):66.
265. Browne LH, Graham PH. Good intentions and ICH-GCP: Trial conduct training needs to go beyond the ICH-GCP document and include the intention-to-treat principle. Clin Trials. 2014;11(6):629-34.
266. Kaur S, Choy CY. Ethical considerations in clinical trials: a critique of the ICH-GCP guideline. Dev World Bioeth. 2014;14(1):20-8.
267. Hossain MS, Dietz KJ. Tuning of Redox Regulatory Mechanisms, Reactive Oxygen Species and Redox Homeostasis under Salinity Stress. Front Plant Sci. 2016;7:548.
268. Tan B, Jin S, Sun J, Gu Z, Sun X, Zhu Y, et al. New method for quantification of gasotransmitter hydrogen sulfide in biological matrices by LC-MS/MS. Scientific reports. 2017;7:46278.
269. Alkadi H. A Review On Free Radicals and Antioxidants. Infect Disord Drug Targets. 2018.
270. Zhou Q, Huang G, Yu X, Xu W. A Novel Approach to Estimate ROS Origination by Hyperbaric Oxygen Exposure, Targeted Probes and Specific Inhibitors. Cell Physiol Biochem. 2018;47(5):1800-8.

271. Cirino G, Vellecco V, Bucci M. Nitric oxide and hydrogen sulfide: the gasotransmitter paradigm of the vascular system. Br J Pharmacol. 2017;174(22):4021-31.
272. Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting Free Radicals in Oxidative Stress-Related Human Diseases. Trends Pharmacol Sci. 2017;38(7):592-607.
273. Paolini M, Valgimigli L, Marchesi E, Trespidi S, Pedulli GF. Taking EPR "snapshots" of the oxidative stress status in human blood. Free Radic Res. 2003;37(5):503-8.
274. Kossmann S, Hu H, Steven S, Schonfelder T, Fraccarollo D, Mikhed Y, et al. Inflammatory monocytes determine endothelial nitric-oxide synthase uncoupling and nitro-oxidative stress induced by angiotensin II. The Journal of biological chemistry. 2014;289(40):27540-50.
275. Mahdi A, Collado A, Tengbom J, Jiao T, Wodaje T, Johansson N, et al. Erythrocytes Induce Vascular Dysfunction in COVID-19. JACC Basic Transl Sci. 2022;7(3):193-204.
276. Orrico F, Laurance S, Lopez AC, Lefevre SD, Thomson L, Moller MN, et al. Oxidative Stress in Healthy and Pathological Red Blood Cells. Biomolecules. 2023;13(8).
277. Prebensen C, Lefol Y, Myhre P, Luders T, Jonassen C, Blomfeldt A, et al. Longitudinal whole blood transcriptomic analysis characterizes neutrophil activation and interferon signaling in moderate and severe COVID-19. Scientific reports. 2023;13(1):10368.
278. Nakajima S, Kitamura M. Bidirectional regulation of NF-kappaB by reactive oxygen species: a role of unfolded protein response. Free radical biology & medicine. 2013;65:162-74.
279. Bolland H, Ma TS, Ramlee S, Ramadan K, Hammond EM. Links between the unfolded protein response and the DNA damage response in hypoxia: a systematic review. Biochem Soc Trans. 2021;49(3):1251-63.
280. Sehrawat U, Haimov O, Weiss B, Tamarkin-Ben Harush A, Ashkenazi S, Plotnikov A, et al. Inhibitors of eIF4G1-eIF1 uncover its regulatory role of ER/UPR stress-response genes independent of eIF2alpha-phosphorylation. Proceedings of the National Academy of Sciences of the United States of America. 2022;119(30):e2120339119.
281. Bartoszewski R, Gebert M, Janaszak-Jasiecka A, Cabaj A, Kroliczewski J, Bartoszewska S, et al. Genome-wide mRNA profiling identifies RCAN1 and GADD45A as regulators of the transitional switch from survival to apoptosis during ER stress. FEBS J. 2020;287(14):2923-47.
282. Garcia M, Kokkinou E, Carrasco Garcia A, Parrot T, Palma Medina LM, Maleki KT, et al. Innate lymphoid cell composition associates with COVID-19 disease severity. Clin Transl Immunology. 2020;9(12):e1224.
283. Vinkel J, Rib L, Buil A, Hedetoft M, Hyldegaard O. Key pathways and genes that are altered during treatment with hyperbaric oxygen in patients with sepsis due to necrotizing soft tissue infection (HBOmic study). Eur J Med Res. 2023;28(1):507.
284. Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: atypical GRP78 in cell viability, signalling and therapeutic targeting. The Biochemical journal. 2011;434(2):181-8.
285. Zhang Y, Liu R, Ni M, Gill P, Lee AS. Cell surface relocalization of the endoplasmic reticulum chaperone and unfolded protein response regulator GRP78/BiP. The Journal of biological chemistry. 2010;285(20):15065-75.

286. Al-Hashimi AA, Caldwell J, Gonzalez-Gronow M, Pizzo SV, Aboumrad D, Pozza L, et al. Binding of anti-GRP78 autoantibodies to cell surface GRP78 increases tissue factor procoagulant activity via the release of calcium from endoplasmic reticulum stores. The Journal of biological chemistry. 2010;285(37):28912-23.
287. Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. Antioxid Redox Signal. 2009;11(9):2299-306.
288. Aguiar JA, Tremblay BJ, Mansfield MJ, Woody O, Lobb B, Banerjee A, et al. Gene expression and in situ protein profiling of candidate SARS-CoV-2 receptors in human airway epithelial cells and lung tissue. The European respiratory journal. 2020;56(3).
289. Gaudette BT, Jones DD, Bortnick A, Argon Y, Allman D. mTORC1 coordinates an immediate unfolded protein response-related transcriptome in activated B cells preceding antibody secretion. Nat Commun. 2020;11(1):723.
290. Diaz-Bulnes P, Saiz ML, Lopez-Larrea C, Rodriguez RM. Crosstalk Between Hypoxia and ER Stress Response: A Key Regulator of Macrophage Polarization. Front Immunol. 2019;10:2951.
291. Di Conza G, Ho PC, Cubillos-Ruiz JR, Huang SC. Control of immune cell function by the unfolded protein response. Nat Rev Immunol. 2023.
292. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012;13(4):227-32.
293. Bansal A, Kumar S, Rai N, Kumari S, Kumar V, Kumar A, et al. A Pilot Study on Blood Components in COVID-19 Affected Subjects: A Correlation to UPR Signalling and ER-Stress. Indian J Clin Biochem. 2023;38(3):374-84.
294. Shaban MS, Muller C, Mayr-Buro C, Weiser H, Meier-Soelch J, Albert BV, et al. Multi-level inhibition of coronavirus replication by chemical ER stress. Nat Commun. 2021;12(1):5536.
295. Wingelaar TT, van Ooij PAM, van Hulst RA. Oxygen Toxicity and Special Operations Forces Diving: Hidden and Dangerous. Front Psychol. 2017;8:1263.
296. Catalogna M, Sasson E, Hadanny A, Parag Y, Zilberman-Itskovich S, Efrati S. Effects of hyperbaric oxygen therapy on functional and structural connectivity in post-COVID-19 condition patients: A randomized, sham-controlled trial. Neuroimage Clin. 2022;36:103218.
297. Leitman M, Fuchs S, Tyomkin V, Hadanny A, Zilberman-Itskovich S, Efrati S. The effect of hyperbaric oxygen therapy on myocardial function in post-COVID-19 syndrome patients: a randomized controlled trial. Scientific reports. 2023;13(1):9473.
298. Samper-Pardo M, Leon-Herrera S, Olivan-Blazquez B, Gascon-Santos S, Sanchez-Recio R. Clinical characterization and factors associated with quality of life in Long COVID patients: Secondary data analysis from a randomized clinical trial. PloS one. 2023;18(5):e0278728.
299. Hedberg P, Granath F, Bruchfeld J, Askling J, Sjoholm D, Fored M, et al. Post COVID-19 condition diagnosis: A population-based cohort study of occurrence, associated factors, and healthcare use by severity of acute infection. J Intern Med. 2023;293(2):246-58.
300. Giszas B, Trommer S, Schussler N, Rodewald A, Besteher B, Bleidorn J, et al. Post-COVID-19 condition is not only a question of persistent symptoms: structured screening including health-related quality of life reveals two separate clusters of post-COVID. Infection. 2023;51(2):365-77.

301. Balestra C, Germonpre P. Increasing EPO using the normobaric oxygen paradox: a 'not so simple' task. Acta Physiol (Oxf). 2011;203(2):287-8.
302. Burtscher J, Mallet RT, Pialoux V, Millet GP, Burtscher M. Adaptive Responses to Hypoxia and/or Hyperoxia in Humans. Antioxid Redox Signal. 2022;37(13-15):887-912.
303. Alvarez Villela M, Dunworth SA, Kraft BD, Harlan NP, Natoli MJ, Suliman HB, et al. Effects of high-intensity interval training with hyperbaric oxygen. Frontiers in physiology. 2022;13:963799.
304. Salvagno M, Coppalini G, Taccone FS, Strapazzon G, Mrakic-Sposta S, Rocco M, et al. The Normobaric Oxygen Paradox-Hyperoxic Hypoxic Paradox: A Novel Expedient Strategy in Hematopoiesis Clinical Issues. Int J Mol Sci. 2022;24(1).
305. Thews O, Vaupel P. Temporal changes in tumor oxygenation and perfusion upon normo- and hyperbaric inspiratory hyperoxia. Strahlenther Onkol. 2016;192(3):174-81.
306. Burtscher J, Strasser B, D'Antona G, Millet GP, Burtscher M. How much resistance exercise is beneficial for healthy aging and longevity? J Sport Health Sci. 2023;12(3):284-6.
307. Naviaux RK. Metabolic features of the cell danger response. Mitochondrion. 2014;16:7-17.
308. Naviaux RK. Oxidative shielding or oxidative stress? J Pharmacol Exp Ther. 2012;342(3):608-18.
309. Kim H-R, Kim J-H, Choi E-J, Lee YK, Kie J-H, Jang MH, et al. Hyperoxygenation attenuated a murine model of atopic dermatitis through raising skin level of ROS. PloS one. 2014;9(10):e109297-e.
310. Kim HR, Lee A, Choi EJ, Hong MP, Kie JH, Lim W, et al. Reactive oxygen species prevent imiquimod-induced psoriatic dermatitis through enhancing regulatory T cell function. PloS one. 2014;9(3):e91146.
311. Kjellberg A, Bjerin O, Franzen-Rohl E, Bartek J, Jr., Lindholm P. Lemierre's syndrome caused by Fusobacterium necrophorum complicated with multiple brain abscesses-A case report, literature review, and suggested management. Clin Case Rep. 2021;9(12):e05142.
312. Hedetoft M, Bennett MH, Hyldegaard O. Adjunctive hyperbaric oxygen treatment for necrotising soft-tissue infections: A systematic review and meta-analysis. Diving and hyperbaric medicine : the journal of the South Pacific Underwater Medicine Society. 2021;51(1):34-43.
313. Hedetoft M, Madsen MB, Madsen LB, Hyldegaard O. Incidence, comorbidity and mortality in patients with necrotising soft-tissue infections, 2005-2018: a Danish nationwide register-based cohort study. BMJ Open. 2020;10(10):e041302.
314. Madsen MB, Skrede S, Perner A, Arnell P, Nekludov M, Bruun T, et al. Patient's characteristics and outcomes in necrotising soft-tissue infections: results from a Scandinavian, multicentre, prospective cohort study. Intensive care medicine. 2019;45(9):1241-51.
315. Mladenov M, Lubomirov L, Grisk O, Avtanski D, Mitrokhin V, Sazdova I, et al. Oxidative Stress, Reductive Stress and Antioxidants in Vascular Pathogenesis and Aging. Antioxidants (Basel). 2023;12(5).
316. Cillo AR, Somasundaram A, Shan F, Cardello C, Workman CJ, Kitsios GD, et al. People critically ill with COVID-19 exhibit peripheral immune profiles predictive of mortality and reflective of SARS-CoV-2 lung viral burden. Cell Rep Med. 2021;2(12):100476.

317. Waisner H, Grieshaber B, Saud R, Henke W, Stephens EB, Kalamvoki M. SARS-CoV-2 Harnesses Host Translational Shutoff and Autophagy To Optimize Virus Yields: the Role of the Envelope (E) Protein. Microbiol Spectr. 2023;11(1):e0370722.
318. Li X, Zhang Z, Wang Z, Gutierrez-Castrellon P, Shi H. Cell deaths: Involvement in the pathogenesis and intervention therapy of COVID-19. Signal Transduct Target Ther. 2022;7(1):186.
319. Liu Z, Fu Y, Huang Y, Zeng F, Rao J, Xiao X, et al. Ubiquitination of SARS-CoV-2 ORF7a Prevents Cell Death Induced by Recruiting BclXL To Activate ER Stress. Microbiol Spectr. 2022;10(6):e0150922.
320. Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? Nat Rev Immunol. 2011;11(4):289-95.
321. Zhao Y, Shao Q, Peng G. Exhaustion and senescence: two crucial dysfunctional states of T cells in the tumor microenvironment. Cell Mol Immunol. 2020;17(1):27-35.
322. Schmitt CA, Wang B, Demaria M. Senescence and cancer - role and therapeutic opportunities. Nat Rev Clin Oncol. 2022;19(10):619-36.
323. Barbosa LV, Pra DMM, Nagashima S, Pereira MRC, Stocco RB, da Silva FLF, et al. Immune Response Gaps Linked to SARS-CoV-2 Infection: Cellular Exhaustion, Senescence, or Both? Int J Mol Sci. 2022;23(22).
324. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun. 2020;11(1):3434.
325. Judge SJ, Murphy WJ, Canter RJ. Characterizing the Dysfunctional NK Cell: Assessing the Clinical Relevance of Exhaustion, Anergy, and Senescence. Front Cell Infect Microbiol. 2020;10:49.
326. Naviaux RK. Mitochondrial and metabolic features of salugenesis and the healing cycle. Mitochondrion. 2023;70:131-63.
327. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. Trends Immunol. 2015;36(4):265-76.
328. Rasche L, Duell J, Morgner C, Chatterjee M, Hensel F, Rosenwald A, et al. The natural human IgM antibody PAT-SM6 induces apoptosis in primary human multiple myeloma cells by targeting heat shock protein GRP78. PloS one. 2013;8(5):e63414.
329. Misra UK, Pizzo SV. Ligation of cell surface GRP78 with antibody directed against the COOH-terminal domain of GRP78 suppresses Ras/MAPK and PI 3-kinase/AKT signaling while promoting caspase activation in human prostate cancer cells. Cancer Biol Ther. 2010;9(2):142-52.
330. Chang YJ, Huang YP, Li ZL, Chen CH. GRP78 knockdown enhances apoptosis via the down-regulation of oxidative stress and Akt pathway after epirubicin treatment in colon cancer DLD-1 cells. PloS one. 2012;7(4):e35123.
331. Dadey DYA, Kapoor V, Hoye K, Khudanyan A, Collins A, Thotala D, et al. Antibody Targeting GRP78 Enhances the Efficacy of Radiation Therapy in Human Glioblastoma and Non-Small Cell Lung Cancer Cell Lines and Tumor Models. Clin Cancer Res. 2017;23(10):2556-64.
332. Stepien K, Ostrowski RP, Matyja E. Hyperbaric oxygen as an adjunctive therapy in treatment of malignancies, including brain tumours. Med Oncol. 2016;33(9):101.

333. Alpuim Costa D, Sampaio-Alves M, Netto E, Fernandez G, Oliveira E, Teixeira A, et al. Hyperbaric Oxygen Therapy as a Complementary Treatment in Glioblastoma-A Scoping Review. Front Neurol. 2022;13:886603.
334. Li C, Chen YM. Endoplasmic Reticulum-Associated Biomarkers for Molecular Phenotyping of Rare Kidney Disease. Int J Mol Sci. 2021;22(4).
335. Mnich K, Moghaddam S, Browne P, Counihan T, Fitzgerald SP, Martin K, et al. Endoplasmic Reticulum Stress-Regulated Chaperones as a Serum Biomarker Panel for Parkinson's Disease. Mol Neurobiol. 2023;60(3):1476-85.
336. Al Zaidi M, Marggraf V, Repges E, Nickenig G, Skowasch D, Aksoy A, et al. Relevance of serum levels of the endoplasmic reticulum stress protein GRP78 (glucose-regulated protein 78 kDa) as biomarker in pulmonary diseases. Cell Stress Chaperones. 2023;28(3):333-41.
337. Ciplys E, Aucynaite A, Slibinskas R. Generation of human ER chaperone BiP in yeast Saccharomyces cerevisiae. Microb Cell Fact. 2014;13:22.
338. Shen X, Ellis RE, Lee K, Liu CY, Yang K, Solomon A, et al. Complementary signaling pathways regulate the unfolded protein response and are required for C. elegans development. Cell. 2001;107(7):893-903.
339. Verma AK, Diwan D, Raut S, Dobriyal N, Brown RE, Gowda V, et al. Evolutionary Conservation and Emerging Functional Diversity of the Cytosolic Hsp70:J Protein Chaperone Network of Arabidopsis thaliana. G3 (Bethesda). 2017;7(6):1941-54.
340. Jordbruksverket. These are the 3Rs: Jordbruksverket; 2023 [updated 2023-10-11. Information on and links to regulation on 3R]. Available from: https://jordbruksverket.se/languages/english/swedish-board-of-agriculture/animals/the-swedish-3rs-center/these-are-the-3rs.
341. Marchi S, Patergnani S, Pinton P. The endoplasmic reticulum-mitochondria connection: one touch, multiple functions. Biochimica et biophysica acta. 2014;1837(4):461-9.
342. Wang Y, Ruan L, Zhu J, Zhang X, Chih-Chieh Chang A, Tomaszewski A, et al. Metabolic regulation of misfolded protein import into mitochondria. bioRxiv. 2023.
343. Fratantonio D, Virgili F, Zucchi A, Lambrechts K, Latronico T, Lafere P, et al. Increasing Oxygen Partial Pressures Induce a Distinct Transcriptional Response in Human PBMC: A Pilot Study on the "Normobaric Oxygen Paradox". Int J Mol Sci. 2021;22(1).
344. Tessema B, Sack U, Serebrovska Z, Konig B, Egorov E. Effects of Hyperoxia on Aging Biomarkers: A Systematic Review. Front Aging. 2021;2:783144.