A Review of Using Hypoxia as a Therapeutic Modality: Developing Intermittent Hypoxia-Hyperoxia Conditioning Protocol Guidelines for Surgical CABG Patients

by

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Abstract

This document features an exploration of the hormesis effect induced by intermittent hypoxic conditioning (IHC) as a potential therapeutic modality for surgical coronary artery bypass graft (CABG) patients. A brief history of IHC is reviewed along with a detailed review of known cellular mechanisms. Various review and primary studies on IHC are examined and show beneficial clinical outcomes for cardiovascular patients. This information is then used to develop protocol guidelines for incorporation of IHC into the clinical management of CABG patients. These guidelines address proper administration, clinical safety, and patient management. Among the findings of this review are that although more research is warranted to fully understand all the mechanisms and to optimize the administration of IHC, this therapy is likely a safe, non-invasive and non-pharmacological treatment that would be a useful aid to current cardiac management and rehabilitation.

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Nomenclature

Abbreviations

AI	Atherogenic Index
AMPK	Adenosine Monophosphate-Activated Protein Kinase
ATP	Adenosine Triphosphate
CABG	Coronary Artery Bypass Graft
CHC	Chronic Hypoxic Conditioning
CHT	Continuous Hypoxic Training
ECG	Electrocardiogram
EPO	Erythropoietin
F_iO_2	Fraction of Inspired Oxygen
HAT	High Altitude Training
HC	Hypoxic Conditioning
HH	Hypobaric Hypoxia
HIIT	High Intensity-Interval Training
HIF-1alpha	Hypoxic-Inducible Factor-1alpha
HR	Heart Rate
HSP	Heat Shock Protein
HT	Hypoxic Training
IHC	Intermittent Hypoxic Conditioning
IHE	Intermittent Hypoxic Exposure
IHHC	Intermittent Hypoxia-Hyperoxia Conditioning
IHHE	Intermittent Hypoxia-Hyperoxia Exposure
IHHT	Intermittent Hypoxia-Hyperoxia Training
IHIT	Intermittent Hypoxic Exposure During Interval Training

IHT	Intermittent Hypoxic Training
I/RI	Ischemic/Reperfusion Injury
LDL	Low-density Lipoprotein
LHTH	Live High Train High
LHTL	Live High Train Low
LLTH	Live Low Train High
LVEF	Left Ventricular Ejection Fraction
NH	Normobaric Hypoxia
Nrf2	Nuclear Factor Erythroid-2
NYHA	New York Heart Association
OSAS	Obstructive Sleep Apnea Syndrome
PDH	Pyruvate Dehydrogenase
PFK	Phosphofructokinase
PGC-1alpha	Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1alpha
RBC	Red Blood Cells
RIPC	Remote Ischemic Pre-Conditioning
ROS	Reactive Oxygen Species
RST	Repeated Sprint Training
RSH	Repeated Sprint Training in Hypoxia
SaO_2	Saturation of Oxygen in Arterial Blood
SCR	Standard Cardiac Rehabilitation
SRT	Self-Regulated Treatment
TC	Total Cholesterol
VO_2peak	Peak Oxygen Consumption Measured During Exercise

1. Introduction

High altitude training (HAT), hypoxic conditioning (HC), oxygen challenge, and other similar frequently used phrases in research and clinical settings refer to the alteration of the atmospheric concentration of oxygen, whether through partial pressure (hypobaric atmosphere) or fraction of inspired oxygen (F_iO_2), to challenge the body. This hypoxic stimulation initiates numerous cascades, ultimately leading to cellular adaptation [1]. The concept of intentionally challenging the body to elicit a response or resistance is termed "hormesis," and many everyday examples -- such as exercising and vaccinations, and less commonly considered examples such as neuroplasticity and reactive oxygen species (ROS) -- exemplify this phenomenon [2, 3]. Hormesis is based on the unique ability cells have to adapt, condition, and optimize themselves for future insults when exposed to a low dose of harmful stimulus, whereas a high dose of the same stimulus could cause detrimental effects (Figure 1) [2, 3, 4]. The phrase "Dose makes the poison," coined by 16th-century chemist and physician Paracelsus, sums up the defining difference between the beneficial and deleterious effects of a substance; allow just enough cellular strain to elicit an adaptive response, but not enough to injure the cell [2, 4, 5].

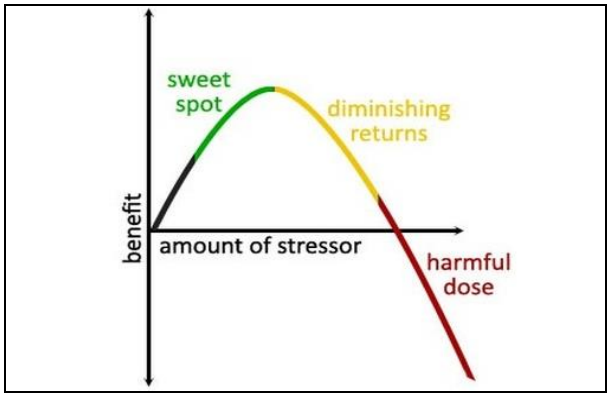

Figure 1: Graphical Representation of Hormesis [6].

Attempting to comprehend the physiologic effects of HC very quickly becomes highly complex; HC consists of an interconnected maze of molecular and cellular cascades that are not entirely understood. While the known mechanisms and pathways are discussed in this thesis, it is of benefit to focus attention on the responses elicited in whole-animal or human studies. Studies investigating athletes' use of HC for increased performance have dominated the research on HC, but there is a relative lack of human clinical trials, resulting in HC often being disregarded in the clinical setting primarily because of concerns regarding its safety and efficacy in patient populations.

Several studies, however, have recently emerged from Europe demonstrating safety and efficacy when using intermittent hypoxic conditioning (IHC) for pre- and post-conditioning in cardiac surgery [7, 8, 9]. The goal of this review is to look at the history of HC and to synthesize the currently available data on IHC, specifically on the cardiovascular system. Further, the document features an attempt to develop protocol guidelines for designing a study for the use of intermittent hypoxia-hyperoxia conditioning (IHHC) in pre- and post-conditioning of coronary artery bypass graft (CABG) patients to determine the clinical efficacy of IHHC.

2. Background

2.1 Brief History of Hypoxic Effects on the Body

Hypoxia and its effects on the human body have been studied as early as the 1800s, shortly after Joseph Priestley discovered oxygen [10]. Balloonist and mountaineers quickly found that ascending to high altitudes without proper acclimation was debilitating and even lethal [10, 11]. It was observed that people who lived in high altitude or who had sufficient time to acclimate, adapted remarkably [10, 11, 12]. These individuals could ascend to much higher altitudes, with greater exercise capacity, and without succumbing to acute mountain sickness [10, 11, 12]. Although the research was not released to the rest of the world until sometime after the Soviet Union fell in 1991, Russian researchers were some of the first pioneers of HC [13, 14].

Dating back to before World War II, the former Soviet Union used altitude and hypobaric chambers to condition their pilots to fly open cockpit airplanes at altitudes higher than 7000 meters [13]. Because of the cost and inconvenience of these methods, Russian researchers studied if intermittent hypoxic gas inhalation would elicit similar acclimation and found that there were promising results [13]. Much more recently, the United States has also started using HC for pilots (awareness under cabin pressure failure), paratroopers (high altitude jumps), amphibious units (increased lung capacity), special forces (acclimating for missions to high altitudes), and increasing exercise capacity for soldiers [15]. It was not until the 1968 Olympics in Mexico City (2240m), where record low endurance event times were recorded, however, that hypoxic training (HT) started to gain more recognition for its ability to stimulate cellular adaptation and to contribute to increased athletic performance [16, 17]. In the last half century since the

1968 Olympics, different HAT and intermittent hypoxic training (IHT) strategies have been heavily researched to assess the changes that occur at the molecular level, as well as how those adaptions translate to performance outcomes.

2.2 Athletic Benefits of Hypoxic Training

There are several HT strategies (Figure 2), and although each variation may emphasize greater molecular and cellular changes in different areas, the goal of all of these strategies is to increase aerobic capacity and efficiency in order to reap performance gains [18].

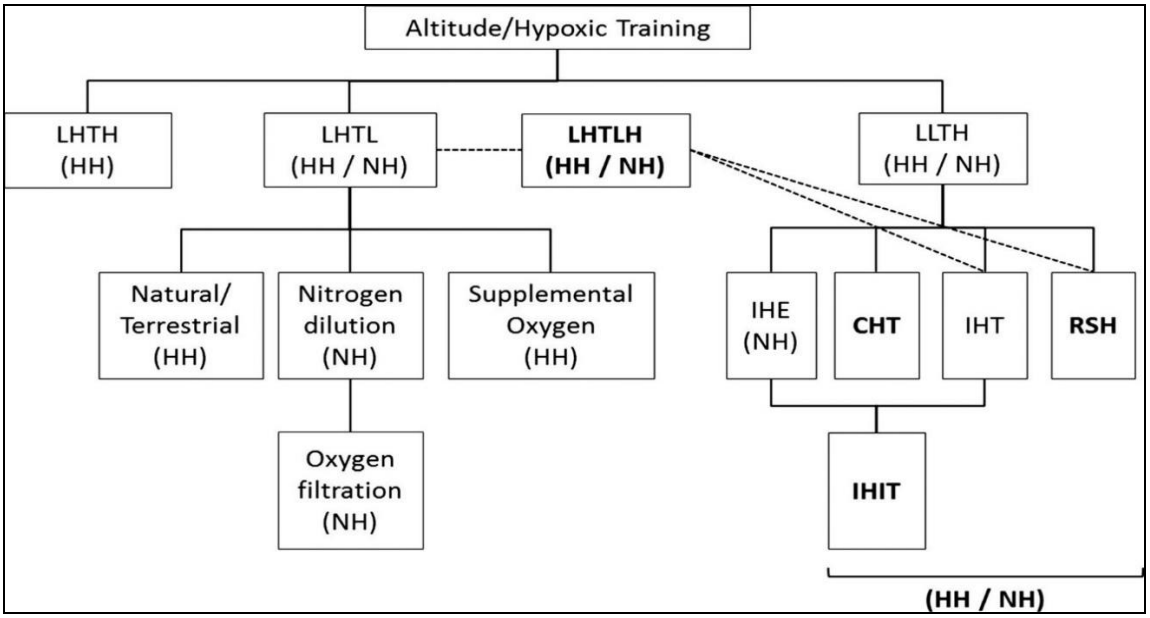

Figure 2: Summary of High Altitude/Hypoxic Training Techniques [18]. IHE=intermittent hypoxic exposure; IHIT=intermittent hypoxic exposure during interval training; CHT=continuous hypoxic training; RSH=repeated sprint training in hypoxia; LH=live high; TH=train high; LL=live low; TL=train low; HH=hypobaric hypoxia; NH=normobaric hypoxia.

2.2.1 High Altitude Training

Initially, research focused on passive acclimation and HAT, and many studies emerged supporting the claim that these methods elicited an increase in erythropoietin (EPO) production, red blood cells (RBCs), and hemoglobin mass and affinity [16, 18, 19, 20, 21, 22]. Different protocols such as the live high train high (LHTH), live low train high (LLTH), and live high training low (LHTL) were developed to find ways to optimize these adaptation processes [16, 18, 20, 21, 22, 23]. LHTL has been thought to be the most beneficial high altitude protocol because it involves prolonged exposure to altitude, thus allowing the body to form the blood-based adaptations mentioned previously, but still allowing the athlete to train at his or her maximal output in normoxic conditions [16, 18, 20, 21, 22, 23]. Many research studies show that these high altitude protocols are effective at increasing aerobic capacity and to some extent aerobic efficiency [16, 18, 20, 21, 22, 23]. These studies also found that these adaptations translate to increased performance outcomes [16, 18, 20, 21, 22, 23].

The disadvantage of terrestrial HAT techniques is that they are often impractical because of the athlete having to live in an area where they can drop several thousand feet for every training session. Also, performance gains seem to vary from athlete to athlete, with the greatest benefits in elite athletes who are most impacted by even small improvement [16, 18, 20, 21, 22, 23]. Because of this, HAT has become either a coincidental training strategy for people who already live at high altitude, or for elite endurance athletes who are dedicated and believe it benefits their performance [24].

2.2.2 Intermittent Hypoxic Training

In the last two decades, research on IHT has emerged as an additional protocol to HAT [18, 22]. Instead of targeting predominantly aerobic capacity, as HAT did, IHT emphasizes aerobic efficiency [18, 22]. Transcription factor hypoxia-inducible factor-1alpha (HIF-1alpha) and several other critical regulating proteins are some of the primary targets of IHT [18, 22]. IHT mechanism of adaptation has many similarities to exercise. Because of this, several studies have found that IHT is optimized when paired with maximal aerobic exercise [25]. In some studies, high-intensity interval training (HIIT) and repeated sprint training (RST) showed similar activation of HIF-1alpha on their own when compared to IHT at submaximal workloads [18, 25, 26]. It was observed, for the same reason that the LHTL protocol was found to be most effective, that maximal work capacity is hard to reach over an extended IHT session because of impaired oxygen consumption and decreased training stimulus [25]. When IHT is paired with short bouts of HIIT, maximal output is tolerated, however, and both IHT and HIIT mechanisms of adaptation compound to elicit greater molecular and cellular stimulus [25]. Recent research has shown that coupling HIIT or RST with IHT has promising results for activating HIF-1alpha and is well tolerated by athletes during training sessions [18, 25, 26]. Studies have also found that IHT during HIIT could not only be an excellent alternative to HAT but that these different training strategies could complement each other [18, 27]. Because the various HT strategies target different mechanisms, if done correctly, combining the two approaches is proposed to be an even more efficient way to bring about adaptations [18, 22].

2.2.3 Hypobaric versus Normobaric Hypoxia

One main difference between HT techniques is the way the hypoxic environment is encountered: hypobaric hypoxia (HH) versus normobaric hypoxia (NH) and chronic hypoxia versus intermittent hypoxia. Research on whether there is a significant difference between the mechanism of HH or NH is divided [28, 29, 30, 31, 32]. Several of the research studies concluded there are too many confounding variables to understand if HH induces different effects than NH, and further, even if there are differences, they are not significant [28, 30, 32]. In opposition to that, it has been proposed that HH elicits superior adaptations when compared to NH in ventilatory responses, fluid balance, acute mountain sickness severity, nitric oxide (NO) metabolism, and performance improvements [31]. Another study found that HH and NH produced similar results at rest, but following exercise, HH leads to greater hypoxemia and better right ventricular function compared to NH [29]. Because the mechanisms are not fully understood, more research is needed to determine to what degree HH and HN differ in inducing an adaptive response [28, 30, 32].

2.2.4 An Era of Hypoxic Training

With identified benefits of IHT came the invention of masks and facilities that would mimic high altitude through a slew of artificially induced normobaric and hypobaric hypoxic environments. These presented a more practical way for athletes to train anywhere they wanted and, because these devices are easily accessible, athletes have recently started using them more frequently for different training techniques (endurance, resistance, HIIT, and recovery). Because of variation in IHT protocols and devices used, some studies have produced varied outcomes, resulting in a push for further

research [16, 18]. While additional studies are essential to fine-tune HT strategies to elicit the most significant physiological adaptations for each athlete's level of fitness, event, and goals, IHT is currently warranted and has promising potential [18].

Many of the studies that have investigated HT have shown similar results regarding cellular changes and performance outcomes. Training in a hypoxic environment, at an intensity that elicits a high anaerobic threshold, increases EPO, RBCs, hemoglobin, myoglobin, glycolytic capacity, mitochondrial density, and capillary density (the mechanisms of these adaptations can be found in Appendix A) more than training in a normoxic environment [20, 27, 33, 34]. Through decreasing demand of oxygen, increasing the carrying capacity of oxygen, and increasing aerobic efficacy, HT translates to increased energy utilization and event performance and has a promising future as a tool for athletes to gain a competitive edge [20, 33].

HT has come a long way in the athletic realm of research, but these promising results also have the potential to translate well to the clinical setting. Various patient populations have elicited similar benefits as seen induced by exercise, which has known benefits on cardiovascular, respiratory, metabolic, and cognitive health. The decades of research on HT for athletic performance serve as a basis for the use of IHC to treat clinical disorders.

2.3 Hypoxic Conditioning in the Clinical Setting

2.3.1 Naturally Occurring Examples of the Body's Response to Hypoxia

Hypoxia has been observed in the clinical setting for quite some time as the result of obstructive sleep apnea syndrome (OSAS). The hallmark of OSAS is numerous (30 to 90) cyclical episodes of extended severe intermittent hypoxia, with hypercapnia and

increased intrathoracic pressure, which leads to excessive oxidative stress and systemic inflammatory response [1, 35]. Initially, mild OSAS may serve as a protective preconditioning mechanism and elicits angiogenesis and increases coronary collateral vasculature [36]. Over time, however, the severity and duration, and the nature of nocturnal exposure of OSAS become maladaptive, and deleterious conditions of hypertension, stroke, coronary artery disease, metabolic dysregulation, and increased cardiovascular mortality have been linked to untreated OSAS [1].

Congenital Heart Disease is another disease state that is associated with hypoxia/hypoxemia and leads to an adaptive response for survival [37]. Increased hematocrit is one of the essential adaptations for increasing oxygen delivery to the tissues; however, it is not without consequences [37]. Erythrocytosis in congenital heart disease patients is associated with stroke, impaired muscle performance, thrombosis, increased shear stress on the blood, and vascular dysfunction and rupture [37]. Another adaptation found in Tetralogy of Fallot patients is increased expression of peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha), leading to mitochondrial biogenesis and increased oxidative phosphorylation to allow for more efficient use of the hypoxemic blood the tissue experiences [38].

Another example of the body's ability to adapt to hypoxia are the millions of people who live at an altitude of more than 4000m [11, 39]. Recently, studies have demonstrated that people living at high altitude have a significantly lower incidence of obesity, type 2 diabetes mellitus, ischemic heart disease, asthma, neuroses, and longer average lifespan when compared to individuals who do not live at high altitude [11, 39].

This shows that people are not only surviving in a moderate state of hypoxia, but they are also thriving in it.

In these examples of naturally occurring hypoxia and hypoxemia, and others such as chronic obstructive pulmonary disease and asthma, there are beneficial adaptations stimulated by hypoxia that initially serve as a survival mechanism [1, 37, 40]. Unfortunately, however, diseased states often progress in severity, and over time, maladaptations lead to severe consequences [1, 37, 40]. Associated co-morbidities and deleterious effects observed in various disease populations make many clinicians wary when they learn about IHC—on the surface, it is a counterintuitive therapy [1, 35]. But as previously discussed and as seen with HT in athletes, when monitored and administered appropriately, HC shows many positive adaptations that are triggered as part of a hormesis effect [1, 35, 41].

2.3.2 Therapeutic Intermittent Hypoxic Conditioning

IHC affects mediators and tissues throughout the body, stimulating a variety of overlapping cellular pathways. These complex cascades are still poorly understood, but additional research is emerging to clarify the ability of these various pathways to induce cardiovascular, metabolic, respiratory and neural benefits [35]. Figure 3 shows a simplified diagram of the significant and known activated HC pathways. Appendix B provides a detailed explanation of these main mechanisms and their interconnected cascades. When it comes to the clinical relevance of a treatment, however, patient outcomes are what matter. Accordingly, the following section seeks to give a summary of the beneficial results observed in studies with cardiovascularly compromised patients.

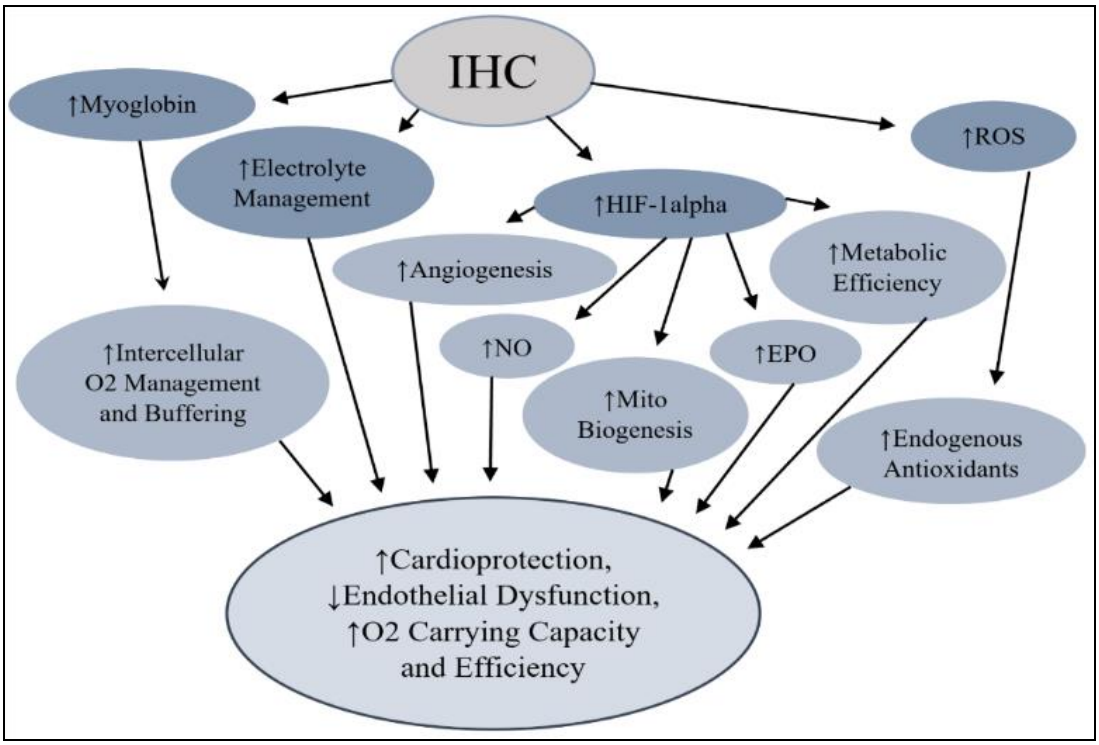

Figure 3: Proposed IHC Stimulus on Mediators of Cardiovascular Health/Protection [5, 35]. ROS = reactive oxygen species.

3.3.3 Clinical Outcomes Observed in Cardiovascular Patients

The number of research studies looking at the impact of HC on patients with compromised cardiovascular systems is limited, but there is a small number of high-quality studies. Several will be examined in detail. Lyamina *et al.* [42] employed an IHC protocol with 37 young males suffering from Stage I hypertension and compared them to a control group of 20 normotensive healthy participants. Four days prior to the study, patients were asked to consume a low nitrate diet, for which guidelines were provided. Twenty consecutive daily IHC sessions consisting of 4 to 10 cycles of 3 minutes of hypoxia (FiO2 10%) were administered. Blood pressure and urinary NO excretion were measured before and after treatment and at a three-month follow-up. The results showed

a statistically significant decrease in blood pressure and an increase in NO levels (Table 1).

Table 1: Summary of Data Collected from Lyamina *et al.* Study [42]. SBP=systolic blood pressure, DBP=diastolic blood pressure.

Lyamina *el al.* (2011)	**Control**	**Pre IHC**	**Post IHC**	**3 Month Follow Up**
SBP/DBP mmHg	121/76	151/95	129/78 (16%↓)	Maintained in 85% of pts
NO Excretion umol/day	63	46	59 (22%↑)	58 (21%↑)

Glazachev *et al*. [7] employed a normobaric IHHC protocol (in place of an exercise based standard cardiac rehabilitation) in post-operative CABG patients. The study enrolled 46 patients in an 8-week non-randomized, controlled trial. Twenty-seven patients carried out 3 IHHC sessions per week consisting of 5 to 7 cycles, lasting for 4 to 6 minutes of hypoxia (10-14%) with 3 minutes of hyperoxia (30%) recovery. The IHHC group was compared to 19 patients in the control group that went through a standard (F_iO_2 21%) cardiac rehabilitation exercise program. The IHHC group showed statistically significant improvements in exercise performance and tolerance, blood pressure reduction, blood and metabolic profiles, and perceived quality of life (Seattle Angina Questionnaire). Outcome measures were taken before and after treatment, and at a one-month follow-up (Table 2). The IHHC group showed similar or better results compared to the control group.

Table 2: Summary of Clinically Relevant Data Collected in Glazachev *et al.* Study [7]. LVEF=left ventricular ejection fraction; TC=total cholesterol, AI=atherogenic index; LDL=low-density lipoprotein.

Glazachev *et al.* (2017)	Before IHHC	After IHHC	1 Month Follow Up
Exertional Angina	12 patients	6 patients (50%↓)	3 patients (75%↓)
Bruce Exercise Time s	280	295 (5%↑)	332 (15%↑)
VO2peak mL O2/min/kg	14.3	16.1 (11%↑)	15.4 (7%↑)
SBP/DBP mmHg	151/85	130/73 (14%↓)	129/75 (14↓)
Resting HR/Max HR bpm	72 / 122	68 / 120 (5% / 2%↓)	67 / 116 (7% / 5%↓)
LVEF%	58	63 (8%↑)	62 (7%↑)
Reticulocytes%	9.0	11.3 (20%↑)	9.2 (2%↑)
TC / AI / LDL mmol/L	5.6 / 3.5 / 4.7	5.1 / 3.2 / 3.4 (9 / 9 / 28%↓)	5.5 / 2.6 / 3.5 (2 / 26 / 26%↓)

Tuter *et al.* [9] employed a normobaric IHHC protocol in pre-operative CABG patients. A total of 127 enrolled patients were divided into three groups: IHHC, RIPC, and control. In the IHHC group, four daily 20- to 30-minute IHHC sessions were administered for 3 to 5 minutes of hypoxia (12%) followed by 1 to 3 minutes up hyperoxia (35% to 40%). The RIPC group underwent three cycles of 10-minute remote ischemic preconditioning (RIPC) followed by 10 minutes of recirculation; this was administered directly before induction. The control group followed the same schedule as the IHHC group with a sham procedure (humidified normoxic air). The lactate and Troponin I levels of these three groups were measured before, 2 hours after, and 24 hours after the operation, along with complications observed peri- and post-operatively. The IHHC group show statistically significant results with lactate and Troponin I levels, along with a trend of a reduction in arrhythmias, indicating myocardial protection (Table 3).

Table 3: Summary of Data Collected in Tuter *et al.* Study [9]. RIPC=remote ischemic preconditioning.

Tuter *el al.* (2018)	**IHHC versus RIPC 24h**	**IHHC versus Control 24h**
Lactate Levels mmol/L	18%↓	17%↓
Troponin I Levels ng/mL	41%↓	46%↓
Arrhythmias	29% ↓	44% ↓

In addition to taking a detailed look at the benefits identified in these three studies, a summary of the general clinical outcomes induced by HC, and found in over 20 studies, are shown in Table 4. Although many different HC protocols were used in studies with various focuses, and varying degrees of beneficial change, Table 4 shows an assortment of possible clinical applications for patients. The patient population included athletes with overtraining syndrome, the healthy, the elderly, the obese, and patients with coronary artery disease, CABG procedures, heart failure, diabetes mellitus, and hypertension. Several significant animal studies are included to show outcomes where patients studies were either not feasible or lacking.

Table 4: Summary of Clinical Results of Hypoxic Conditioning. All results were statistically significantly different when compared to the control group unless stated otherwise, denoted by a "^." N=normobaric; H=hypobaric; CHC=chronic hypoxic conditioning.

Study Subjects	Hypoxic Challenge	Outcomes	References
Patients	N-IHHC	↓ Myocardial Infarction / Injury	[9]
Rats	N-IHC, H-IHC		[43, 44, 45, 46]
Patients	N-IHC, N-IHHC, H-IHC	↑ Cardiac Function	[7, 29, 47^, 48^]
Rats	N-IHC, H-IHC		[43, 45, 46]
Patients	H-IHC	↑ Coronary Blood Flow	[49]
Rats	H-IHC		[43]
Patients	N-IHHC, H-IHC	↓ Arrhythmias / Fibrillation	[9^, 48^]
Rats	N-IHC, IHH		[44, 45, 50]
Patients	N-IHC, N-IHHC	↑ Antioxidant Reserve / ↓Inflammatory Response	[9^, 51]
Rats	N-IHC, H-IHC		[45, 46, 50]
Patients	N-IHC, N-IHHC, H-IHC, H-CHC	↓ Hypertension	[7, 42, 52, 53]
Patients	N-IHC, N-IHHC, H-IHC, H-CHC	↑ Hypoxia / Ischemic Tolerance	[9, 29, 52, 54, 55, 56]
Patients	N-IHC, N-IHHC	↑ Efficiency O_2 Utilization	[9, 52]
Patients	N-IHC, N-IHHC	↑ Exercise Capacity / Efficiency / Tolerance	[7, 8, 47, 52, 54, 57, 58, 59, 60]
Patients	N-IHC	↑ Vascular Function	[48, 51, 60]
Patients	N-IHC	↓ Angina	[7, 57, 58, 59, 60]
Patients	N-IHC	↑Glycemic Control	[56, 61]
Patients	N-IHC, N-IHHC, H-IHC	↓Total Cholesterol / LDL / Triglycerides	[7, 58, 59, 60, 62]
Patients	N-IHC, N-IHHC	↑ Perceived Quality of Live	[7, 42, 47, 57]
Patients	N-IHC	↑ RBCs / Hemoglobin / EPO	[7, 47^, 52, 63]
Patients	N-IHC	↑HIF-1alpha	[56, 63]

As seen in Table 4, there are numerous possible benefits of HC in diseased patients. Several significant findings in these studies are worth highlighting. Most notably, HC has been shown to be incredibly effective at increasing exercise capacity and tolerance in patient populations who have trouble exercising [7, 8, 47, 52, 54, 57, 58, 59, 60, 64]. Along with this, multiple studies also show a substantial decrease in angina, an increase in hypoxic tolerance (up to an 8% rise in saturation of oxygen in arterial blood (SaO_2) during hypoxia at the end of the protocol), and an increase in O_2 utilization [7, 9,

52, 54, 55, 56, 58, 59, 60]. With hypertensive patients, an increase in NO production was seen, resulting in a considerable drop in blood pressure [7, 42, 52, 53]. Lipid profile on numerous patient studies decreased dramatically, and a decrease in arterial stiffness and vascular health was also seen [7, 48, 51, 58, 59, 60, 62]. Although subjective, multiple studies showed improvement with the quality of life questioner pre- to post-protocol [7, 42, 47, 57]. In two patient studies, trends of increased LVEF% were seen, and in another, over 40% reduction in Troponin I levels was seen after a CABG procedure [7, 9, 47]. This supports numerous animal studies that have shown increased cardioprotection (as much as a 50% reduction in infarct) and increased cardiac function after hypoxic conditioning [43, 44, 45, 46]. All these beneficial effects, although some seemingly small when isolated, can have compounding effects on the cardiovascular health of patients. It is evident that more patient studies, looking at clinically relevant outcomes, need to be conducted, but the preliminary research points to a helpful aid in the management of a variety of patient populations.

2.3.4 Remote Ischemic Pre-conditioning

Although similar in principle, it is important to understand the difference between IHC and RIPC. Ischemic pre-conditioning works by the same concept of hormesis, but instead of inducing hypoxia through decreasing the F_iO_2 diffused in the lungs, it occludes the blood vessel from supplying any blood to the tissue [5]. Intermittent ischemic conditioning has shown promising results in animal studies and was trialed in open-heart surgeries for a period for its ischemic-reperfusion-injury (I/RI) protection [5]. This was accomplished by cross-clamping the aorta several times for 3 to 5 minutes at a time before the final cross-clamp would be applied, to enable access to work on the heart [5].

Because of the impracticality of this method and the detrimental effects of dislodging plaque on the aorta, it did not become a widely used technique [5].

RIPC was established to work around the obstacles associated with ischemic preconditioning; it uses the same technique but only occludes the arm or leg [5]. This is accomplished through a blood pressure cuff inflated to 200 mmHg for 5 to 10 minutes and then being released; various protocols call for different durations and cycles of occlusion [5]. The thought is that the mechanisms stimulating I/RI protection in the occluded arm or leg will distribute once circulation resumes [5]. One of the principal mechanisms of RIPC that differs from IHC is the CO_2 accumulation and resultant acidemia [5]. There is controversy over whether this aids in I/RI protection or is detrimental, but at this point, there is not enough research on the effects of CO_2 to draw a conclusion [5]. Another area of difference is the application in clinical settings. RIPC is simple and can be administered directly before surgery in a timely manner, yet patients often have discomfort from this technique [5]. As more research emerges on RIPC, it seems to potentially have a role as an alternative effective means for short term I/RI protection [5].

2.4 Need for Clinical Studies on Protocols for Surgical Cardiac Patients

Apprehension among clinicians about the safety of subjecting cardiac patients to IHC and the concern of whether using IHC in cardiac patient management is feasible are the two main barriers keeping IHC from the clinical setting. Although in recent years, there has been more research in patient studies emerging regarding the various adaptions of therapeutic IHC, there have been few studies to look at the pre- and post-conditioning effects in surgical cardiac patients, especially in the United States. The absence of

practical protocols for clinicians to use has consigned IHC to a theoretical status, and limited its development as a therapeutic modality for clinicians to consider. Consequently, there is a need to look at appropriate IHC protocol parameters and safety in order to develop guidelines for a safe and effective protocol.

2.5 Project Statement

Because of the potential therapeutic benefit of IHC in cardiac patients, additional high-quality clinical studies should be carried out on this topic. In order to do so, a well-designed protocol needs to be put in place. The goal of this project was to present the currently available research on IHC and to develop a pre- and post-operation Intermittent Hypoxia-Hyperoxia Conditioning (IHHC) protocol guideline that could be implemented by a clinical site for CABG patients.

3. Methods

In the process of developing this protocol guideline, the following steps were taken. Over 200 articles were searched and reviewed using terms such as “HC”, “HC for cardiovascular patients”, “HT for athletes”, “mechanisms of HC”, “hypobaric versus normobaric hypoxia”, “cardiac rehabilitation”, etc. Articles were also found by following references used by various review articles and hypoxic conditioning primary studies. Articles were reviewed for information regarding the history, mechanisms, safety, and efficacy of HC, particularly with regard to cardiovascular patients.

Additional information was obtained by reaching out to several authors of recent review articles and research studies on HC and cardiovascular-impaired patients. Two HC device manufacturing companies were also contacted (Hypoxico and Ai Mediq S.A.) to inquire about more information concerning their devices and the research they have conducted with cardiovascular patients. A physical therapist was also consulted regarding cardiac rehabilitation for post-CABG patients.

Once all available data were examined, articles most pertinent to developing a HC protocol for CABG patients pre- and post-operation were used. The most current and appropriate data on safe and effective dosing, duration, frequency, and length of various HC strategies were synthesized. In addition, numerous studies were examined for the safety of HC in cardiovascular patient populations. These findings were then used to create the protocol guidelines for pre- and post-conditioning in CABG patients.

4. Results

4.1 Finding the Balance of Hypoxic Conditioning

It is apparent that the dosing, duration, and frequency of hypoxia play a significant role in whether it elicits beneficial or deleterious results [4]. If too little stimulus is given, then no results are seen, and the therapy is a waste of time and money. On the other hand, too much stimulus applied leads to unsafe therapy (Figure 4).

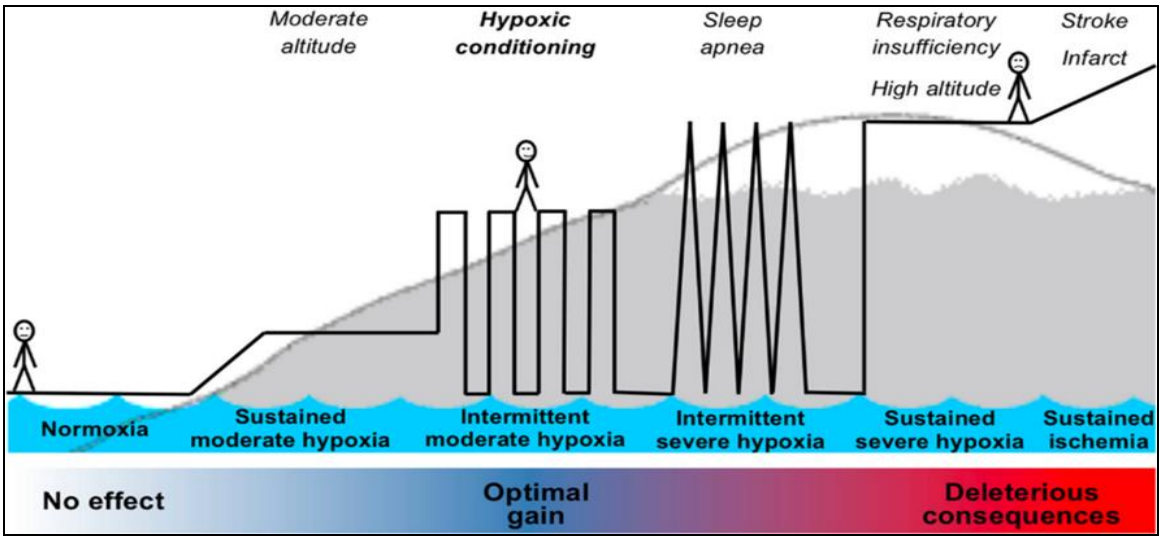

Figure 4: Dose-Response of Hypoxic Exposure [1]. Moderate hypoxia is approximately 10% to 15% F_iO_2 or 2500m to 5000m.

Because hypoxia can lead to maladaptations, it is essential to understand the appropriate administration of IHC. Multiple review articles have examined the various HC protocols in numerous primary studies and reached similar conclusions on what is considered optimal dosing of hypoxia (Table 5) [4, 41, 65, 66]. When considering the treatment of patients, IHC is widely accepted as the most clinically feasible and

beneficial [4, 41, 65, 66]. IHC allows for relatively short treatments that can be integrated into patient care simply and inexpensively.

Table 5: Adaptive versus Maladaptive Protocols for Hypoxic Conditioning [4, 41, 65, 66].

	Adaptive	Maladaptive
Length	Need 1 to 2 weeks to start seeing results for pre-conditioning; 4 to 8 weeks for postconditioning	Varies based on dose, frequency, and duration
Duration of hypoxia	Several minutes to several hours or days (lower F_iO_2 shorter duration)	The durations depend on the severity of the dose
Frequency	1 to 15 cycles a day (with adequate recovery between)	> 30 cycles a day (with inadequate recovery between)
Dose	10% to 16% F_iO_2	< 10% F_iO_2
Examples	Sports training, weight loss training, acclimation to high altitude, therapeutic pre- and post-conditioning for cardiac surgery	Obstructive sleep apnea syndrome, congenital heart disease, asthma, chronic obstructive pulmonary disease
Proposed optimal IHC protocol for surgical cardiac patients	3 to 7 minutes of hypoxia; 4 to 7 cycles; 10% to 14% F_iO_2 depending on patient's tolerance to IHC; minimum of 1 to 2 weeks for pre-conditioning and 8 weeks for post-conditioning	N/A

As seen in Table 5, the administration of HC can vary greatly, but within the acceptable parameters, beneficial results have been observed, and treatments have been found to be completely safe. Along with decades of research on athletic training protocols, numerous recent studies on cardiac surgical patients have shown IHC to be safe and well tolerated [7, 8, 9, 47, 57, 58, 63, 67]. As mentioned earlier, IHC mimics exercise, to a degree, so when administering IHC, parameters such as heart rate (HR), electrocardiogram (ECG), S_aO_2, and patient response should be monitored in agreement

with cardiac rehabilitation guidelines [68]. During IHC, it is acceptable for an HR increase of 30% to 50% from baseline, for S_aO_2 to drop at most to 80%, and for respiratory rate to increase up to 30 to 40 breaths per minute [7, 9, 66, 68, 69]. Clinical signs of intolerance, such as a significant increase in blood pressure, dizziness, angina, ECG abnormality, skin pallor, or patient discomfort, should also be monitored by a physician [7, 9, 42, 66, 68, 69]. As long as these signs are monitored and the appropriate protocol parameters are followed, IHC has been shown to be safe in cardiac patients.

Although safety parameters have been found, the variation in protocols serves to show the need for research studies to assess optimal administration for specific patient response. Ai Mediq company has attempted to solve part of this dilemma by developing an HC device, ReOxy, that uses Self-Regulated Treatment (SRT) software [69]. ReOxy displays three trending lines on its screen: S_aO_2, HR, and F_iO_2 level [69]. Using the data trends from the hypoxic test, the SRT system calculates the F_iO_2 to expose patients to and autoregulates during the session to keep the patients in the zone of maximal therapeutic strain [69]. Hypoxic tests need to be administered every 5 to 6 sessions to recalibrate patient treatment; once a patient reaches tolerance of 10% F_iO_2, the F_iO_2 should not be reduced anymore [66]. A recommendation by Ai Mediq and authors of recent cardiac surgery pre- and post-conditioning studies, is to increase the F_iO_2 to 30 to 40% during reoxygenation recovery periods, coining the term intermittent hypoxia-hyperoxia training (IHHT) [66, 69]. This procedure not only serves to speed recovery time but also causes a moderate increase in ROS to induce a greater endogenous antistress response [66, 69]. The ReOxy device has helped tremendously with bringing about patient-specific protocols for clinicians. ReOxy is currently a class 2B recommendation, which states that

the mechanism and applications are not well defined yet, but that the benefit is greater than the risk [70]. ReOxy is CE marked and in full compliance with the Medical Device Directive in Europe and is currently seeking Food and Drug Administration approval in the United States [69].

4.2 Study Design

This section provides protocol guidelines for clinicians to use when designing an IHC study for their institution. It is understood that different institutions and clinicians will have different resources and procedures for conducting a prospective research study, so this section is meant to aid in that development by giving key strategies for a successful IHC protocol. This protocol features two phases, pre-conditioning and post-conditioning. Pre-conditioning is meant for preparing the body for the stress response provoked by cardiopulmonary bypass (CPB) and arresting the heart. Post-conditioning is used primarily as an aid to stand cardiac rehabilitation (SCR) to help increase exercise capacity and tolerance, as well as to elicit cardiovascular health. A culmination of research from the methods section, multiple recent and successful research study strategies [7, 8, 9, 58, 63], and recommendations from Ai Mediq S.A. on the appropriate use of the ReOxy device [69] were used for developing these study guidelines.

4.1.2 Target Patient Population

This should be a prospective single-center, single-surgeon double-blind research study. Patients should be selected consecutively as they seek consultation in undergoing an elective coronary artery bypass graft (CABG) procedure. The study director and participants should be unaware of group allocation because of the double-blind nature of the study. Participants should be asked not to change their drug or nutritional intakes and

to maintain current daily exercise habits. The study should continue until 90 patients have been selected and adhered to the study protocol. Table 6 itemizes the exclusion criteria for the study.

Table 6: Exclusion Criteria for IHHC Study Guideline. NYHA=New York Heart Association.

Exclusion Criteria
• Unstable clinical condition • NYHA functional class IV • Decompensated heart failure • Acute coronary syndrome in the previous month • Pre-operative percutaneous coronary intervention • Occlusive atherosclerotic disease of upper and lower limbs • CABG without CPB • Grade 3 hypertension at rest SBP >180 and/or DBP >110 mmHg • Additional simultaneous procedures • History of exercised induced syncope or severe angina • Inadequate time to participate in a minimum of 6 IHC sessions pre-operative • Unable to tolerate the initial hypoxic test • Cross-clamp time over 60 minutes

4.2.3 Intermittent Hypoxia-Hyperoxia Conditioning Protocol

This study should have three study groups: IHHT, intermittent hypoxia-hyperoxia exposure (IHHE), and a control. IHHT will be administered during exercising (during post-conditioning outpatient rehabilitation), whereas IHHE will be administered while sitting. Once patients are randomly placed into one of the three study groups, a hypoxic test to assess the patient's tolerance and sensitivity to hypoxia should be conducted. The control group should be administered a sham hypoxic test while resting. The ReOxy hypoxia-hyperoxia device should be used for all sessions because of its unique operating

system, as recommended by Ai Mediq [69]. The hypoxic test should be conducted with a physician present and last 10 minutes [66]. During this test, the F_iO_2 will be reduced to 12% and the patient's S_aO_2 and HR, along with clinical signs of intolerance, such as dizziness, angina, ECG abnormalities, skin pallor, and patient's response, should be closely monitored [66]. If S_aO_2 falls below 80% or HR rises higher than 30% to 50% of the patient's baseline HR, the device will automatically regulate the amount of F_iO_2 up to 30% to 40% and allow the patient's saturations to rise to 98% [66]. Once at a S_aO_2 of 98% or higher, the F_iO_2 will drop down to 12%, and this will continue for 10 minutes as the ReOxy device collects data on the three trending values of S_aO_2, HR, and F_iO_2 [66, 69]. These data are then analyzed and give a detailed summary for a patient-specific IHHC protocol [66, 69]. If the physician at any time assesses clinical signs of intolerance, the test should be ended immediately, and the patient should discontinue participation in the study.

Pre-conditioning sessions should start the day after the patient consults for surgery and consents to treatment. During the pre-conditioning phase, 6 to 8 treatment sessions should be given before surgery, with the last session being administered the day before surgery. Treatment sessions should last between 30 and 45 minutes, each session completing 4 to 7 cycles. A cycle should consist of 3 to 7 minutes of hypoxia (10% to 14% F_iO_2 depending on patient tolerance) followed by a 3-minute hyperoxia recovery period. This should be the same hypoxic protocol through both phases.

Post-conditioning treatments will start one day after the patients are extubated if they are considered stable. Both IHHT and IHHE groups will be re-tested and will initially start as IHHE at rest. Once the patients in the IHHT group start their outpatient

SCR, they will re-test during exercise, and their IHHT protocol will start. The IHHT should be administered during the patient's aerobic exercise period, in their SCR protocol, on either a treadmill or a stationary bicycle. Post-conditioning IHHC treatments should be continued 3 to 4 times per week alongside patient's SCR protocol. The control group should follow the same regime as the IHHE group but with sham hypoxic sessions (humidified normoxic air).

4.1.4 Outcome Measures for the Efficacy of the Protocol

Seven assessments of the outcome measure should be taken throughout the study. Multiple outcome measures should be measured during the duration of this study to assess cellular adaptations, as well as the functional clinical outcomes (Table 7).

Table 7: Outcome Measures for IHHC Protocol Guideline. VEGF=vascular endothelial growth factor; PFK=phosphofructokinases; PDH=pyruvate dehydrogenases; AMPK=adenosine monophosphate-activated protein kinase; HSP=heat shock protein.

Clinical Outcome Measures	Research Outcome Measures
• RBCs and Hemoglobin Levels • Troponin-I Levels • Lactate levels • Total Cholesterol/Triglycerides • Atherogenic index • Blood Pressure • LVEF% • VO_2peak • Time of exercise until exertion • Exertional angina or syncope • Inotropic usage post-op • Post-op arrhythmias • Post-op time to extubation • Length of stay in the hospital	• Expression of Oxidative Stress • Expression of Endogenous Antioxidants • Expression of HIF-1alpha • Expression of EPO • Expression of VEGF • Expression of PFK or PDH • Expression of AMPK • Expression of PGC-1alpha • Expression of HSP • Levels of Endogenous NO

If a researcher should decide to develop a study based on these recommendations, it is likely that not all these outcome measures will be measured because of equipment availability and cost. However, each of these outcome measures provides information about various functions of the IHHC's mechanisms and are valuable for assessing and analyzing IHHC's effects on CABG patients.

4.1.5 Potential Study Flow Chart

A progression of a potential study design is briefly summarized in a flow chart (Figure 5) which gives visual organization to the protocol.

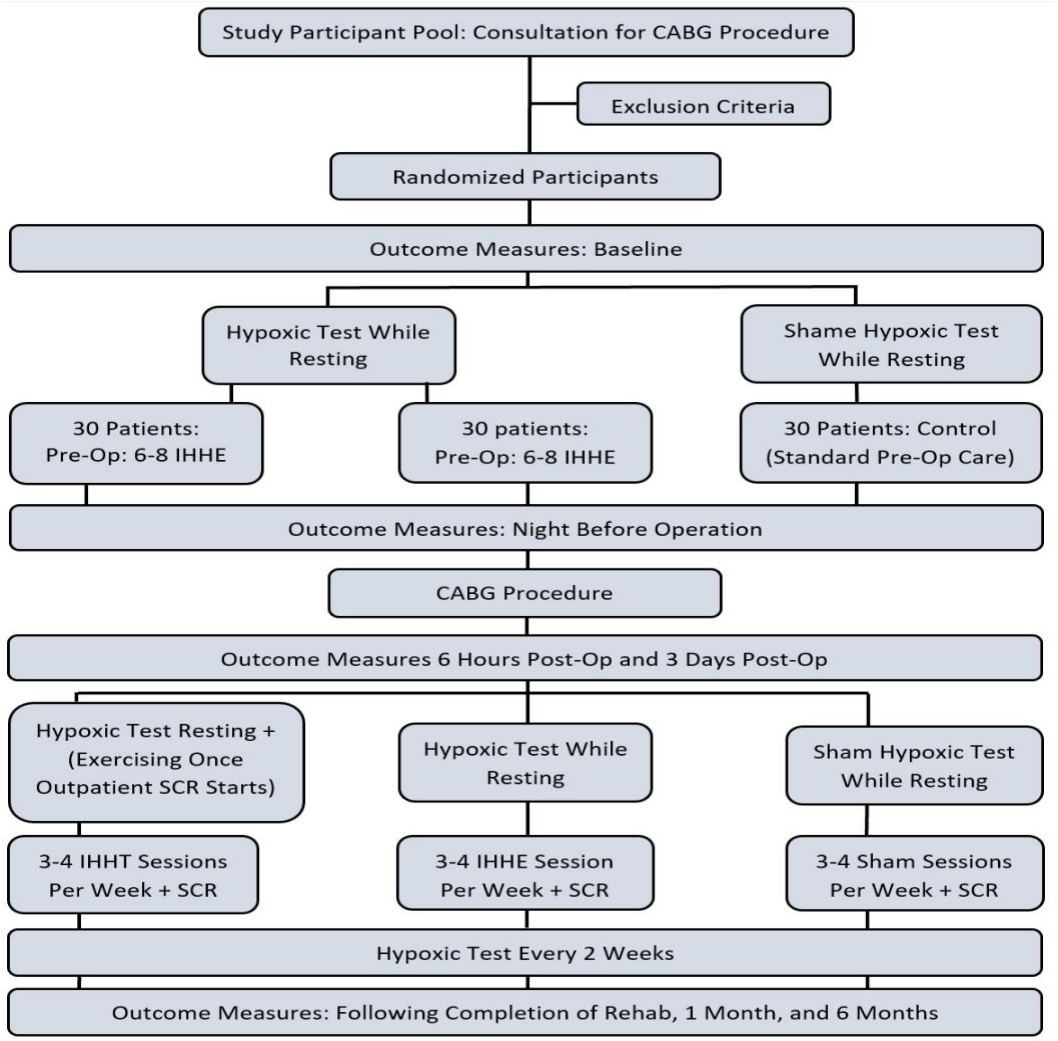

Figure 5: Potential IHHC Study Design.

5. Discussion

IHHC is a non-invasive, non-pharmacological, and inexpensive treatment that has been shown to be safe and effective in several studies and multiple patient populations. When dealing with the multifaceted and interconnected physiologic pathways of the body, it is vital not to oversimplify. A danger with some therapies, which selectively target singular signaling cascades, which are not fully understood, is the unintended consequences of altering the body's natural adaptations mechanisms.

One of the benefits of IHHC is that induced hypoxia stimulates numerous pathways that regulate each other. These, in turn, make slight changes to bring about greater resistance or adaptation throughout the body. This holistic approach has many similarities to exercise, which speaks for itself, and not only can aid in attenuating the stress response from surgery and CPB, but also in aiding cardiac rehabilitation. In previous studies, an IHHC protocol alone was compared against SCR, but because of the enhanced benefits seen by combining IHT with exercise, this protocol guideline suggests using IHT as an aid instead of a replacement. This combined approach also translates beyond surgery as an adjunct to exercise in maintaining cardiovascular health through continued home therapy.

Multiple research studies have emerged showing the potential uses and benefits of HT in various patient populations. The protocol guidelines discussed in this document provide clinicians a background of HC along with strategies of how to implement it in patient management. A considerable amount of research, to understand all the mechanisms and appropriate uses in the clinical setting, is still required for IHC to be considered a standard of care. Nevertheless, the preliminary results show that IHC should

be considered as a viable therapy in the clinical setting and has promising potential for short- and long-term adaptations in the cardiovascular system. It is hoped that this protocol guideline will be useful in reducing systemic stress induced by CPB and in aiding in the recovery and maintenance of good health.

5.1 Limitations

Some foreseen limitations of a study are variations in management styles by cardiologists, anesthesiologists, perfusionists, intensivists, and physical therapists through the course of the patient's operation and rehabilitation. There is also the potential that the selected outcome measures are not comprehensive in assessing the effectiveness of IHHC therapy. Another unknown is the incomplete understanding of how a patient's medications and vitamin supplements affect the IHHC adaptive mechanisms, thus altering outcomes [35]. As a single-center, single-surgeon study, with many exclusion criteria, the ability is limited to confidently infer that the effects of IHHC translate to other cardiac procedures and patient populations.

5.2 Future Recommendations

Future research that could be of use is broadening the patient population of IHHC to various cardiac procedures, as well as to intensive care unit patients that are unable to mobilize, and pediatric patients. Comparing different frequencies of administering IHHC is important to understand how many sessions it takes to see meaningful outcomes in limited time. It is additionally important to assess different outcome measures to understand more about the intricate pathways of IHHC, as well as other clinical benefits patients are experiencing. Acetazolamide is sometimes administered for high altitude

acclimation and acute mountain sickness and has been proposed as a useful aid to HC as well [71].

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Appendix A

Mechanisms of Hypoxic Training Adaptations

Traditional HAT is typically encountered as sustained HH exposure leading to passive acclimation. As discussed previously, one of the main changes is the increase in EPO production leading to the hematopoietic effect of increased RBCs and hemoglobin in the blood, which allows for greater oxygen-carrying capacity.[1] This adaptation occurs as a compensatory measure from living at high altitude. IHT, on the other hand, is typically encountered as short bouts of moderate NH during exercise, which triggers multiple cascading events in the muscle tissue and blood from the elicited stress response.[2] The primary target of IHT is not increasing oxygen-carrying capacity, but rather increasing oxidative efficacy by increased expression of HIF-1alpha, glycolytic enzymes, PGC-1alpha, angiogenesis, and myoglobin mass and affinity.[3] HIF-1alpha is a transcriptional activator of EPO, glycolytic enzymes, VEGF, myoglobin, and increases mitochondrial biogenesis.[4] Researchers agree that HIF-1alpha plays a key role as one of the critical stimuli for adaptations that are made during IHT.[5] Glycolytic enzymes that are up-regulated are PFK, PDH, AMPK, and citrate synthase.[6] These are each rate-limiting enzymes in the glycolytic pathway that promote adenosine triphosphate (ATP) production, the body's energy currency, and are markers for glycolytic capacity and efficiency.[7] In the oxidative pathway, cytochrome c oxidase is altered to optimize oxygen consumption at low oxygen concentrations.[8] Increases in mitochondrial density and biogenesis are seen from these changes and the increased transcriptional expression of PGC-1alpha.[9] With an increase in mitochondria, there is an increase in aerobic capacity and efficiency for the cell to function at the highest level.[10] HIF-1alpha's up-regulation of

VEGF, one of the main inducers of angiogenesis, increases capillary density, thereby decreasing the diffusion distance that oxygen has to enter the muscle cell, which in turn improves tissue function at low oxygen concentrations.[11] Myoglobin functions much like hemoglobin, but it is an intramuscular binding molecule instead of traveling in the blood like hemoglobin.[12] This increase in myoglobin affinity and concentration in the muscle cells is thought to increase oxygen storage and transport efficiency within the cell, leading to decreased oxygen consumption.[13]

Appendix B

Mechanism of Therapeutic Hypoxic Conditioning Adaptations

IHC triggers a multitude of sensors throughout the body that cause cascading alterations in the cells. These sensors include adrenergic receptors, opioid receptors, and sarcolemma and Ca^{++} channel receptors.[1] An immediate protective role accomplished by the sympathetic nervous system, in the presence of hypoxia, is vasoconstriction of peripheral vessels and increased cardiac contractility.[2] This ensures that aortic blood pressure does not fall and adequate perfusion reaches the brain and myocardium, despite the decreased arterial oxygen content.[3] These sensors also aid in the activation of multiple mediators and effectors, along with direct stimulation from IH.[4] Interestingly enough, it was found that a significant reduction in IHC mediated effect is seen when patients have been taking beta-blockers, opioidergic blockades, and antioxidant supplementation before the IHC treatment, suggesting that the medications disrupted the body's homeostatic regulation of adrenergic and opioid receptors and endogenous antioxidant system.[5]

Mediators activated are transcriptional factors (HIF-1alpha, heat shock protein (HSP), PGC-1alpha, and nuclear factor erythroid-2 (Nrf-2)), protein kinases, NO synthase, cytoglobins, and ROS.[6] All these mediators and effectors have multiple triggers for up-regulation and act as co-activators to each other, so when looking at cascading pathways (Figure B-1), it is important to remember that many aspects are not fully understood yet. With that said, looking at the major activators and their proposed effects helps with the overall understanding of this intricately designed system.

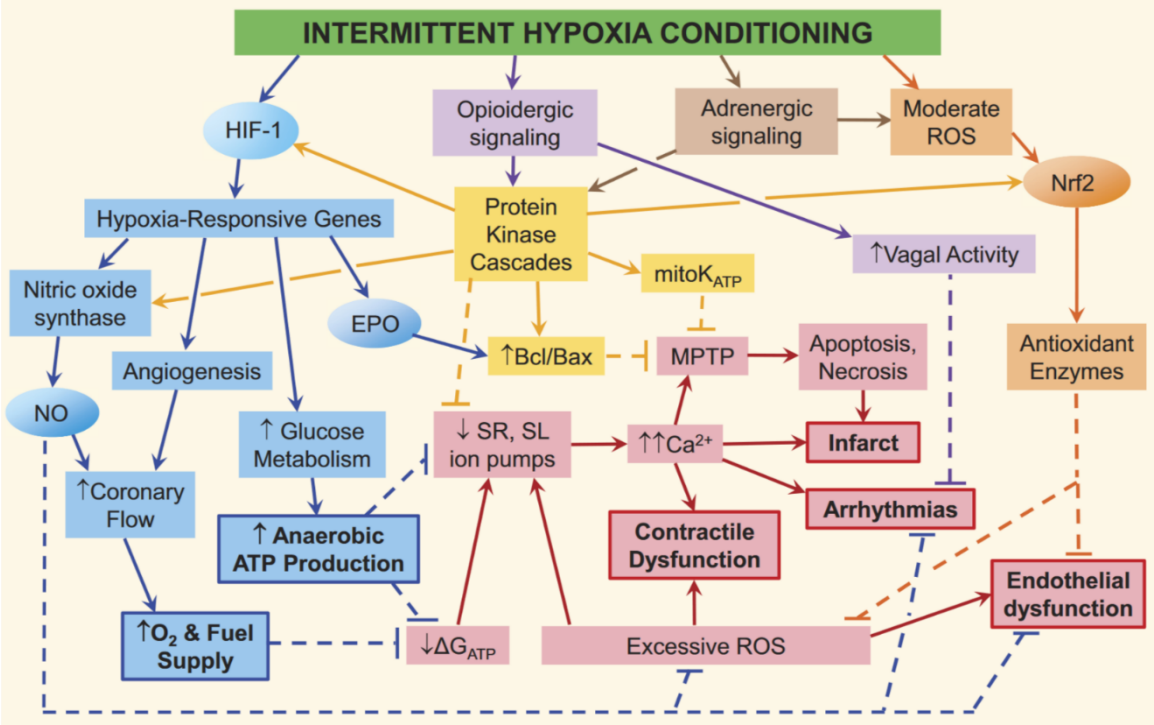

Figure B-1: Detailed Cardioprotective Signaling Pathways Induced by IHC.[7]

Hypoxic Inducible Factor

HIF-1alpha, as mentioned briefly with regard to its effects on athletic performance, is a key player in many of the downstream effectors and promotes both short- and long-term adaptations.[8] Because HIF-1alpha is highly sensitive to hypoxia and fosters tolerance to hypoxia through aiding in the increased expression of EPO, angiogenesis, increased glucose metabolism, PGC-1alpha, NOS synthases, HSP, and cytoglobins, it can be viewed as the "master switch" for many of the cascading pathways.[9]

Erythropoietin

EPO aided in the discovery of HIF-1alpha because of the correlation of parallel increased expression in both EPO and HIF-1alpha.[10] EPO is best known for its

hematopoietic effect, but research has emerged showing that EPO has a less known role in I/RI protection and vascular endothelial health.[11] It has been shown that EPO contributes to myocardial resistance to I/RI and protection from atherosclerotic build-up by dramatically decreasing lipid accumulation through increased cholesterol efflux.[12] Neuroprotective effects have also been noted, through playing a part in synapto- and neurogenesis and increased motor plasticity.[13]

Angiogenesis

Angiogenesis has a significant role in cardiovascular health, but its regulation is paramount due to the detrimental propagation of tumors if left unchecked.[14] Hypoxia and HIFs are a central regulator of angiogenesis through a multitude of pathways (Figure 7).[15] VEGF has long been thought to be the principal regulator of angiogenesis, and because of this, therapies have targeted VEGF for its proangiogenic properties.[16] The problem with this is that, while VEGF is one of the main proangiogenic initiators, it is only one of many factors, and when complex systems are dumbed down, they usually do not work as advertised. Because there are many other regulatory steps in angiogenesis, when only VEGF is targeted, vessel growth and remodeling are often abnormal, tortuous, and leaky.[17] Another problematic factor is that VEGF does not regulate vascularization, which can lead to serious consequences of tumor growth.[18] In contrast, HIFs regulate angiogenesis on a substantially larger scale mediating VEGF, angiopoietins, platelet-derived growth factor, fibroblast growth factor, interleukins, and multiple other molecules which each induce the “angiogenic factors” seen in Figure B-2.[19] Equally important is the ability of HIF to self-regulate and have checks and balances, thus making

IHC activation of HIFs not only better for angiogenesis but also safer in combating unwanted tumor vascularization.[20]

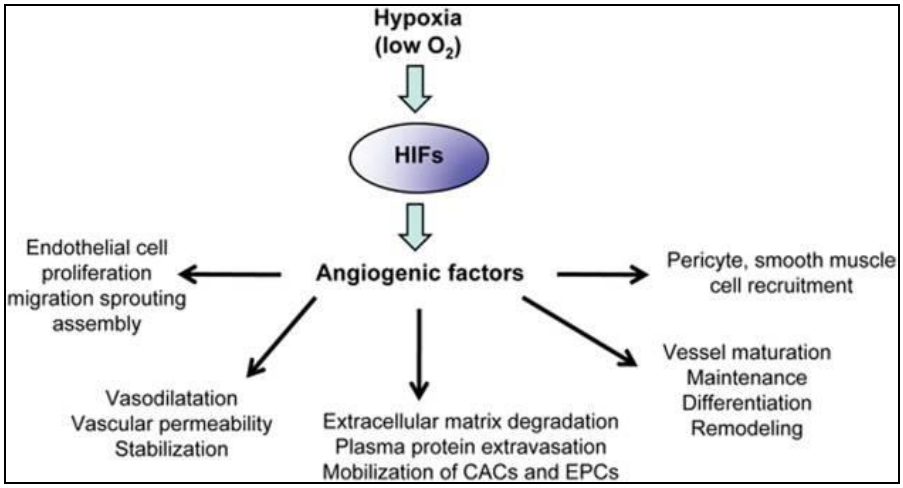

Figure B-2: HIF's Effect on Angiogenesis.[21]

Glucose Metabolism

Glycolysis and oxidative phosphorylation are the body's primary source of energy, and during hypoxia, these processes are severely inhibited because of oxygen's pivotal role. Aerobic glycolysis produces almost 20 times more ATP than anaerobic glycolysis, making the absence of oxygen a significant problem for cellular energy supply.[22] Under hypoxic conditions, HIF-1alpha facilitates increased capacity and efficiency in the glycolytic processes by up-regulating various rate-limiting enzymes, glucose transporters, and increasing glycogen stores, which allow for rapid restoration of ATP and phosphocreatine after periods of hypoxia.[23] HIF-1alpha up-regulates PFK and PDH, which increases anaerobic glycolysis and energy efficiency, even with the accumulation of lactate.[24] Citrate synthases and cytochrome c oxidase, often used as

indicators of aerobic glycolysis and oxidative phosphorylation, respectively, are also up-regulated when exposed to the stimulus of hypoxia, indicating increased aerobic activity.[25] Patients with elevated blood glucose have been shown to have reduced tolerance to hypoxia.[26] HIF-1alpha increases expression and activation of glucose transporters, which accept glucose into the cell after being activated by insulin, in order to induce glycolysis and utilize glucose more efficiently during a hypoxic event.[27] AMPK is another regulatory protein that is induced by hypoxia and has been shown to have multiple cardioprotective roles as well as an ability to increase PGC-1alpha expression.[28] PGC-1alpha is a transcriptional co-activator, and both PGC-1alpha and HIF-1alpha stabilize each other and increase each other's expression.[29] PGC-1alpha is a crucial regulator of mitochondrial biogenesis and causes a shift from glycolytic fibers to increased mitochondrial-rich oxidative fibers and increased glycogen deposit.[30] With all these adaptations to the glycolytic system, another cardioprotective mechanism that could arise is the sustained cytosolic Ca^{++} and sarcoplasmic reticulum Ca^{++} sequestration, since glycolysis is the preferred energy source for intracellular Ca^{++} management, leading to decreased I/RI.[31] These cascading events are still not fully understood, but from the currently available research, these interactions are believed to be the reason why glucose metabolism is up-regulated, and increased resistance to hypoxia and I/RI are seen.

Nitric Oxide

NO is one of the vital mediators to cardiovascular health.[32] Because of NO's reactive nature, however, overproduction can also have detrimental effects.[33] NO has been shown to protect the myocardium by increasing coronary flow, decreasing inflammation and maintaining endothelial function, preserving calcium utilization, and

reducing myocardial oxygen consumption.[34] This contrasts with the adverse effects of NO's overproduction that lead to increased I/RI, aggressive free radicals, denaturing of proteins, and acute hypotension.[35] Figure B-3 shows the proposed mechanisms by which hypoxia regulates NO with an intricate array of cascades that allows NO to induce beneficial effects but not be overproduced or expressed.[36]

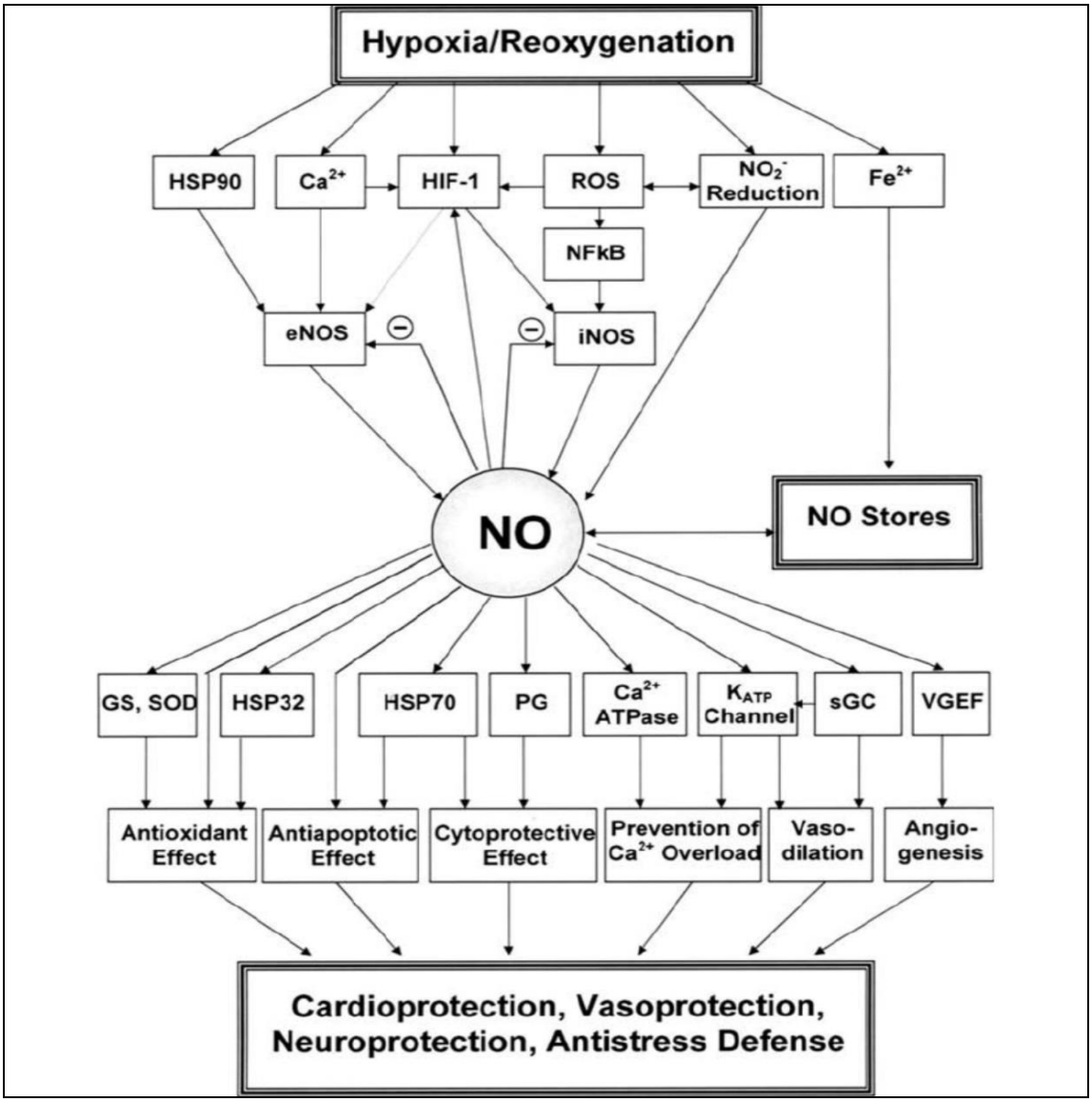

Figure B-3: Nitric Oxide's Proposed Response to Hypoxia/Reoxygenation.[37] iNOS=inducible NOS; eNOS=endothelial NOS; SOD=superoxide dismutase; GS=glutathione, PG=prostaglandins.

Heat Shock Protein

Known as the cellular chaperones for stress-induced protein deformity, HSPs are vital for the correction of the malformation of proteins.[38] HSPs play a key role in cellular protection and are up-regulated by hypoxia, as well as mediators such as NO and HIF-1alpha.[39] HSPs also aid in regulating the expression of NO and HIF-1alpha once activated.[40] HSPs play a significant role in attenuating free radicals and excess stressors on cellular structures and also facilitate reconstruction of damaged cellular processes.[41]

Reactive Oxygen Species

ROSs are a double-edged sword: they are the culprit for many disease processes but are also vital to cellular health.[42] ROS has been studied extensively regarding its role in maladaptations and adaptations. Over formation overwhelms the body's endogenous antioxidant system, creating excess stress on proteins and cells, while under formation does not stimulate antioxidant production and other cellular mediators enough, leading to degeneration.[43] ROS up-regulates multiple transcription factors, including HIF-1alpha and Nrf2.[44] While HIF-1alpha's role is extensive in cardioprotection, Nrf2 also has a large role.[45] Nrf2 is a key regulator of endogenous antioxidant production, which is an essential defense against oxidative stress and IRI.[46] Moderate IHC induces ROS production similar to that of aerobic exercise and leads to beneficial cellular signaling and increased production of endogenous antioxidants.[47] As seen in Figure B-4, ROS poduction is a sliding scale that is homeostically regulated and should not be viewed entirely as a negative compound.[48]

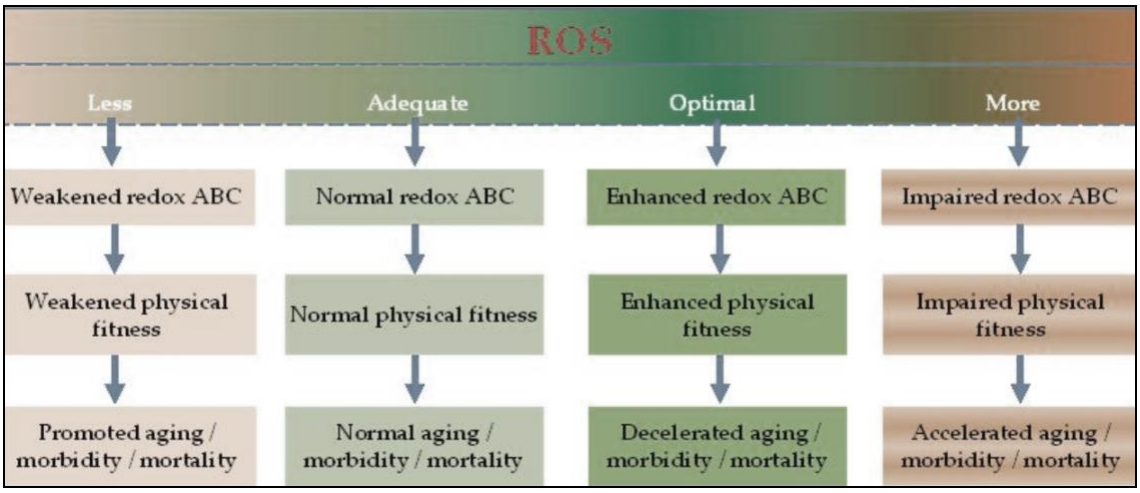

Figure B-4: Simplified Diagram of ROS Production's Effects.[49] ABC=allostatic buffering capacity

Cytoglobins

Myoglobin and neuroglobin are both oxygen carrying and storing proteins within muscle and neural cells.[50] Recently, another function that has been proposed is that they are free radical scavengers and play a protective role against neural and myocardial infarcts.[51] Both myoglobin and neuroglobin are increased in the presence of hypoxia and HIF-1alpha and help to greatly improve oxygen utilization within muscle and neural cells.[52]

Although all these mechanisms that are induced by hypoxia are not fully understood, this summary of key cellular mediators and effectors shows the great need to further investigate how IHC stimulates changes of transcription factors, proteins, and hormones within tissue throughout the body. With a better understanding of these pathways, IHC can be used to elicit beneficial adaptations and aid in combating the rampant cardiovascular disease around the world.

Appendix A

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Thesis Approval Form

Master of Science in Perfusion—MSP

Milwaukee School of Engineering

This thesis, entitled "A Review of Using Hypoxia as a Therapeutic Modality: Developing Intermittent Hypoxia-Hyperoxia Conditioning Protocol Guidelines for Surgical CABG Patients," submitted by the student Daniel Neuman, has been approved by the following committee:

Faculty Advisor: _____ Date: _____

Dr. Ron Gerrits, PhD

Faculty Member: _____ Date: _____

Kirsten Kallies, MS, LP, CPP

Faculty Member: _____ Date: _____

Gary Shimek, MLIS