UNIVERSITY OF CALIFORNIA, SAN DIEGO

Limits of Cardiac Function by Oxygenation

A Thesis submitted in partial satisfaction of the
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by

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The Thesis of Eilleen Siue Yin Ao-ieong is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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DEDICATION

To my family and friends for their unconditional love and support

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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This work could no be completed without the support from everyone in the Microhemodynamic and Functional Cardiovascular Engineering laboratory. Thanks to Professor Pedro Cabrales for his guidance during the completion of this thesis project, Allan Barra for his wonderful help in animal surgery, Cynthia Walser and Daniel Ortiz for their helpful input throughout the project.

ABSTRACT OF THE THESIS

Limits of Cardiac Function by Oxygenation

by

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Master of Science in Bioengineering

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Professor Pedro Cabrales, Chair

Study aimed to examine the limitation of cardiac function (CF) by oxygenation and strategies to improve CF in two cases of reduced oxygen supply: 1) hemodilution by hemorrhagic shock (HS) 2) hypoxia. CF indices where obtained by changes in pressure and volume (PV) through the insertion of a miniaturized conductance catheter into the left ventricle (LV) in a Golden Syrian hamster model. 1) HS was simulated by extracting 30 % of blood volume and equal volume reinfusion with either Dextran 70 (Dxt70) or polymerized hemoglobin (PolyHb), the latter is a Hemoglobin (Hb)-based oxygen carrier(HBOC). HS resulted in the fall of CF, decrease cardiac output (CO), stroke volume (SV) and stroke work (SW), with slow recovery to baseline after reinfusion. PolyHb did not show beneficial effects in the CF and resulted in vasoconstriction, with a 116% and 46% increase

in systemic vascular resistance (SVR) for 11.5g/dL and 8.5g/dL PolyHb respectively. 2) Effects of hypoxia in the heart were investigated by subjecting animals to 30 min stepwise changes of 15%, 10% and 5% O2. Before hypoxia, hamster was given a vehicle or 100mg/kg of 5-hydroxymethyl-2-furfural (5-HMF). 5-HMF produced no significant differences on moderate hypoxia. At 5% O2, 5-HMF resulted in increased SW, SV, CO and ejection fraction (EF) of 38%, 53%, 30% and 37% from normoxia; vehicle group resulted in a slight decrease from normoxia. Tissue hypoxia was examined by pimonidazole staining, 5-HMF reduced tissue hypoxia by 64%. Overall 5-HMF reduced the amount of hypoxic tissue and improved CF during acute hypoxia.

Chapter 1

Introduction

The role of the heart in our body is essential: to drive, oxygenate and feed the circulatory system, and thus the rest of our cells and organs. In situations such as pulmonary disease, pulmonary embolism, high altitude, cardiac arrest and trauma, the body can be exposed to situations of hypoxemia: low oxygen content in blood. This thesis project we will go in depth on how the function of the heart is affected in situations of low oxygen delivery such as HS and hypoxia, and possibilities to ameliorate cardiac stress.

1.1 Hemorrhagic Shock

During HS, there is a decrease in the hematocrit and arterial oxygen content. Profound HS often results in decreased oxygen availability and impairment in myocardial contractile function leading to cardiac failure[1, 2, 3, 4]. The first line of treatment for HS is fluid resuscitation. Colloids and crystalloids have been routinely used to reestablish the hemodynamics. Blood transfusion can be used to reestablish oxygen carrying capacity and hypovolemia. Nevertheless, this practice is still associated with adverse effects such as risk of infections [5] . Development of acellular HBOCs can provide an alternative to improving tissue oxygenation and reestablishing lost volume. HBOCs offer the advantage of having low immunological response, long shelf life and reduced risk of infection when compared to traditional blood transfusion. In addition, treatment with hyperoxic ventilation

has shown promising results in reversing peripheral tissue hypoxia and preserving CF after hemodilution [6]. Benefits associated with hyperoxic ventilation are due to the increased dissolution of oxygen in plasma; the high concentration of plasma oxygen leads to its preferential utilization by the tissues. Although CF is preserved, hyperoxic ventilations results in exceeding coronary vasoconstriction and reduction of myocardial function [6]. The coupling of hyperoxic ventilation with HBOCs after normovolemic hemodilution needs to be investigated as it might offer potential benefits in improving tissue oxygenation. In Chapter 2, we will investigate how the CF is affected when HBOCs and plasma expanders paired with assisted ventilation are used to treat HS. The objective will be to establish the role of acellular oxygen carrying capacity on CF during anemia, including enhanced oxygen and targeted oxygen delivery.

1.2 Hypoxia

The second part of this thesis project will focus on the limitations of the CF during hypoxic hypoxia. In this case, the CF is limited by the lack of oxygen available in the lungs, which can happen during obstructive pulmonary disease or from being at high altitudes. This can progress to hypoxemia, a state of low arterial oxygen supply. The cardiovascular system will often compensate by increasing CO, heart rate (HR) and coronary blood flow [7]. Although the heart has proven to be adaptable to oxygen changes, the cardiac tissue eventually becomes hypoxic and its ability to cope becomes impaired. The latter is even worst for those suffering from a history of cardiac disease, as they are more sensitive to changes in oxygen [8]. In Chapter 3, we aim to investigate the limitations of the CF by oxygenation and methods to ameliorate hypoxia in the cardiac tissue. Previous studies have shown the benefits of a left shift in the hemoglobin oxygen affinity curve during acute hypoxia in the microcirculation [9]. By increasing the affinity of hemoglobin to oxygen we aim to increase oxygen loading and increase arterial PO2, allowing a better overall oxygenation of the tissues. 5-HMF is a non toxic, orally bioavailable, and highly specific low molecular weight 5 carbon-ring-aromatic, that can be used

to allosterically increase the affinity of hemoglobin to oxygen [10]. The objective for this section will be to firstly determine the limitations of the CF during different degrees of hypoxia, and secondly, determine the effects of increasing the hemoglobin oxygen affinity through 5-HMF in the CF at different degrees of hypoxia.

1.3 Approach

Several methods need to be addressed in order to understand the cardio-mechanical and physiological changes in the LV. The cardiac performance can be assessed by different indices such as cardiac contractility, CO, SV, SW and EF among others. A myriad of techniques have been develop to assess such indices, ranging from thermodilution, echocardiography to magnetic resonance techniques. For this project we chose to use a miniaturized PV catheter as it has the advantages of providing real time measurements, high temporal resolution and low cost. The 1.4F PV conductance catheter (SPR-839, Millar Instruments, TX) can be inserted in the left ventricle of small rodents through the carotid artery (closed chested method); the catheter consists of two electrodes that generate an electric field inside the cardiac chamber[12]. The two sensing electrodes will measure a potential difference that is inversely proportional to the conductive material in the site. In addition, a manometer located in the center will measure respective changes in LV pressure. The conductive material will consist of both blood and muscle, nevertheless, we assume that the time varying signal consist on only changes in the blood pool. Thus the time varying component can be related to changes of blood volume in the cavity during the cardiac cycle. However, this value is not calibrated and must be converted to absolute volume. The main equation that relates conductivity in relation to volume is the following:

$$V = \frac{1}{\alpha(\rho L^2(G - Gp)} \tag{1.1}$$

Where V is the volume, ρ is the resistivity of blood, L is the distance between sensing electrodes, G is the measured conductance, Gp is the parallel conductance due to conductivity of the muscle wall and α is a gain coefficient (a volume correction factor). To convert this relative volume value to absolute volume, a calibration

must be done by creating a conductance/volume calibration curve with conductance measurements of blood in preset known volume cylinders. The factor α is applied when techniques such as echocardiography and MRI are available to obtain better approximations when using the cuvette method. In addition, a parallel conductance, accounting for the muscle contribution to the total conductance, can be obtained through a hypertonic saline calibration. The advantage of using a PV catheter over other approaches is its ability to measure LV performance independently from loading conditions, in addition to its ability to provide continuous real time measurements.

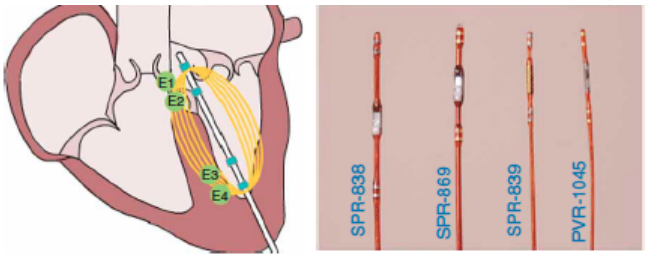


Figure 1.1: Representation of conductance catheter . Left: Positioning of catheter in LV. Right: Different models of conductance catheter [11].

In addition to CF indices, the health of tissue can be assessed by quantifying levels of hypoxia. Measuring hypoxia in the tissue can help understand the extend of the damage and the consequences of hypoxemia. Hypoxia in the cardiac tissue can be determined through immunohistochemical assays. Pimonidazole hydrochloride (Hypoxyprobe-1, Hypoxyprobe, Burlington MA) is a widely used and accepted hypoxia marker. Hypoxyprobe-1 is stable and has the advantage of having high water solubility, allowing the administration of small volume and avoiding hemodilution and rheological distributions. Pimonidazole is activated in hypoxic cells by forming covalent adducts with thiol groups present in proteins. The reagent MAb1 (Monoclonal antibody 1) binds to the adducts making them detectable through immunochemical methods in fixed tissues. By injecting Hypoxyprobe-1 intravenously, tissue samples can be extracted after corresponding procedure and fixed into histological preparations for immunochemical analysis. Positive Pimonidazole staining will be used as a quantification of hypoxic tissue.

Chapter 2

Hemorrhagic Shock

2.1 Abstract

Study aimed to establish the role of acellular HBOC on CF during anemia. Anesthetized Golden Syrian hamsters under mechanical ventilation with air were hemorrhage 30% of the total blood volume followed by equal volume resuscitation with Dxt70 or PolyHb (Hemopure, OPK Biotech, Boston, MS). Blood oxygen saturation of >90% was maintained throughout experiment. CF was assessed through the use of a miniaturized conductance catheter inserted into the left ventricle with continuous PV readings. HS resulted in a fall of CF, decrease CO, SV and SW, with slow recovery to baseline after reinfusion. PolyHb did not show beneficial effects in the CF and resulted in vasoconstriction, with a 116% and 46% increase in SVR for 11.5g/dL and 8.5g/dL PolyHb respectively. Targeted oxygen delivery showed no significant differences in CF. Further research is needed to understand and improve the current state of HBOCs.

2.2 Introduction

HS is a condition that results in reduced tissue perfusion and inadequate delivery of oxygen and nutrients to maintain necessary cellular function. Hypovolemic shock, the most common of HS, is characterized by the loss of blood volume due to trauma or bleeding. The inherent decrease in red blood cells (RBCs) results

in the reduction in tissue oxygenation and an increase in anaerobic metabolism. The ability of the heart to pump blood becomes impaired, furthermore severe HS can lead to multiorgan dysfunction [4, 12].

The first line of treatment to HS is to reestablish the hemodynamics by restoring the volume with the use of plasma expanders (PEs) or/ and allergenic blood transfusion [13]. Safety on the latter has always been a matter of concern. The American Red Cross test for Hepatitis B and C viruses, human immunodeficiency virus (HIV), human T-cell lymphotropic syphilis, West Nile viruses and for the agents of Chagas disease[14, 15, 16, 17]. Nevertheless, there is always the risk that donated blood may contain yet to be identified infectious agents. As new infectious agents emerge, the cost per unit blood will increase due to necessary additional screening. In addition, there are concerns regarding the safety of blood transfusion following extended storage time resulting in storage lesion [19, 20]. To further compound the problem, the availability of human blood is even more limited in emergency situations such as in war zones or after natural disasters [21]. Needless to say, transfusion-related adverse events, both short- and long-term rank among the costliest contributors to health care expenditures [18].

Alternatively, PEs are often used to substitute volume loss during trauma. Their long shelf life, reduced risk of infection and lack of immunologic reaction make them an attractive alternative to blood transfusion. Many times, they are also used in combination with blood transfusion. However, the use of PEs are often limited by side effects such as nephrotoxicity in the case of dextran and extravasation in the case of albumin [22]. Furthermore, PEs do not provide the targeted oxygen carrying capacity that is lost during HS. Therefore, it has been a long-term goal for scientists and engineers to develop a safe and efficacious universal RBC substitute (i.e. O2 bridge). HBOCs are being developed as RBC substitutes for use in transfusion medicine. Interestingly, design of these materials has evolved from an initial approach involving massive infusion of cell-free Hb, to dually restore blood volume and the compromised O2 carrying capacity of blood, to a more elaborate approach, where Hb is chemically modified to avoid adverse reactions that are associated with its infusion in the cell-free state. Therefore, Hb polymerization

represents a simple chemical modification approach that can reduce the toxicity of cell-free Hb. However, despite significant commercial development, recent clinical trial results of PolyHb solutions hamper further development [23, 24, 25]. The key issues observed during phase III clinical trials of PolyHb solutions include vasoconstriction, systemic hypertension, cardiac events and oxidative stress-induced tissue toxicity [26, 27].

There still exist a lack of understanding on the mechanism of PolyHb action. In particular, there is a lack of comprehension on its intrinsic effects in the heart and its function. Given the occurrences of transient ischemic attacks, strokes and myocardial infarctions, the effects of PolyHb on CF must be investigated [28]. The purpose of this study was to establish the role of acellular oxygen carrying capacity on CF during anemia, including enhanced oxygen and targeted oxygen delivery.

Four resuscitation fluids were investigated for the purpose of this study: Dxt70 a conventional colloidal PE; high concentration PolyHb (11.5 g/dL , PolyHb 11.5); and low concentration PolyHb (8.5g/dL, PolyHb 8.5).

2.3 Methods

Animal handling and care followed the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Golden Syrian hamsters (80-90g; Charles River Laboratories, Boston, MA) were anesthetized with sodium pentobarbital (40mg/kg, i.p.) before surgery. Catheters were inserted in jugular vein and left femoral artery for blood extractions, fluid infusion and blood pressure monitoring. Tracheotomy was performed and cannulated with a polyethelyne-90 tube for mechanical assisted ventilation (TOPO dual mode ventilator, Kent Scientific, CT). The closed chest method was used to evaluate CF in these experiments [11]. A 1.4F PV conductance catheter (SPR-839, Millar Instruments, TX) was inserted through the exposed right carotid artery and slowly advanced passing through the aortic valve and into the LV. Changes in PV in the LV were instantaneously and continuously monitored. Isofluorane vaporizer (Isofluorane Vapor 19.1, Drägerwerk AG Lübeck) was connected to ventilator for administration of

anesthesia throughout the experiment. Animals were given 0.6%vol isofluorane throughout the experiment, if response to toe pinch was observed, isofluorane was increase by 0.1%vol.

After surgery, animal was connected to ventilator administering air with an average respiration rate of 90 breaths per minute and a peak inspiratory pressure of 20cmH2O. Experimental setup can be observed in Figure 2.1

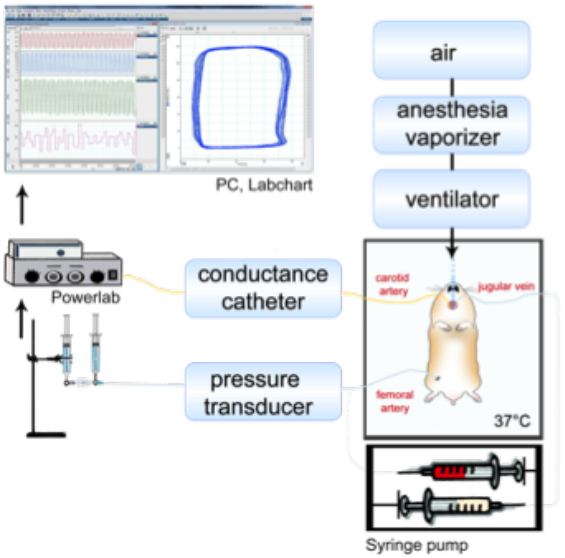


Figure 2.1: Representation of experimental setup for HS. Hamster was maintained in a 37 °C heated bed. Tracheotomy was performed to connect hamster to mechanical ventilator administering air and anesthetic (isofluorane). Conductance catheter was inserted through carotid artery and advanced to the LV. Femoral artery was cathaterized for blood extraction and pressure measurements. Jugular vein was cathaterized for infusions. CF and MAP measurements were sent to Powerlab and saved for analysis.

After PV catheter insertion, animal was allowed to rest for 30 minutes to get acclimatized to the settings before baseline measurements were taken. Animal was then subjected to 30% HS in 3 minutes. Shock was maintained for 10 minutes. 30% Volume infusion with resuscitation fluid was administered at an infusion rate of 100uL/min. CF and systemic values (mean arterial pressure (MAP), HR, hematocrit (Hct) and Hb) were monitored and analyzed at baseline, 10 min-

utes after shock, and 10, 20, 30, 45 and 60 minutes post resuscitation. MAP and HR were obtained through the femoral line (Powerlab, ADInstruments, Colorado Springs, CO), Hct was estimated from centrifuged heparinized capillary tubes and Hb content was determined photometrically (B-Hemoglobin; Hemocue, Stockholm Sweden). Arterial blood gases were measured at baseline, 10min after shock, 10 and 60min post resuscitation (RapidLab 248; Bayer, Norwood, MA). Animal was euthanized with bolus of pentobarbital sodium (Euthasol) followed by a bilateral thoracotomy. Timeline of the protocol can be observed in Figure 2.2

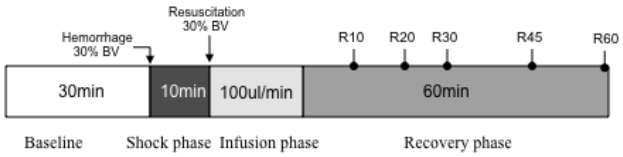


Figure 2.2: Timeline of HS protocol. After surgery, hamster is maintained under anesthesia for 30min to reach a steady state for baseline measurements. 30% of the blood volume is extracted and animal is allowed to rest for 10min before equal volume infusion with resuscitation fluid. Infusion is given at 100ul/min. CF was measured at 10, 20, 30, 45 and 60min into recovery (R10, R20, R30,R45,R60). Blood Samples taken at the end of baseline phase, at the end of shock phase and at R10 and R60.

2.4 Results

Inclusion Criteria: Golden Syrian hamsters weighing 80-90g. MAP>75mmHg at baseline, SV>17uL, Hc*t* >45%, HR>350bpm, CO>7000uL/min at baseline.

11 Golden Syrian hamster were used in this study. Sample size of 4 was used for Dxt70 and PolyHb 11.5. Sample size of 3 was used for preliminaries studies with PolyHb 8.5. Samples of 7-13 loops at steady state were analyzed using PVAN software (PVAN 3.6, Millar Instruments, TX) for CF indices: SV, SW, CO, EF, cardiac contractility (dPdt/VEd). Systemic vascular resistance (SVR) was

calculated by assuming zero central venous pressure

$$SVR = \frac{MAP}{CO} \tag{2.1}$$

The internal energy utilization (IEU) was used as a measure of internal metabolism of the LV and was calculated as following: [29].

$$IEU = ([Ves - V0] * Pes)/2 \tag{2.2}$$

Ves is the end systolic volume, V0 is the ESPV volume axis intercept and Pes is the end systolic pressure. Using the assumption that end systolic pressure volume curve intercept is small, V0 was set to zero for the calculations. Results were analyzed using a two way ANOVA followed by Bonferoni post test.

2.4.1 Systemic and Blood Chemistry

pH showed no significant differences throughout the procedure and between treatment groups. Overall, PO2 levels remained higher than physiological levels, >80mmHg. Ventilation with air was successful in increasing oxygen solubility. As expected, there is a drop in the level of Hct and Hb after HS. In Figure 2.3(a), the Hct stabilizes after infusion. In Figure 2.3(b), the Hb concentration is partially recovered after infusion for the PolyHb groups when compared to Dxt 70. No statistical difference between hemoglobin levels was observed between high and low concentration PolyHb. Details of the results can be seen in Table A.1 in the appendix

Animals resuscitated by PolyHb 11.5 had a better recovery in MAP at R10 when compared to Dextran 70. However animals resuscitated with PolyHb 8.5 had a significantly higher MAP when compared to Dxt 70 at R45 and R60. No difference was detected between the two PolyHb solutions. HR in Figure 2.3(d), dropped during HS. This was followed by an irresolute efforts of recoveries and overall decrease from baseline from all groups.

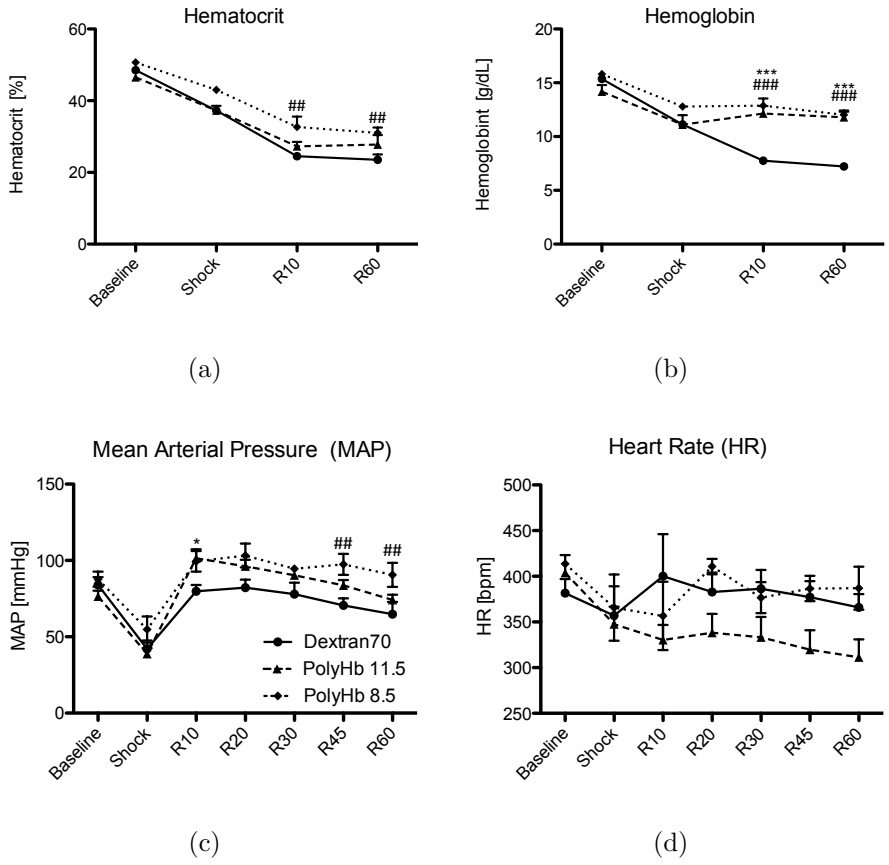


Figure 2.3: Systemic and blood measurements during HS. *P< 0.05, **P< 0.01, ***P< 0.001 Dxt70 Vs PolyHb 11.5. # P< 0.05, ##P< 0.01, ### P< 0.001 Dxt70 Vs PolyHb 8.5

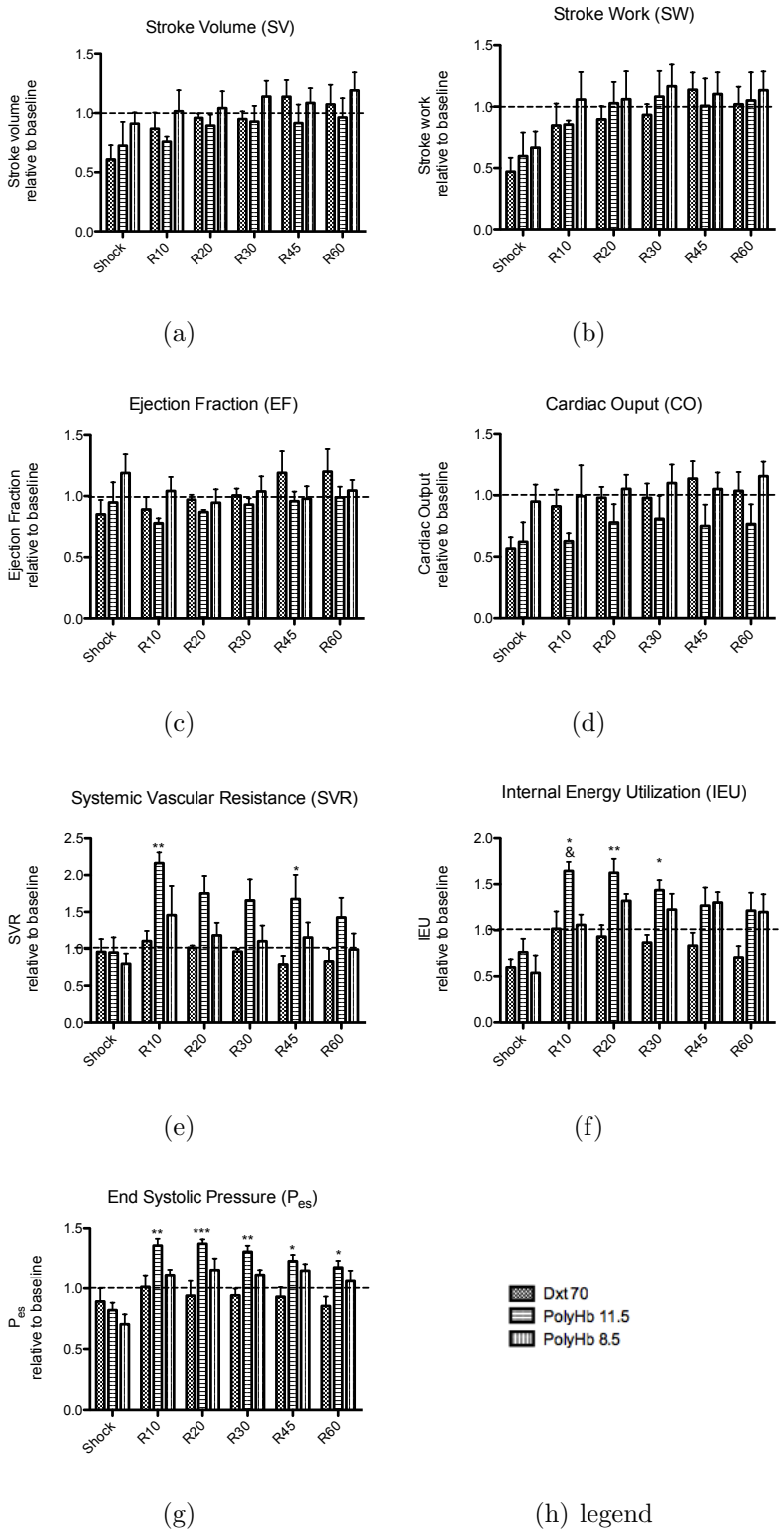
2.4.2 Cardiac Function

All cardiac indices were normalized against baseline and summarized in Figure 2.4. Overall, SV and SW decrease during shock, and recovered after resuscitation for all groups. CO decrease during shock, Dxt 70 showed the fastest recovery from shock, whereas changes on both PolyHb groups were more gradual during recovery. Both EF and dP/dtmax/Ved show no clear developments during shock and recovery and remained unchanged from baseline levels. PolyHb did not show significant difference when compared to Dxt 70 in in ventricular performance (CO, SW, dP/dtmax / Ved, EF).

However, PolyHb was observed to have immense hypertensive properties

with a vast increase in resistance. SVR for PolyHb 11.5 increased by 116% from baseline and PolyHb 8.5 increased 46% from baseline at R10. Dxt70 showed to have no significant effect in SVR and remained at baseline levels. SVR for PolyHb groups slowly dropped with recovery time. IEU showed a slight drop during shock. Dxt 70 group recovered to baseline levels after infusion. PolyHb groups showed increased IEU during recovery. IEU for PolyHb 11.5 has 65% increase from baseline at R10 with a slow drop with recovery time. IEU for PolyHb 8.5 remained similar to baseline levels at R10, but increased by 32% from baseline at R20 without noticeable drop with recovery time.

Pes slightly dropped during shock. Dxt70 recovered to baseline levels, whereas PolyHb 11.5 increased by 35% from baseline at R10 and slowly dropped to baseline. PolyHb 8.5 had a slight 11% increase from baseline at R10 and remainsed there even 60min into recovery.



(a) (b) (c) (d) (e) (f) (g) (h) legend

Figure 2.4: CF measurements during HS *P< 0.05, **P< 0.01, ***P< 0.001 Dxt70 Vs PolyHb 11.5. & P< 0.05, &&P< 0.01, &&& P< 0.001 PolyHb 11.5 Vs PolyHb 8.5

2.5 Discussion

In this study it was observed that infusion with PolyHb was effective in partially reestablishing MAP and hemoglobin levels after HS. However this resulted in high SVR, Pes and IEU when compared to baseline in the PolyHb group. These effects were observed to be concentration dependent. In addition, ventilation with air proved to increase the solubility of O2 in blood.

During recovery, group treated with PolyHb seemed to have a better recovery in MAP when compared to Dxt70. Although all groups showed a decrease in MAP with recovery time, PolyHb 8.5 showed the least and slowest decrease during the 60 min recovery period.

The indices in CF did not show significant differences between the groups. One explanation for the inconclusiveness of these results relates to limitations in the model. Although all animals were subjected to 30% HS, they reacted differently to the shock. For example SV for PolyHb 8.5 was higher than the other groups during shock. After recovery, this group carried on that advantage, and remained the highest of the group. Because of these irregularities, it is difficult to draw conclusions about CF. There is also a limitation in the lack of accuracy and precision from the calibration of the volume.

Meanwhile, measurements that were more heavily weighted in changes of LV pressure showed less variance. The IEU was highest at R10 for the PolyHb 11.5 group. At this stage it was significantly different from the lower concentration PolyHb 8.5 and Dxt70. IEU for PolyHb 11.5 remained highest, 60% above baseline with a decreasing trend as recovery time increased. On the other hand PolyHb 8.5 increased from baseline at R20 and remained 30% higher from baseline. The IEU of both PolyHb concentrations approached the same level, 30% above baseline, whereas IEU for Dxt70 never recovered to baseline levels. IEU relates to the metabolic activity of the LV. By restoring oxygen transport through the use of PolyHb, it is reasonable to expect a recovery in the metabolic activity of the tissue. IEU also relates to the efficiency of the cardiac activity.

PolyHb 11.5 caused a 116% increase in the SVR at R10. This effect is observed to be concentration dependent as a lower dose of PolyHb caused a 46%

increase in SVR, and Dxt70 had no effect in SVR. SVR slowly dropped with recovery time. At R60 PolyHb 11.5 was still 55% above baseline, whereas PolyHb 8.5 dropped to baseline levels. This increase in SVR concurs with the vasoconstrictive side effects seen in earlier studies and contributes to an overall systemic hypertensive state.

There are two major theories to explain the occurrence of Hb-induced vasoconstriction. In the first and most popular theory, it is hypothesized that vasoconstriction is caused by the ability of cell-free Hb to scavenge nitric oxide (NO) produced by the endothelium, thus inhibiting relaxation of the smooth muscle cell layer [30, 31]. In the second theory, it is hypothesized that cell-free Hb oversupplies O2 to the surrounding vasculature via Hb-facilitated diffusion of O2 [32]. An auto regulatory response then induces vasoconstriction in order to limit the available surface area for O2 transport through the blood vessel wall [33, 34]. Regardless of the exact mechanism for the development of vasoconstriction, vascular resistance increases and this translates into the development of systemic hypertension [35, 36]. Both vasoconstriction and hypertension are further exacerbated by extravasation of cell-free Hb through the blood vessel wall (i.e. tissue deposition of Hb), since the tissue deposited cell-free Hb is in closer proximity to the smooth muscle cell layer and endothelial cell layer, thereby facilitating disruption of normal NO signaling [31, 37, 38]. It is also important to note that the iron atom in the heme prosthetic group of Hb is able to participate in redox chemistry which leads to the formation of reactive O2 species (ROS). ROS induce cellular toxicity (nucleic acid, lipid and protein damage) via oxidative stress and decrease the O2 carrying capacity of cell-free Hb via the formation of oxidized Hb (i.e. methemoglobin, metHb), which cannot transport O2 [34, 39]. Therefore, the formation of ROS and free iron derived from extravasated cell-free Hb may induce tissue damage that can account for the cases of myocardial infarction observed in phase III clinical trials of small sized commercial PolyHbs such as Hemopure and PolyHeme [40].

We can agree that PolyHb causes a tremendous increase in SVR and vasoconstriction, especially at the early stages after administration. Although vasoconstriction was not observed to significantly affect CF, clinical use of PolyHb should

be given with careful consideration due to potential long term side effects. Future research should focus on the capabilities and limitations of PolyHb.

Chapter 3

Hypoxia

3.1 Abstract

Acclimatization to high altitude requires time to complete all the physiological adaptation mechanisms that influence oxygen (O2) transport and utilization during chronic hypoxia. Increasing Hb-O2 affinity would increase arterial O2 saturation for a given O2 tension, and may preserve O2 metabolic needs during hypoxia, as long as CF is preserved. In this study, we used 5-HMF, a naturally occurring low-molecular-weight five-carbon-ring aromatic aldehyde that allosterically shifts Hb-O2 affinity curve to the left, with limited toxicological side effects. This study was designed to evaluate the effects of using 5-HMF in the CF during hypoxia. Anesthetized Golden Syrian hamsters under mechanical ventilation received 100mg/kg i.v of 5-HMF, control animals received only the vehicle. All animals were subjected to step wise increased hypoxia by reducing the fraction of inspired O2 (FiO2) from 21% to 15%, 10%, and 5% every 30 minutes at atmospheric pressure. CF was assessed using a closed chest method with a miniaturized conductance catheter inserted into the LV for continuous PV measurements. Hypoxic areas in the heart were studied histologically using pimonidazole staining. 5-HMF improved cardiac indices calculated from PV measurements, including SW, SV, cardiac CO and EF compared to the vehicle group during acute hypoxia. At 5% FiO2, SW, SV, CO and EF were increased from normoxia by 25%, 36%, 20% and 18% in the 5-HMF group, whereas these cardiac indices decreased or remained

unchanged for the vehicle group. Statistical analysis showed significant difference between groups in SV and EF. There was no change in heart chronotropic activity between the groups. Changes in CF were driven by volume dependent indices. Overall, 5-HMF improved CF during acute hypoxia.

3.2 Introduction

Exposure to hypoxia has long been associated with changes in the cardiovascular function and cardiac stress. Changes in the oxygen tension has shown to impact the HR, CO, contractility and vascular resistance [41, 42]. The cardiac tissue can be exposed to hypoxia in several occasions such as cardiac arrest, pulmonary disease or at high altitude.

Often the heart will compensate for this decrease in environmental oxygen by increasing the HR and CO as a measure preserve CF and tissue oxygenation [43]. However, the normal myocardium is known to have good tolerance to hypoxic conditions. Experiments where subjects where exposed to simulated altitude of 4500m by breathing from a low oxygen reserve showed that there was an increase in coronary blood flow to maintain the oxygenation of the cardiac tissue [8]. However subjects with a history of cardiac diseases showed the opposite reaction. At a simulated altitude of 2500m, these patients where observed to have a 18% reduction in coronary blood flow, whereas the healthy counterpart had an increase of 10% in coronary blood flow [8]. Thus, acclimatization to low oxygen requires higher oxygen demands by the cardiac tissue. The fate of the cardiac tissue will be ultimately determinaned by the myocardial oxygen consumption (MVO2), which in turn is defined by intramyocardial tension, HR, contractile state, basal resting metabolism and the external work of the heart [44]. In cases of acute hypoxia, the myocardium can be exhausted and fail. In addition, some may experience sign of altitude sickness and cardiac fatigue, often expressed in the form of palpitations, tachycardia and shortness of breath. Thus, it is important to investigate the effects of hypoxia in the cardiovascular system.

The delivery and transport of O2 is carried on by RBCs, where Hb re-

versibly binds to O2 . When the FiO2 is reduced (hypoxia), there is a decrease in arterial PO2 and blood O2 saturation and the oxygenation of tissues is dramatically affected [45]. In high altitude, exposure to moderate hypoxia will lead to a right shift in the oxyhemoglobin dissociation curve facilitating O2 offload into the tissues[46]. Changes in the affinity will often depend on the acid base status of the blood (Bohr and Haldane effect). Moreover, during acclimatization, the decrease in affinity will depend on the concentration of phosphates in RBC, in particular the increase of intraerythrocytic diphosphoglycerate (2,3-DPG) that serves as a heteroallosteric effector to Hb [47].

Nevertheless, advantages of the right shift in the oxygen saturation curve are not absolute and can be relative to the altitude. This is because natural adaptive right shift in the O2 saturation curve is most apparent in the steeper portion of the oxyhemoglobin dissociation curve, such that enhanced release of oxygen will only be advantageous if venous blood corresponds to this portion of the curve[48]. A right shift might also entail difficulties in securing oxygen at the pulmonary level. At altitudes above 3500m the advantages of a right shift seem minimal [48].

In previous studies we have investigated the advantages of pharmacologically increasing the oxygen affinity, ie. left shift in the oxygen saturation curve [9]. In this study we aim to gain insight in its effects on the CF. We propose that by pharmacologically increasing the oxygen affinity, we will increase alveolar oxygen concentration, improve O2 loading and increase arterial SO2. This allows a better retention of the oxygen in the coronary circulation, enabling a better overall oxygenation of this tissue by spreading the distribution of oxygen further into the cardiac tissue.

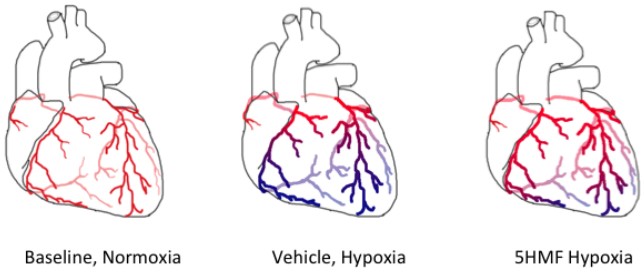
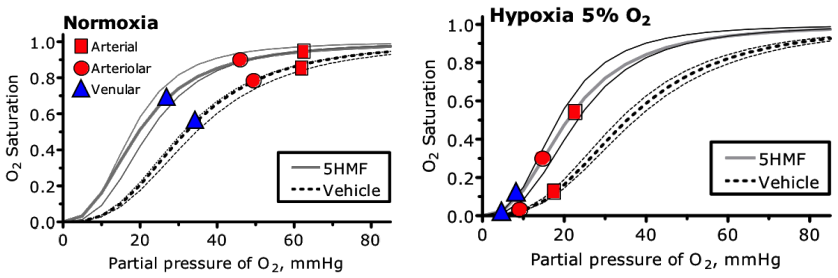


Figure 3.1: Representation of the oxygenation in the coronary circulation during normoxia and hypoxia. Red represents oxygenated blood, blue represents deoxygenated blood. Left: during normoxia, cardiac tissue is well oxygenated. Center: during hypoxia, oxygen is quickly offloaded into the tissues. Right: during hypoxia with 5-HMF pretreatment, there is an increases in oxygen loading in the blood, arterial oxygen concentration is higher.



(a) normoxia (b) hypoxia

Figure 3.2: Changes in blood O2 equilibrium during normoxia and hypoxia. During 5% O2, oxygen saturation will be higher with left shift in oxyhemoglobin dissociation curve [9].

The Hb O2 affinity can be manipulated by the use of allosteric effectors. One such effector is 5-HMF, which has been previously used to moderately increase Hb O2 affinity, increase tissue PO2, improve O2 delivery and hemodynamics during hypoxia [9, 49, 50].

5-HMF is a naturally occurring low molecular weight 5 carbon-ring-aromatic, that covalently binds to the cleft making a Schiff-base Hb adduct with the N-terminal Val 1 nitrogen which destabilizes the T state and stabilized the R state of hemoglobin [51]. In addition, 5-HMF is non toxic, orally bioavailable and highly

specific to intracellular hemoglobin [10]. In a previous study, it was determined that samples treated with 5-HMF resulted in a 12.6mmHg downshift at the 50% pO2 saturation point, proving to be a successful left-shifter in the SO2 curve [9]. It was demonstrated that hasmters treated with 5-HMF resulted in an increase of the MAP, HR and periosvascular pO2, in addition to the preservation the microvascular blood flow during acute hypoxia (5% O2) [9]. In this study we aim to further inquire into the mechanical response of the heart, through the use of miniaturized conductance catheter to measure the ventricular PV changes during hypoxic stress.

3.3 Methods

Animal handling and care followed the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the local animal care committee. Male Golden Syrian Hamsters (80-90g; Charles River Laboratories, Boston, MA) were used for this study. Hamsters were anesthetized with sodium pentobarbital (40mg/kg, i.p.) before surgery. Jugular vein and left femoral artery were catheterized for blood extractions, fluid infusion and blood pressure monitoring. Tracheotomy was performed and cannulated with polyethelyne-90 tube for mechanical assisted breathing using pressure controlled ventilator (TOPO dual mode ventilator, Kent Scientific, CT). A 1.4F PV conductance catheter (SPR-839, Millar Instruments, TX) was inserted into the LV using the closed chested method [[11]. Briefly, PV catheter inserted through the exposed right carotid artery and slowly advanced passing through the aortic valve and into the LV. Isofluorane vaporizer (Isofluorane Vapor 19.1, Drägerwerk AG Lübeck) was connected to ventilator and administered throughout the experiment. Animals were given 0.6%vol isofluorane throughout the experiment. If response to toe pinch was observed, isofluorane was increase by 0.1%vol. Hamster were kept on a 37°C heated pad throughout experiment. After surgery, hamsters were kept under normoxia for 30min to obtain baseline measurements. Saline vehicle or 100mg/kg of 5-HMF (Sigma Aldrich, St, Louis, MO) was administered

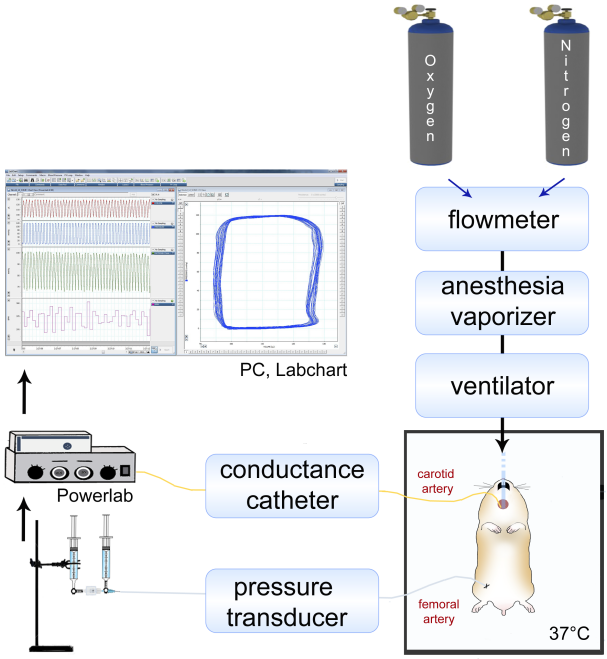


Figure 3.3: Representation of experiemental setup for hypoxia. Hamster was maintained in a 37 °C heated bed. Tracheotomy was performed to connect hamster to mechanical ventilator administering anesthetic (isofluorane). Gas cylinders containing 100% O2 and 100% N2 were connected to a flowmeter to adjust combinations of oxygen delivery. Flowmeter provided gases to anethesia vaporizer and ventilator. Conductance catheter was inserted through carotid artery and advanced to the LV. Femoral artery was cathaterized for blood samples and pressure measurements. Jugular vein was cathaterized for infusions. CF and MAP measurements were sent to Powerlab and saved for analysis.

20 min before hypoxia. Hamsters were then subjected to 30min challenge of 15%, 10% and 5% O2 hypoxia. CF measurements were taken 25 min into respective hypoxic stage. A representation of the timeline is described in Figure 3.4 .

Systemic Parameters such as MAP and HR were recorded continuously from the femoral artery (PowerLab 8/30, ADInstruments, CO). Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tube. Hb content was determined spectrophotometrically (B-Hemoglobin; Hemocue, Stockholm, Sweden). Arterial blood was collected in heparinized glass capillaries (50ul) and immediately analyzed for arterial oxygen partial pressure (PO2), arterial car-

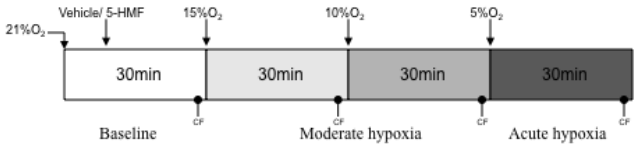


Figure 3.4: Timeline of hypoxia protocol. After surgery, hamster was maintained under anesthesia to reach a steady state for baseline measurements for 30min. This was followed by 30min of 15%, 10%, and 10% hypoxia. CF measurements and blood samples were obtained 25min into each hypoxic stage .

bon dioxide partial pressure (PCO2) and pH (Rapidlab 248; Bayer, Norwood, MA). Ventricular PV were continuously measured (PowerLab 8/30, ADInstruments, CO).

Immunohistochemistry staining of pimonidazole bound to hypoxic zones in the heart were studied. Hypoxyprobe-1 Green Kits (pimonidazole and corresponding monoclonal antibody conjugated to fluorescein) (Hypoxyproble, Burlington, MA) was injected 5 min before 10% hypoxia treatment. Animals' heart was immediately excised after euthanasia and fixed with a 10% gluteraldehyde solution. Heart was then sliced into 6 section noted in Figure 3.5. Six randomized areas per slide were analyzed for positive pimonidazole staining

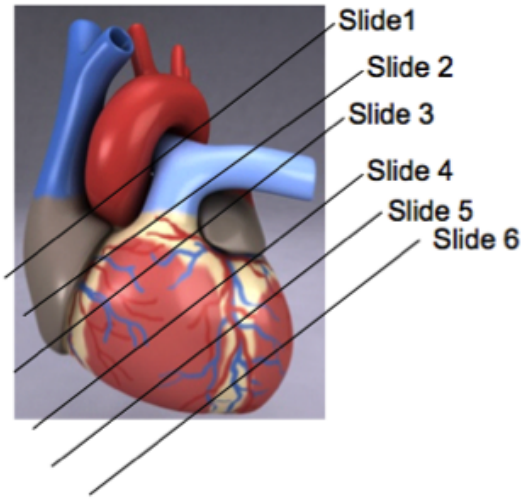


Figure 3.5: Representation of the location of slides used for pimonidazole staining.
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3.4 Results

Inclusion Criteria: Golden Syrian hamsters weighing 80-90g. MAP>75mmHg at baseline, SV>17uL, Hct >45%, HR>350bpm, CO>7000uL/min at baseline.

8 Golden Syrian hamsters were used in this study. Sample size of 4 was used for vehicle and 5-HMF groups respectively. Samples of 7-13 loops at steady state were analyzed using PVAN software (PVAN 3.6, Millar Instruments, TX) for CF indices: SV, SW, CO, EF, cardiac contractility (dPdt/VEd), and arterial elastance (Ea). SVR was calculated as stated in equation 2.1. IEU was calculated as stated in equation 2.2. LV mechanical efficiency (VME) was defined and calculated as:

$$VME = \frac{SW}{PVA}, \tag{3.1}$$

$$PVA = (IEU + SW) \tag{3.2}$$

where PVA is the total pressure volume area.

3.4.1 Systemic and Blood Chemistry

Hypoxia caused no significant changes in the Hct and Hb. There were no significant changes observed in the pH of blood throughout hypoxia. In Figure 3.6(a) and 3.6(b) , both pO2 and pCO2 decreased with hypoxia. 5-HMF resulted in slightly higher pO2, however, no statistical difference was observed between the groups. A summary of the blood composition and chemistry are shown in Table A.2 in the appendix. In Figure 3.6(c), MAP slightly increases when first exposed to moderate hypoxia of 15% O2 and decreases as hypoxia becomes more acute. At 10% O2, MAP for the 5-HMF group decreases at slower rate when compared to vehicle group. At 5% O2, both groups converge to similar MAP. In Figure 3.6(d) no significant changes in HR are observed between hypoxia levels or treatement groups.

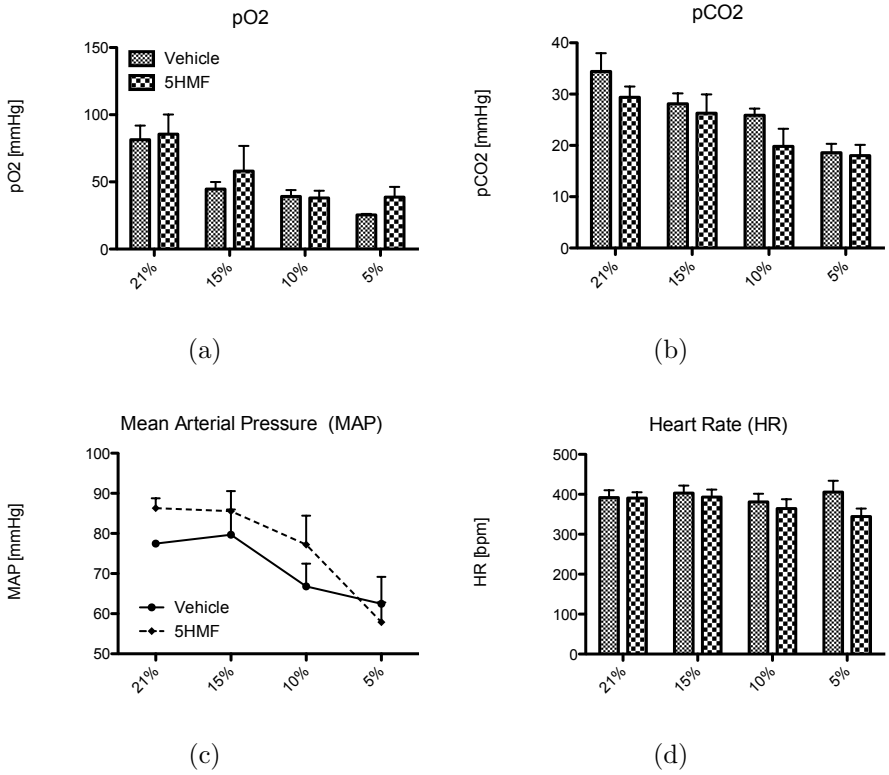

Figure 3.6: Systemic and blood measurements during different levels of hypoxia

3.4.2 Cardiac Function

All CF indices were normalized against baseline and are summarized in Figure 3.7. Most evident changes in the CF can be observed during acute hypoxia (5% O2). As expected, the CO for the vehicle group showed an 18% increase from normoxia at 15% O2 . However CO decreases as hypoxia become more acute. At 5% O2, CO for vehicle is relatively similar to baseline. For 5-HMF group, CO remains similar to normoxia at 15% O2, CO increases 20% from normoxia at 10% and 5% O2. No statistical differences in CO were detected between the groups at each hypoxia stage. No significant changes were observed for the EF of the vehicle groups as hypoxia became more acute. For the 5-HMF group an increase in EF is observed at at 10% O2 and most significantly at 5% O2 when compared to the vehicle group. An increase of 18% from normoxia is observed for 5-HMF at 5% O2. The SV significantly increased to 35% from normoxia for the 5-HMF group during 5% O2, whereas it decreased for the vehicle group during acute hypoxia. Similar trend is observed with the SW, for the 5-HMF group there is an increase after 10% O2 that is sustained with the 5% O2. However, the vehicle group has an earlier response expressed as an increase at 15% O2, but that is not sustained as hypoxia becomes more acute. Differences in SW nevertheless show no statistical significance. dP/dtmax/Ved, an index of contractility, showed no significant difference between treatment groups or hypoxia level. The vascular response is also most evident during acute hypoxia. The AE and SVR significantly decreased to 62% and 57% from normoxia for the 5-HMF group at 5% O2. Whereas for the vehicle group, AE and SVR increased and remain unchanged from that at normoxia respectively. There were no significant changes in the calculated value of IEU between hypoxia stage or treatment groups. LVE at 5% O2 increased to 19% from normoxia for 5-HMF, and remained at baseline levels for vehicle group.

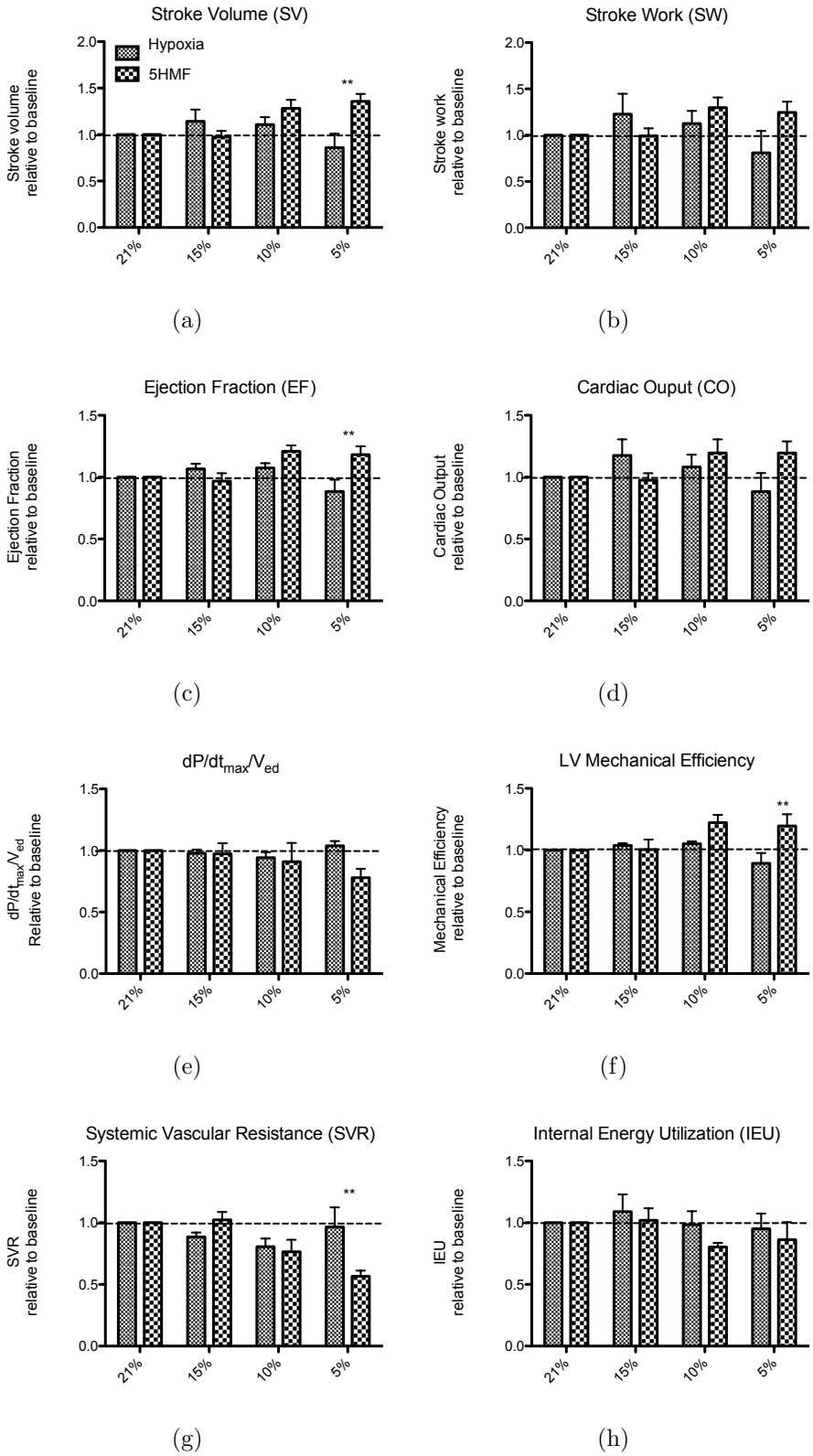

Figure 3.7: CF measurements during different hypoxia stages. *P< 0.05, **P< 0.01, ***P< 0.001 vehicle Vs 5-HMF.

3.4.3 Pimonidazole Staining

A total of 4 hearts and 6 hearts were used to analyze the vehicle and 5-HMF groups respectively. Resulting in a total sample size of 24 and 36 sample areas per slide for each group respectively. Pimonidazole positive area represents hypoxic tissue. Tissue becomes more hypoxic the closer it is to the apex of the heart (Slide 6). The area of positive pimonidazole staining at the top of the heart (slide1) is 41.38± 6.60% and 27.11± 5.31% for the vehicle and 5-HMF group respectively. Whereas in the apex of the heart it is 74.42± 5.50% and 48.08± 5.49 % respectively, almost double the amount of affected tissue as it progresses to the apex. Animals treated with 5-HMF show significantly less hypoxic tissue per slide when compared to vehicle group. On average, 5-HMF reduced hypoxic tissue by 3/5. Detailed results of the pimonidazole staining are in Table A.3 in the appendix.

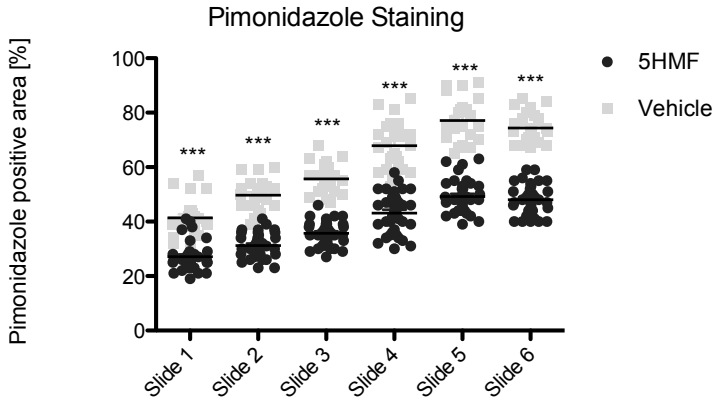

Figure 3.8: Positive pimonidazole staining represents amount of hypoxia in cardiac tissue. Amount of staining increases the closer it is to the apex of the heart. *P< 0.05, **P< 0.01, ***P< 0.001 vehicle Vs 5-HMF. .

3.5 Discussion

This study draws several important observations. Exposure to hypoxia had an expected effect on the vehicle group: an increased in CO at first exposure to hypoxia (15% O2). This correspond to a moderate level of hypoxia comparable

to altitudes of 2400m where the increase in cardiac activity is a common response in order to preserve tissue oxygenation [43]. Nevertheless, as hypoxia increases to 10% O2 and later to 5% O2, the cardiac tissue starts to yield. The mechanical ability of the heart to pump blood is impaired. This is most evident in the decline of CO, EF, SV, SW and the considerable amount of hypoxic cardiac tissue for the vehicle group. On the other hand, groups treated with 5-HMF had no significant changes in CF at 15% O2. At 10% and 5% O2, indices of CF such as CO, EF, SV, SW increased. The heart compensates by increasing its SV rather than its HR. However results in HR were unexpected, as they were not observed with earlier microhemodynamic study where there was an increase in HR for both vehicle and 5-HMF group at 10% O2[9]. One explanation for the lack in chronotropic response can be that its effects are counteracted by the cardio-depressive properties of the isofluorane. We see a slight increase in MAP at 15% O2, however as O2 levels decrease, MAP decreases as well. There are no significant differences between the treatments groups. By 5% O2, both treatments converge to 60mmHg. These results are difficult to interpret as we do not exactly know how much of the decrease can be attributed to the effects of the hypoxia and how much are attributed to the anesthetic. Further discussion on these limitations will be extended in Chapter 4.

AE and SVR significantly drop for the 5-HMF group, whereas it increases or remains relative to normoxia in the vehicle group. The drop in SVR has been previously observed in other studies and is consistent with the concept that O2 transport is regulated in specific vascular bed [9, 52]. The AE expresses the ventriculo-arterial coupling, the decrease in AE for the 5-HMF group at 5% O2 shows to be significantly lower than baseline and when compared to the vehicle group. Although the SV increases the end systolic pressure is unresponsive to those changes. This is consistent with the finding in Yalcin et al, where the group treated with 5-HMF had a significant increase in the diameter of venules and arterioles accompanied by increased blood flow at 5%O2 [9]. The vasodilation that is observed can explain the low AE, the increased diameter and bloodflow can improve oxygenations of tissues.

Histology shows that 5-HMF was effective in protecting the tissue from

becoming more hypoxic. The positive pimonidazole staining increases towards the apex of the heart in both vehicle and 5-HMF group. This is expected as the coronary circulation leaves the aortic sinus, the blood's oxygen will be consumed by the cardiac tissue. Regions furthest from the aortic sinus will encounter most significant deoxygenation. The apex region of the heart performs the most work and results in higher metabolic demands that explain faster oxygen depletion. By increasing the oxygen affinity, 5-HMF was successful in increasing O2 loading and offer a better oxygenation of the cardiac tissue, this is evident as the total average of positive staining in the vehicle group was 61.04 6.58%, versus the 39.07 5.54% for the 5-HMF group. By pharmacologically shifting the SO2 curve to the left, we were able to reduce hypoxic tissue by 36% during 10% to 5% O2 hypoxia.

The principal finding is that at levels of acute hypoxia (5% O2), 5-HMF treatment improves CF and lessens hypoxic cardiac tissue. However no improvements in MAP were observed. At moderate levels of hypoxia (>15%O2) the benefits of 5-HMF are not evident, and the hamster seem to adapt to the low oxygen environment successfully regardless of the treatment. This was expected as benefits for SO2 left shift are most evident at altitudes of 3500m and above, which are relative to oxygen levels of 13.6% O2 and below [48].

One of the limitations of this study is using a hamster as an animal model. Hamsters are fossorial animals, thus their adaptation to low oxygen environments is widely different from humans. This is why they have low arterial PO2 at normoxia. However, hamsters are still sensitive to hypoxia and hypercapnia [53, 54]. At low oxygen concentration, their respiratory system responds by increasing the frequency of respiration with no changes in tidal volume and little activation of stretch receptors [54] . However, in this protocol, animals were connected to mechanical ventilator running at a constant frequency with slight increase in peak inspiratory pressures as oxygen concentrations decreased. When studying the CF we must also understand that small animals have a high HR when compared to larger mammals. This results in shorter times for endocardial perfusion and higher fraction of inspired O2. Another important note, is that because their innate and baseline HR it already high, there is a limited rise in CO when needed. The CO

response can potentially be different in larger mammals as there is more flexibility in that account. Walker et al describes that the hamsters early acclimatization to high altitude different from humans. That is, it characterized by early respiratory alkalosis that can contribute to a left shift in SO2 curve and is later counteracted by the effects of 2,3-DPG [54]. On the counterpart, humans are more sensitive to hypocapnia, where the Bohr effect can limit the benefits of 5-HMF treatment. We assume that the effect of erythropoietin and 2-DPG do not take place within this experimental timeline due to the minimal changes in hematocrit and pH. In the future, it would be valuable to investigate the benefits of 5-HMF in a heart failure animal model, as this condition is most affected in hypoxic environment. In the past, the protective effect of 5-HMF were proven to affect the microhemodynamics response and the microvascular O2 transport. In this study we prove that treatment with 5-HMF resulted in the protective effects of the heart tissue and the preservation and improvement of CF indices during acute hypoxia.

Chapter 4

Limitations and future directions

4.1 Anesthesia

The choice, use, and implementation of anesthesia have been a topic of much concern for *in-vivo* experimentation, particularly in CF studies. Anesthetics ranging from barbiturates to inhalants have been associates with cardio and respiratory depressive side effects [55, 56, 57]. This becomes a challenge when examining the CF of animal models, as it might impact the parameters being measure in this realm. Three common anesthetics where studied to determine the best anesthetic regimen for CF studies: Ketamine-xylasine(KX), pentobarbital and isofluorane.

Ketamine is a dissociative anesthetic that can be administered intravenously. Ketamine acts as a potent noncompetitive N-methyl-D-aspartate (NMDA) antagonist, with anesthetic, analgesic, amnesic and cataleptic effects [58]. Ketamine can produce transient stimulating effects on the heart by increasing CO, HR and MAP [58, 59]. However, it is a poor muscle relaxant, and is often used in combination with xylazine, a 2-adrenergic agonist, to provide better muscle relaxation and sedation [55]. KX, is often the anesthetics of choice for small rodent surgery, nevertheless it results in long lasting cardiodepressive effects, including reduce HR and CO.

Pentobarbital, a short lasting barbiturate, works by depressing the central nervous system. It depresses the respiratory center and affects the hemodynamics of the heart. Nevertheless, it has been proven to be less cardiodepressive than

KX [55]. Low dose, continuous intra-venous administration of pentobarbital can offer prolong anesthesia while conserving the capacity of cardiovascular regulation [60]. However, dose dependence is extremely important with this substance, as it can have diverging effects in the cardiovascular and pulmonary system if given in excess [56]. For instance dosage necessary for sedation in surgical procedure are often high, causing respiratory depression [61]. Another alternative to anesthesia is the use of inhalants such as isoflurane, which provide a better depth control of sedation [56]. Isofluorane, like other anesthetics, have cardiodepressive effects. Nevertheless, its use results in better HR and oxygen saturation levels when compared to pentobarbital, offering a more suitable option to evaluate CF [62]. In addition, it provides a better baro-reflex response when compared to pentobarbital resulting in more physiologically relevant information for experiments [62]. The use of inhalant anesthetics require specialized equipment, such as a vaporizer and a ventilator. The administration of the inhalant itself requires a mask, chamber or access to the respiratory tract in order to be administered. This is a challenge when performing the surgery, as access to the chest becomes obstructed by the use of any of these tools.

In order to circumvent the surgical issue, an injectable anesthetic was considered to perform the surgery and insert the catheter before a low dosage of isofluorane was administered for the subsequent experimental procedure. A preliminary study using KX or pentobarbital for the surgical section of the experiment was examined. Briefly, Golden Syrian hamster was anesthetized with either 200/10 (K/X) mg/kg concentration of KX or 40mg/kg of pentobarbital through intraperitoneal injection. Catheterization of left femoral artery and tracheotomy and was performed while animal was sedated [11]. MAP and HR was tracked as animal recovered from surgery, as animal began to react to toe pinch, isofluorane was administered through a ventilator. The use of KX resulted in prolonged cardio depression, with a MAP of 50mmHg with no recovery, including low sustained heart rate for 2 hours. Pentobarbital resulted in less cardio depression and its short lasting effects (30-60min) provided a better recovery. Thus, the latter anesthetic was chosen for the surgical procedure.

It is important to note that this brief analysis was aim to minimize the cardio-depressive effects and find an optimal anesthesia treatment for these studies. Nevertheless, we have to consider that there are still side effects to low dose isoflurane. When interpreting indices in CF, it is critical to understand that they will not be at physiological level. To differentiate between the effects of the anesthetics and the effects of the experimental procedure remains a challenge. By finding a low dose, steady state treatment, and normalizing results to a baseline, we hope to be able to find some relevant difference despite anesthetic side effects.

4.2 Small animal model

Another limitation in the studies was the use of the hamster model. Small mammals have inherently different cardiovascular function when compared to larger humans. Including a faster heart rate, shorter action potentials and difference in electrophysiological handling. Our main concern is the fact that hamsters are fossorial animals, and have adapted to live in burrow environment. Thus, hamster inherently can withstand low oxygen environments and conditions of extreme hypoxia. Nevertheless the hamster has been extensively as an animal model for cardiovascular diseases and is still responsive to hypoxia [63]. Using a small animal model has the advantages of being cost-effective and an efficient tool due to its short generation span, accelerated lifespans and housing cost.

4.3 Methods

There were two main limitations concerning the methodology of this study: the accuracy of volume calibration and the inability to provide occlusion measurements. Volume was attained in relation to the conductance of blood. However, the conductance of blood changes with the hematocrit. This became a challenge in HS experiments, as the hematocrit of the test subjects was dynamic. To overcome this problem, different curves at different hematocrits and dilution solutions were created. It was observed that the change in conductance with hematocrit

was linear at the hematocrit range of interest (30-55%) allowing the interpolation of calibration curves. Thus, in HS experiment, the CF of each stage was obtained with a unique calibration curve pertaining its measured hematocrit and dilution solution (Dextran or PolyHb). However, hematocrit measurements were not obtained for all data points (R20, R30 and R45). Interpolation was used to estimate the corresponding hematocrit. The calibration strategy is not the most accurate, by normalizing the results to baseline we aim to focus on the difference between time points and stages instead of the physiological fidelity of the results.

Two of the most powerful indices in CF are the end diastolic and end systolic pressure volume relationship (EDPVR and ESPVR). The slope of the ESPVR represents end systolic elastance (Ees), a measure of myocardial contractility. Whereas the EDPVR describes the passive properties of the myocardium. In order to obtain these curves, the inferior vena cava must be occluded to provide transient changes in the preload. The inferior vena cava can be easily accessed using an open chest surgery [11]. However, this surgical method is more invasive, less stable, and results in noisy PV loops when compared to the closed chest method. In the closed chest approach it is possible to reach the vena cava by compressing the vessel through the diaphragm in an open abdominal cavity [11]. However, the latter method requires considerable experience and expertise and was not reproducible in this study. Due to the lengthy and sensitive nature of procedures such as HS and hypoxia, the closed chested method was chosen because of its stability, reliability and safety. The use of ESPVR to obtain Ees is often considered a gold standard for myocardial contractility; difficulties in obtaining transient changes in preload without clinical implications remains a challenge. There have been several efforts in obtaining Ees from single beat estimations from curve fitting techniques [64, 65]. However studies have shown these estimations showed no significant response to dopamine or dobutamine challenge when compared to those from multi-beat estimations [66, 67]. Another alternative to cardiac contractility is using dPmax/dt/Ved, which have shown to be relative to changes in Pes-Ves relations in dobutamine challenge [68]. However, dPmax/dt/Ved, is very susceptible to volume calibration, as the physiological value of Ved, instead of the normalized

value, is key to the calculation of contractility. In the case of HS, where different calibration curves were used for each data point, the accuracy of contractility will be hindered by this bias. Obtaining reliable information about the contractility of the heart still remains a challenge for this methodology.

4.4 Future directions

In the future we would like to focus on improving the experimental model and obtaining results for cardiac failure cases. In the methodology, we want to aim at fine-tuning the accuracy of volume calibration in order to achieve physiologically pertinent results. This can be achieved by using the α factor in the conductance equation, which can be obtained by doing a comparative study with another PV method such as an echocardiography. However this requires resources and specialize equipment.

It would interesting to examine the limitation of oxygenation in a failing heart model. Heart failure (HF) is the inability of the heart to pump enough oxygen to the body. Causes for this condition range from coronary artery disease to high blood pressure. The prevalence and magnitude of this disease is immense. The American Heart Association reported that 5.1 million people in the United State suffered from HF[60]. It remains one of the leading cause of death, half of those that develop HF die within 5 years of diagnosis[69]. There are obvious hemodynamics and metabolic adaptations that have taken place in a failing heart. The response of the heart to low oxygen environment, either from HS or hypoxia will be relevant.

As we mentioned in the beginning , many environments and diseases can expose the heart to low oxygen enviroments. Although this project only invetigated two of such cases: HS and hypoxia, it would be attractive to model conditions such as sleep apnea, and how intermittent levels of hypoxia can affect the CF and contribute to heart disease.

4.5 Conclusion

The heart is an indispensable organ and several situations can expose it to a low oxygen environment. It is important to understand its limitations and alternatives to ameliorate this condition. We have examined two cases of low oxygen environment. During HS, not only do we have changes in oxygen delivery but clear changes in hemodynamics. Volume reinfusion is used to reestablish the hemodynamic, however targeted oxygen delivery showed no significant improvements in CF. In addition, PolyHb showed immense vasoconstriction. It is evident that we are still a long way from creating the ideal blood substitute. Future research must focus on the understanding and improvement of HBOCs. During acute hypoxic hypoxia, we see deterioration of the CF in addition to detriments of the cardiac tissue. We were able to prove that by increasing hemoglobin oxygen affinity through the use of 5-HMF we improved both CF and tissue hypoxia during acute hypoxia.

Appendix A

Table A.1: Blood composition and blood chemistry for HS

		Dxt 70		PolyHb 11.5		PolyHb 8.5	
		Mean	SD	Mean	SD	Mean	SD
Hematocrit [%]	Baseline	48.50	2.38	46.50	1.29	50.67	1.53
	Shock	37.25	2.63	37.25	2.22	43.00	1.00
	R10	24.50	0.58	27.25	2.50	32.67	5.03
	R60	23.50	3.00	27.75	5.38	31.00	2.65
Hemoglobin [g/dL]	Baseline	15.35	0.73	14.18	1.24	15.80	0.53
	Shock	11.13	0.49	11.10	1.80	12.80	0.46
	R10	7.75	0.65	12.15	1.40	12.87	1.16
	R60	7.23	0.74	11.80	1.01	12.03	0.68
pH	Baseline	7.40	0.09	7.33	0.10	7.41	0.16
	Shock	7.37	0.08	7.31	0.10	7.36	0.09
	R10	7.37	0.06	7.33	0.16	7.36	0.10
	R60	7.38	0.05	7.34	0.14	7.35	0.17
pCO_2 [mmHg]	Baseline	42.13	12.24	51.10	22.93	43.73	25.28
	Shock	22.10	4.46	31.15	10.12	32.03	9.57
	R10	32.50	6.36	36.30	13.17	36.87	10.21
	R60	34.88	11.38	36.55	16.72	38.73	13.51
pO2 [mmHg]	Baseline	86.43	28.61	81.95	8.72	95.20	14.80
	Shock	131.03	17.25	113.35	33.19	126.93	29.91
	R10	127.93	13.92	139.90	25.82	126.77	46.29
	R60	101.93	49.95	139.03	37.04	99.33	35.23
PHb [g/dL]	R10			5.30	0.56	5.05	1.06
	R60			5.13	0.38	4.85	0.78

Table A.2: Blood composition and blood chemistry for Hypoxia

		Control		5-HMF	
	$[\%]O_2$	Mean	SD	Mean	SD
Hematocrit [%]	21	50.00	1.83	51.50	2.38
	15	48.50	1.73	51.00	2.45
	10	48.00	2.45	50.25	2.06
	5	48.50	2.08	49.50	0.58
Hemoglobin [g/dL]	21	15.30	0.22	16.00	1.21
	15	14.88	0.63	15.78	1.30
	10	14.75	0.25	15.68	1.13
	5	14.93	0.66	15.43	0.85
pH	21	7.47	0.09	7.46	0.05
	15	7.48	0.06	7.47	0.06
	10	7.50	0.06	7.45	0.10
	5	7.40	0.05	7.29	0.22
pCO_2 [mmHg]	21	34.43	7.10	29.38	4.22
	15	28.13	4.03	26.28	7.34
	10	25.88	2.61	19.83	6.82
	5	18.58	3.48	18.03	4.19
pO_2 [mmHg]	21	81.48	20.97	85.48	29.27
	15	44.65	10.48	57.88	37.53
	10	39.18	9.63	37.88	10.98
	5	25.40	1.57	38.53	15.34

Table A.3: Percentage of positive pimonidazole staining

		Control		5-HMF	
	$[\%]O_2$	Mean	SD	Mean	SD
Hematocrit [%]	21	50.00	1.83	51.50	2.38
	15	48.50	1.73	51.00	2.45
	10	48.00	2.45	50.25	2.06
	5	48.50	2.08	49.50	0.58
Hemoglobin [g/dL]	21	15.30	0.22	16.00	1.21
	15	14.88	0.63	15.78	1.30
	10	14.75	0.25	15.68	1.13
	5	14.93	0.66	15.43	0.85
pH	21	7.47	0.09	7.46	0.05
	15	7.48	0.06	7.47	0.06
	10	7.50	0.06	7.45	0.10
	5	7.40	0.05	7.29	0.22
pCO_2 [mmHg]	21	34.43	7.10	29.38	4.22
	15	28.13	4.03	26.28	7.34
	10	25.88	2.61	19.83	6.82
	5	18.58	3.48	18.03	4.19
pO_2 [mmHg]	21	81.48	20.97	85.48	29.27
	15	44.65	10.48	57.88	37.53
	10	39.18	9.63	37.88	10.98
	5	25.40	1.57	38.53	15.34

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