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## Microvascular Responsiveness to Cardiopulmonary Bypass

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree  
in Medical Biophysics

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## **Abstract**

Cardiopulmonary bypass can result in multiple organ failure due to mechanisms of ischemia reperfusion injury and the systemic inflammatory response syndrome. The primary objective of this thesis was to investigate and monitor the microvasculature in cardiac surgery patients using multiple methodologies and real-time monitoring techniques. The purpose of our first study was to determine whether pulsatile blood flow during bypass improves microvascular perfusion compared to non-pulsatile flow. We found that changes in sublingual mucosal microcirculation using orthogonal polarization spectral imaging correlate with indices of thenar muscle tissue oxygen saturation and its recovery during a vascular occlusion test using near-infrared spectroscopy in both groups. There were significantly fewer normally perfused vessels, along with impaired microvascular responsiveness and elevated levels of lactate in the non-pulsatile group. Although these technologies help to better understand the pathophysiology of acute circulatory failure, a need exists for improved monitors that can continuously track real-time changes in the microcirculation. Our subsequent studies involved the application of a custom broadband continuous wave near-infrared monitor to determine the feasibility of tracking microvascular hemoglobin content as a surrogate for red blood cell (RBC) flow in skeletal muscle during non-pulsatile bypass. We measure changes in optical density at the isosbestic wavelength as an index of change in hemoglobin over time. The changes in optical density relative to baseline values were continuously monitored throughout the procedure, and showed a positive correlation with various interventions during bypass and with potentially negative outcomes. In our third study we applied continuous wavelet transform analysis to the near-infrared data to reflect the dynamic variability in RBC distribution within the microvasculature as an indicator of autoregulation. We showed signal power composition varied within and between patients at all

time points, and shifting of power distribution from high to low frequency ranges, and vice versa, in relation to specific events during the procedure. These studies support the potential for clinical devices that can be easily interpreted by a clinician in real-time to guide therapeutic targets and improve clinical outcomes. Our current research and related future work is an important first step and compelling pre-requisite for such a monitor.

**Keywords:** Ischemia reperfusion injury, systemic inflammatory response syndrome, cardiopulmonary bypass, microcirculation, pulsatile flow, non-pulsatile flow, orthogonal polarization spectral imaging, near infrared spectroscopy, vascular occlusion test, tissue oxygen saturation, microvascular hemoglobin content, optical density, continuous wavelet transform, autoregulation

## **Summary for Lay Audience**

To repair the heart during surgery, the pumping of the heart must be stopped. To keep the patient alive they are connected to a machine that oxygenates the blood and pumps it throughout the body. However cardiopulmonary bypass can cause inflammation affecting the smallest blood vessels of the body called the microcirculation. My objective was to investigate the effects of bypass on these small vessels using different types of imaging devices. During bypass blood can be pumped continuously without a pulse or with an artificial pulse added. In our first study we investigated whether blood flow to the microcirculation changes when comparing flow modes. We found that pulsatile flow improved blood flow through the microcirculation under the tongue compared to non-pulsatile, and pulsatility also improved how quickly blood flow was restored to a muscle at the base of the thumb following a brief period of occlusion by a blood pressure cuff. As these technologies require the intervention of a health care provider and provide only a snapshot of the patient's condition, our second study investigated new technology that would continuously collect information. This device was placed on the thigh muscle and monitored red blood cell hemoglobin levels as blood flowed through the microcirculation under non-pulsatile flow conditions. We successfully tracked changes in hemoglobin levels in relation to key events occurring during bypass. Since hemoglobin carries oxygen, our third study investigated if the microcirculation changes its pattern of delivery during the course of surgery. As the microcirculation regulates the delivery of blood and oxygen to the tissue it causes small oscillations in hemoglobin levels over a range of frequencies. Our analysis measured the strength of different frequency ranges generated by the microcirculation. We showed that power versus frequency varied within each patient at various time points, and that differences also existed

between patients. We noticed power shifting from one frequency to another during particular interventions which correlated with patient outcomes. In summary, we successfully monitored the microcirculation during bypass. These studies demonstrate the potential for new microvascular monitoring, and are an important first step in development of such a monitor.

## Co-Authorship Statement

This thesis contains the following manuscripts that have been published and are in preparation for submission:

**Chapter 1:** This introduction chapter was written by Michael O’Neil.

**Chapter 2:** This chapter represents work based on a manuscript entitled “*Microvascular Responsiveness to Pulsatile and Nonpulsatile Flow During Cardiopulmonary Bypass*,” published in the *The Annals of Thoracic Surgery*. Michael O’Neil is first author for this work; co-authors are Rene Alie, Mary Lee Myers, John Murkin, Linrui Guo, and Christopher Ellis. Michael O’Neil was responsible for study concept and experimental design, data collection, data analysis, statistical analysis, data interpretation, preparing the figures, and drafting the manuscript. Rene Alie assisted with randomization protocol and data collection. Mary Lee Myers allowed for patient enrollment, and John Murkin was involved with study design and granted access to the near infrared spectroscopy device system. Ray Guo also contributed to study concept, experimental design, and allowing patient enrollment. Supervisor Christopher Ellis was responsible for study concept, experimental design, data interpretation and general oversight.

**Chapter 3:** This manuscript is in preparation but has not been submitted. Michael O’Neil is first author for this work; co-authors are John Paul Mousseau, Asher Mendelson, Ajay Rajaram, Mamadou Diop, Ali Hage, Linrui Guo, and Christopher Ellis. Michael O’Neil was responsible for study design, patient enrollment, data collection, data analysis, statistical analysis, preparation of figures, and drafting the manuscript. John Paul Mousseau assisted with data collection. Asher Mendelson helped design and build the NIRS system used in this study, along with clinical

direction for experimental design and data collection. Ajay Rajaram, under the supervision of Mamadou Diop also contributed in the designed and build of the NIRS device. Ali Hage assisted with statistical analysis. Christopher Ellis conceptualized the study, secured funding, designed the wavelet software used for analysis, and provided guidance with study design and data interpretation.

**Chapter 4:** This manuscript is in preparation but has not been submitted. Michael O’Neil is first author for this work; co-authors are John Paul Mousseau, Asher Mendelson, Ajay Rajaram, Mamadou Diop, Ali Hage, Linrui Guo, and Christopher Ellis. Michael O’Neil was responsible for study design, patient enrollment, data collection, data analysis, statistical analysis, preparation of figures, and drafting the manuscript. John Paul Mousseau assisted with data collection. Asher Mendelson helped design and build the NIRS system used in this study, along with clinical direction for experimental design and data collection. Ajay Rajaram, under the supervision of Mamadou Diop also contributed in the designed and build of the NIRS device. Ali Hage assisted with statistical analysis. Christopher Ellis conceptualized the study, secured funding, designed the wavelet software used for analysis, and provided guidance with study design and data interpretation.

**Chapter 5:** This summary chapter was written by Michael O’Neil.

## **Dedication**

I would like to dedicate this thesis to my beautiful children, my son Gavin and my daughter Avery. Confronted with unfortunate circumstances at such a young age, it was their courage, acceptance and resiliency that inspired me to persevere throughout this journey. When I began this quest they were so young and without a clue, whereas today we can joke about how I am eligible for both a student and seniors discount. I am so blessed to be your dad, and have cherished every moment raising such loving and caring children. Many sacrifices were made along the way, and I hope my efforts towards completion of this thesis justifiably represent all of your love and support. I love you both so very much.

## **Acknowledgement**

“The Long and Winding Road” was a song by The Beatles that became the groups last number one hit. Some believe the lyrics were about the unattainable, the door you never quite reach, or the road you never get to the end of. Often times the unattainable cannot be achieved for reasons out of your control, but with acceptance and the determination to change the things within your control, your destination can be reached.

There were certainly no shortage of road blocks and detours in my quest towards completion of this thesis, but fortunately I was surrounded by many amazing people providing support and encouragement. I would first like to thank my supervisor Dr. Chris Ellis for his guidance, compassion, knowledge, and life lessons that picked me up when I was running on empty and seemingly stranded. More importantly his calming demeanor allowed for me to be myself, and free to ask questions regardless of their repetitive or basic nature. I am very grateful for being granted this opportunity and having travelled down this long and winding road with Dr. Ellis.

The same can also be said for my co-supervisor Dr. Ray Guo, who has been very instrumental in providing opportunity, intellectual stimulation, and support during both my professional and academic career. I have the utmost respect for Dr. Guo, and am very grateful for having spent many years in the operating room with him in pursuit of the same common goal.

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sharing similar interests and engaging in stimulating conversations that have helped motivate and mould me as a scientist.

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## **List of Abbreviations**

2,3-DPG: 2,3 Diphosphoglycerate

ABO: Blood groups

ACT: Activated clotting time

B1: Metabolic band

B2: Endothelial band

B3: Neurogenic band

B4: Myogenic band

B5: Respiratory band

B6: Cardiac band

CPB: Cardiopulmonary Bypass

CWT: Continuous wavelet transform

ECG: Electrocardiogram

EEP: Energy equivalent pressure

ECC: Extra-corporeal circulation

FR: Flow rate

FCD: Function capillary density

GWPS: Global wavelet power spectrum

HR: Heart rate

HL: Heart-lung

Hct: Hematocrit

Hb: Hemoglobin

IVVM: Intravital videomicroscopy

ICU: Intensive care unit

I/R: Ischemia reperfusion

LDF: Laser doppler flowmetry

MAP: Mean arterial pressure

MHC: Microvascular hemoglobin content

MFI: Microvascular flow index

MIS: Minimally invasive surgery

NIRS: Near infrared spectroscopy

NO: Nitric oxide

NP: Non-Pulsatile

OPS: Orthogonal polarization spectral imaging

pH: Acid/base

pCO<sub>2</sub>: Partial pressure of carbon dioxide

pO<sub>2</sub>: Partial pressure of oxygen

PPV: Proportion of perfused vessels

P: Pulsatile

RBC: Red blood cell

RAP: Retrograde autologous priming

SDF: Sidestream dark-field imaging

SHE: Surplus hemodynamic energy

SIRS: Systemic inflammatory response syndrome

StO<sub>2</sub>: Tissue oxygen saturation

SVR: Systemic vascular resistance

VOT: Vascular occlusion test

XC: Cross clamp

$\Delta$ OD: Delta optical density

## **CHAPTER 1: INTRODUCTION**

The main function of the microcirculation is to deliver oxygen and nutrients to the tissues as well as the removal of carbon dioxide and other products of metabolism. It also serves to regulate blood flow and tissue perfusion based upon oxygen supply and demand. In cardiac surgical patients undergoing cardiopulmonary bypass (CPB), the microcirculation may become dysfunctional as a result of the surgical procedure, interventions by the perfusionist and the detrimental effects related to CPB. As a result, the ability to monitor the microvasculature in the clinical setting is of utmost importance, with the ultimate goal of returning the patient to a more normal physiological state following CPB. Given the need for improved microvascular monitoring the objective of this thesis was to investigate the microcirculation using various technologies across three scientific experiments.

### **1.1 PURPOSE AND HYPOTHESIS**

#### **Chapter 2: Microvascular Responsiveness to Pulsatile versus Non-Pulsatile Flow during Cardiopulmonary Bypass**

**Purpose:** Based on our current knowledge of pulsatile (P) flow during CPB and microcirculatory changes under certain pathological conditions, the purpose of this study was to determine whether pulsatility generated by the roller pump improves microcirculatory blood flow and tissue hemoglobin oxygen saturation (StO<sub>2</sub>) compared to non-pulsatile (NP) flow in high-risk cardiac surgical patients. We investigated whether changes in sublingual mucosal microcirculation using orthogonal polarization spectral (OPS) imaging correlate with indices of thenar muscle StO<sub>2</sub> and its recovery during vascular occlusion test (VOT) using near infrared spectroscopy (NIRS) under P and NP conditions.

**Hypothesis:** Based on previous work in the literature we hypothesize that NP flow will cause significant microvascular alterations and impaired vasoreactivity during and after CPB. The use of P flow will maintain a more normal perfusion profile due to the extra energy and shear stress generated from the roller pump thus improving tissue oxygenation and the ischemia reperfusion response associated with VOT.

### **Chapter 3: Variability in Microvascular Hemoglobin Levels during Cardiopulmonary Bypass**

**Purpose:** The purpose of this study was to apply a custom broadband continuous wave NIRS monitor to determine the feasibility of microvascular hemoglobin content (MHC) monitoring of the peripheral circulation in cardiac surgical patients undergoing NP CPB.

**Hypothesis:** The use of high-resolution isosbestic NIRS will successfully provide continuous monitoring of MHC in real time. We hypothesize that changes seen in MHC will correlate to various interventions during the course of CPB which may give insights into microvascular function.

### **Chapter 4: Continuous Wavelet Transform Analysis**

**Purpose:** The purpose of this study is to apply a continuous wavelet transform (CWT) analysis to the delta optical density ( $\Delta OD$ ) data collection to reflect the time-dependent variability of MHC at key time points during CPB, thus providing dynamic information on the microvascular regulation of oxygen supply to match tissue O<sub>2</sub> demand.

**Hypothesis:** We hypothesize that CWT will reveal changes in signal power at various frequency bands associated with microvascular origin, both within and between each patient during the course

of CPB. This information may provide insight on the dynamic variability with the microvasculature as MHC adapts to oxygen supply and demand.

The remainder of this introduction is an overview of the circulatory system, CPB and detrimental effects, along with the current technologies available in monitoring the microcirculation at the bedside during cardiac surgery.

## **1.2 CIRCULATORY SYSTEM**

### **1.2.1 Systemic Circulation**

The systemic circulation, also known as the peripheral circulation, is a continuous closed loop system providing channels through which blood travels as it delivers nutrients and oxygen to organs and tissues of the body. The vascular system consists of arteries, arterioles, capillaries, venules and veins. Arteries are thick-walled muscular vessels used to carry blood away from the heart, whereas veins are thinner walled, do not contain the musculature that arteries do, and return blood to the heart. Arterioles are very small arteries with diameters  $< 200\mu\text{m}$ . They distribute blood flow within the organ through successive diverging branches that become progressively smaller until the smallest arterioles (terminal arteriole) are reached from which the capillaries branch. The capillaries are the smallest vessels of the vascular system, with a length of approximately 0.5 to 1 mm and  $3\text{-}9\mu\text{m}$  in diameter, that penetrate every organ and tissue of the body. The fluid that does not leave the vascular system in the capillary bed is returned through the venules ( $< 300\mu\text{m}$  in diameter) to the veins and on to the heart. The microcirculation consists of all arterioles, capillaries, and venules.

### **1.2.2 Endothelium**

The channels of the vascular system are lined with flat cells called endothelium, a selectively permeable barrier regulating exchange between blood and tissues. They have pores in

their endothelial lining that expand and allow substances to pass between the blood and tissues by a process of diffusion. The endothelium plays an important role in modulating vascular tone by synthesizing and releasing a variety of vasodilator (nitric oxide, prostacyclin) and vasoconstrictor (thromboxane A<sub>2</sub>, prostaglandin H<sub>2</sub>, endothelin 1) factors, and is also important in the regulation of oxygen through the communication and integration of signals along the microvascular network (Ellis et al., 2005). Dysfunction of endothelial cells is integral to the pathogenesis of many human diseases, as they are important for normal capillary function. Within tissue, arteriolar networks control the distribution and magnitude of capillary perfusion in response to local tissue needs (Bagher & Segal, 2011). One mechanism by which tissue oxygen needs are sensed is due to a decrease in oxygen saturation within the red blood cell (RBC). This activates the release of adenosine tri-phosphate (ATP) from the RBC and the subsequent upstream signalling of the endothelium, resulting in vasodilation of arteries that control blood into arteriolar networks (Ellis et al., 2012; Ellsworth et al., 2016).

### **1.2.3 Blood**

The volume of blood present in the circulatory system is called total blood volume, which averages from 4-8 liters in the adult. Cellular elements like the RBC, white blood cells, and platelets comprise 45% of the blood volume. The number of cells in the blood at any time is constant, because the production and destruction of the cells are balanced. Plasma is a viscous fluid that occupies the other 55% of blood volume, which is comprised of 90% water and 10% solid matter such as carbohydrates, proteins, lipids, salts, vitamins and enzymes.

### **1.2.4 Red Blood Cell**

Red blood cells are flexible, biconcave discs with a diameter of 7 microns and a thickness of 2 microns, and have an average life span of approximately 120 days after being released into the circulation through pores within capillaries of the bone marrow. Adult humans have roughly 20-

30 trillion RBC's at any given time, constituting approximately 70% of all cells. Its flexibility enables the RBC to undergo changes in shape necessary for travel through the capillaries of the body. If there are too many RBC's in the circulation the condition is referred to as polycythemia. In this condition the viscosity of blood is much higher, which can increase the workload on the heart and result in circulatory problems for the patient leading to blockage of the microvasculature in organs such as the lungs and kidneys (Han et al., 2020). A low RBC count is termed anemia, indicating a lack of RBC's in the circulation to transport sufficient amounts of oxygen to the tissues. If tissue hypoperfusion exists, serum lactate levels increase secondary to decreased oxygen delivery and increased anaerobic metabolism (Ranucci, De Toffol, et al., 2006).

### **1.2.5 Hemoglobin:**

The primary function of RBC's is the transport of oxygen and carbon dioxide, a process made possible by a complex protein molecule inside the red cell called hemoglobin (Hb). During circulation through the lungs, Hb becomes saturated with oxygen, and then travels to the capillary beds of tissue and organs to release oxygen. When the concentration of Hb in the blood is low, tissues may not receive adequate amounts of oxygen and trigger an increased workload on the heart. The amount of oxygen that tissues receive depends on three factors. The amount of blood flow to the tissues, the level of Hb concentration in the blood, and the affinity of Hb for oxygen. If any one of these is abnormal the body automatically compensates, as is the case when Hb concentration is low, the heart rate (HR) will increase to deliver more oxygen to the tissues.

### **1.2.6 Role of 2,3-DPG**

The affinity of Hb for oxygen is regulated by three intracellular factors: hydrogen ion concentration or pH, carbon dioxide, and the chemical 2,3-diphosphoglycerate (2,3-DPG). If one of these three factors are not within normal limits, Hb will not release oxygen as readily to the tissues. The chemical 2,3-DPG is a molecule bound to Hb, and functions to lower hemoglobin's

affinity for oxygen so that Hb releases oxygen to the tissues more easily. Without 2,3-DPG, Hb would release little oxygen to the tissues. The discovery of the role of 2,3-DPG in oxygen release to tissues has provided new insights about stored blood. This can seriously affect patients undergoing CPB that require blood transfusion. Levels of 2,3-DPG in stored blood may decrease over time, a serious situation for patients receiving large volumes of stored blood, because the amount of oxygen then released to tissues is minimal. Adding 2,3-DPG to stored blood is of little value because the red cell membrane is impermeable to 2,3-DPG. However, rejuvenation of stored RBC's with a solution containing pyruvate, inosine, phosphate and adenine can restore levels of ATP and 2,3-DPG, shift the oxyhemoglobin dissociation curve to the right, lower the affinity of oxygen for Hb and increase oxygen release capacity (Aujla et al., 2018).

### **1.2.7 Oxy-Hemoglobin Dissociation Curve**

Oxygen transport is the movement of oxygen from the atmosphere to the cellular mitochondria. This movement depends on the availability of oxygen, cardiac output, hemoglobin level, tissue perfusion, and the ability of the tissues to extract oxygen. The oxygen-carrying capacity of hemoglobin is influenced by pH, partial pressure of carbon dioxide (PCO<sub>2</sub>), temperature, 2,3-DPG concentration, and hemoglobin concentration. The oxy-hemoglobin dissociation curve may be shifted to the right by these factors (easier dissociation of oxygen from hemoglobin) or to the left (more firmly attached oxygen). Metabolic acidosis, hypercarbia, hyperthermia, increased 2,3-DPG, and anemia shift the curve to the right. Metabolic alkalosis, hypocarbia, decreased 2,3-DPG, and hypothermia shift the curve to the left (Dickson, 1995).

### **1.2.8 Hemolysis**

Hemolysis or destruction of the RBC membrane can be a serious problem. It may be caused by a number of factors such as an immune response to transfusing ABO incompatible blood (Simmons & Savage, 2015), bacterial or viral infections causing sepsis (Effenberger-Neidnicht &

Hartmann, 2018), and red cell membrane stress during CPB caused by high suction pressures during blood recovery, vacuum assisted venous drainage, and an over-occlusive roller pump (Goksedef et al., 2012). When RBC's rupture, Hb is released into the plasma where it can no longer transport oxygen, and the cell stroma may block the microvasculature of the lung and kidneys causing these organs to fail. Increasing levels of plasma free Hb due to hemolysis can also scavenge and deplete nitric oxide (NO) levels (Gow & Stamler, 1998; Reiter et al., 2002), therefore decreasing its bioavailability leading to pulmonary and systemic vasoconstriction (Minnecci et al., 2005; Sertório et al., 2013).

### **1.3 EXTRA-CORPOREAL CIRCULATION**

#### **1.3.1 Definition**

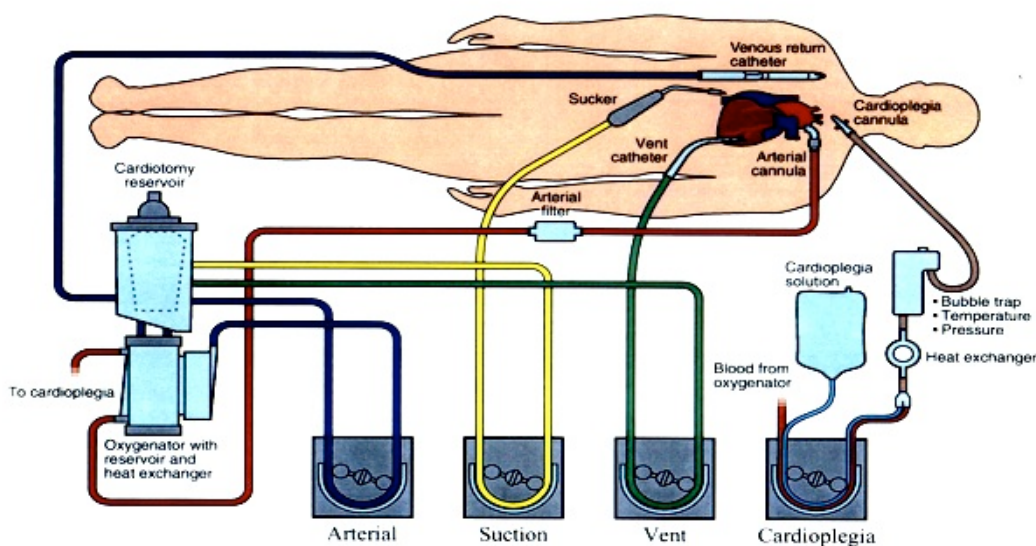
Extra-corporeal circulation (ECC) is a term used to describe the technique of circulating blood outside the body. The primary purpose of ECC is to perfuse vital organs and maintain function by ensuring adequate oxygen transport. Cardiopulmonary bypass is a form of ECC replacing function of the heart and lungs in cardiac surgical patients. A heart-lung (HL) machine and its various components are utilized to perform the act of CPB. The goal of CPB is to support the systemic circulation of the patient while allowing the surgeon the ability to operate on the heart during a chemically induced cardiac arrest.

#### **1.3.2 History of CPB**

The first successful clinical cardiac procedure utilizing the HL machine was in 1953, when Dr. John H. Gibbon made history by using a mechanical pump to maintain the circulation of a young female patient for repair of an atrial septal defect (DeBakey, 2003). The use of CPB by ECC revolutionized the approach to cardiovascular surgery and was considered to be a key component in its advancement.

### 1.3.3 Blood Path during CPB

A venous cannula placed in the right atrium of the heart diverts de-oxygenated blood by gravity into a cardiectomy reservoir to be filtered and stored (Fig.1.1). As the roller pump rotates in a counter-clockwise direction, blood is pulled from the venous reservoir and propelled forward into a heat exchanger to be either heated or cooled. Under positive pressure, blood is then passed through the membrane oxygenator to be ventilated and oxygenated. As the last line of defense, oxygenated blood is purged of any air or debris by an arterial line filter before reaching the patient. Finally, blood exits the extra-corporeal circuit via an arterial cannula placed in the ascending aorta for infusion into the systemic circulation.



**Figure 1.1:** Schematic of an Extra-Corporeal Circuit. © Terumo Cardiovascular Systems Corporation

## **1.4 CARDIOPULMONARY BYPASS CIRCUIT**

The cardiopulmonary bypass circuit includes a number of components that are necessary for circulation and oxygenation of the patient's blood. However, each device can potentially be linked to microvascular injury over the course of surgery due to their design and physiological effects on the blood.

### **1.4.1 Oxygenators**

Oxygenators provide an environment for carbon dioxide and oxygen exchange similar to that in the alveolar-pulmonary capillary unit (Fig.1.2). The total surface area of the natural lungs vary from 50 to 75 square meters. The oxygenator is a hydrophobic microporous hollow fiber membrane, made up of materials such as polymethyl pentene and polypropylene, and represents the largest source of non-endothelial surface area in the extracorporeal circuit, ranging in size between 2 to 4 square meters. Hydrophobic allows gas exchange to occur but prevents blood from passing through the membrane pores. Oxygenators are designed to optimize gas transport by minimizing the gas transport distance (diffusion distance) in the blood, maximizing the effective area for gas diffusion, and increasing the blood transit time in the oxygenator. It also very important to decrease formed element trauma by minimizing shear stresses and providing smooth blood contacting surfaces. Finally, the amount of fluid to prime the oxygenator should be as small as possible to minimize hemodilution, along with a large capacity reservoir to permit easy viewing of the blood levels at all times. Hollow fibre membrane oxygenators also have built in arterial line filters to purge any air or debris during CPB. Gaseous microemboli that forms during CPB can cause post-operative brain injury, and complications through blood vessel occlusion and neurological damage if not adequately purged from the system (Su & Undar, 2010). The pressure drop across the oxygenator is also another concern as it can increase the resistance within the circuitry. Blood forced through the oxygenator at a higher pressure can result in stress injury,

activate the inflammatory response, increase cellular damage and affect patient recovery following surgery (Guan et al., 2009). The loss in hemodynamic energy due to an increased pressure drop can decrease perfusion to the patient, as well as dampen the pulse wave when pulsatile (P) flow is applied during CPB (Undar et al., 2006).



**Figure 1.2:** Capiiox FX 25 Oxygenator and Venous Reservoir. © Terumo Cardiovascular Systems Corporation

### 1.4.2 Pump Tubing

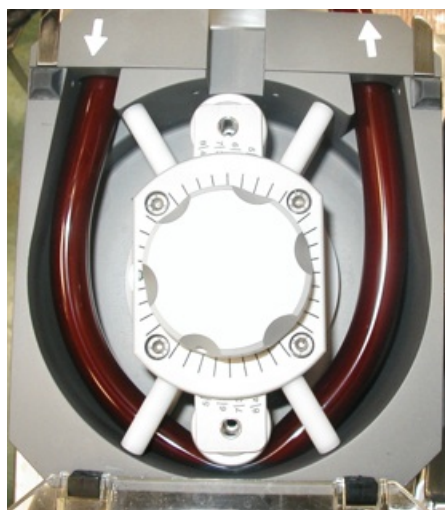
Tubing used within the extra-corporeal circuit is made up of polyvinyl chloride. Minimizing blood trauma and resistance to flow, as well as avoiding blood leakage are the primary considerations in the proper selection of tubing and connectors. With exposure of plasma proteases to nonendothelial lining of the CPB circuit, the contact system and alternate pathway of complement are activated (Kirklin et al., 1983; Westaby, 1983). This can result in vasoplegia syndrome characterized by profound vasodilation and the loss of systemic vascular resistance leading to hypotension (Omar et al., 2015). The CPB circuit has been shown to induce complement

and leukocyte activation, the release of endotoxin and inflammatory mediators such as cytokines, nitric oxide, oxygen free radicals, and platelet activating factors (Wan et al., 1997). The contact between the blood and the various artificial surfaces within the circuit can result in protein denaturation. Denatured proteins may adhere to RBC membranes and increase red cell adhesiveness, aggregate formation, and impairment of the microcirculation (Pop et al., 2002). Contact with foreign surfaces also results in the denaturation of lipoproteins, with the release of free lipid globules into the circulation that may ultimately cause vascular occlusion (Brondén et al., 2006). To reduce the side effects of contact activation to foreign surfaces, several CPB circuits with a heparin coating or surface modifying agents are available that improve in hospital and follow up outcomes (Reser et al., 2012). Heparin coated circuits in concert with minimal anticoagulation together have been documented to be safe and result in a very satisfactory clinical course (Hanedan et al., 2020).

### **1.4.3 Arterial Pumps**

There are two types of pumps commonly used to propel blood throughout the extra-corporeal circuit: kinetic pumps and positive displacement pumps. The centrifugal pump is one example of the kinetic type that essentially works as a vortex generator. The mechanism of moving fluid is by kinetic energy, as the forced rotation of an impeller device drives blood through the impeller vanes to the periphery upon expulsion. An example of the positive displacement type is the roller pump (Fig.1.3). A rotor with twin rollers placed at 180 degrees to each other is mounted within a semi-circular housing. A fixed amount of blood is displaced toward the outlet due to the rotation of the non-occlusive double roller head resulting in unidirectional flow. The roller pump also has the ability to deliver both pulsatile (P) and non-pulsatile (NP) flow, and is the most common type of pump used in most institutions. Evidence favouring one pump over the other remains controversial, as randomized clinical trials showed no differences in terms of hematological data, post-operative

blood loss, blood transfusions, neurological outcomes, and mortality (Asante-Siaw et al., 2006; Saczkowski et al., 2012). However, the majority of studies involved patients undergoing short surgical procedures that required normothermia or mild hypothermic conditions (Mlejnsky et al., 2015; Saczkowski et al., 2012). Another study targeting patients requiring prolonged CPB and deep hypothermic circulatory arrest showed that the centrifugal pump was associated with a reduction in the systemic inflammatory response syndrome (SIRS) compared to the roller pump (Mlejnsky et al., 2015). Several advantages have been documented by the use of a centrifugal pump, such as improved blood and air handling, and elimination of the risk of over-pressurizing the CPB circuit (Klein et al., 1998).



**Figure 1.3:** The arterial roller pump head capable of delivering both pulsatile (P) and non-pulsatile (NP) flow during cardiopulmonary bypass (CPB). Photo by M. O’Neil.

#### **1.4.4 Suction Pumps**

The suction pumps are used to scavenge any blood within the surgical field or chest wall during surgery and return it to the cardiotomy reservoir to be reintroduced into the circulation. Due to the blood and air interface, the use of suction during CPB surgery is associated with a pronounced SIRS and a resulting coagulopathy, as well as exacerbating the micro embolic load to the patient (Carr et al., 2020). The pericardial suction blood returned to the CPB circuit is contaminated by tissue contact, and this tissue factor is an important activator of the coagulation cascade and a principle cause of hemolysis (De Somer et al., 2002). Therefore, it is essential to avoid high negative pressures and reduce the blood to air interface during suctioning in order to minimize these side effects. The separation of cardiotomy suction blood from the CPB circuit has been shown to minimize the SIRS and hemolysis following CPB (Skrabal et al., 2006).

#### **1.4.5 Cardioplegia Pump**

The cardioplegia pump is used to inject a potassium based solution directly into the coronary arteries following aortic cross clamping. Hyperkalemia results in membrane depolarization preventing conduction of action potentials resulting in diastolic cardiac arrest (Ali et al., 2018). This chemically induced cardiac arrest allows the surgeon to operate on a motionless heart (Fig.1.4). Cardioplegia is not only for achieving cardiac arrest, but also for maintaining myocardial protection to counteract global ischemia during aortic cross clamping. Intermittent doses of cardioplegia solution are given throughout surgery to maintain arrest, provide nourishment to the cardiac muscle, and protect the myocardium from intraoperative ischemic damage and reperfusion injury (Sachl et al., 2019). A single cardioprotective strategy may not be adequate for all types of cardiac surgery. The composition of the cardioplegia solution, temperature, and the delivery techniques often change to fit the patient and surgery. The use of blood cardioplegia appears to attenuate the CPB induced SIRS which may in turn improve tissue perfusion and

oxygenation. In a study using incident dark field imaging of the sublingual microcirculation, blood cardioplegia resulted in recovery of microcirculatory changes after termination of CPB when compared to crystalloid cardioplegia (Aykut et al., 2021).



**Figure 1.4:** Quest Myocardial Protection System 2. <https://www.questmedical.com/mps/>

### 1.4.6 Vent Pumps

These pumps are used to vent or evacuate the aorta and left ventricle of blood for many reasons. In particular, cases involving aortic regurgitation can have damaging effects on the myocardium without left ventricular venting (Samuelsson et al., 2011). During cardiac arrest the purpose of left ventricular venting is to prevent distension and muscle damage of the ventricle, reduce myocardial rewarming and oxygen demand, prevent ejection of air, and to facilitate surgical exposure. It is also important to vent the aorta upon cross clamp removal, especially during open cardiac procedures such as aortic valve replacements to prevent air from ejecting to the brain.

## 1.5 CONDUCT OF CPB

The readiness to respond to both common and unusual problems that arise during surgery are the primary elements in the conduct of CPB for the perfusionist. Aside from the risk of mechanical failure, CPB is also associated with a number of stressors that can result in physiological changes impacting the microcirculation. These include varying degrees of hemodilution, hypothermia, hypotension, cardiac arrest, and the use of NP flow. Along with contact activation of blood with the artificial surfaces of the CPB circuit, periods of ischemia-reperfusion injury (I/R), and endotoxin release, these stressors can induce a SIRS. The release of inflammatory mediators and leukocyte activation can result in endothelial injury causing microvascular alterations and inadequate tissue perfusion and oxygenation (Kara et al., 2016).

The perfusionist holds many roles during the maintenance of CPB. Not only troubleshooting the HL machine and some of the ancillary equipment, but also to meet the physiological needs of the patient for a successful postoperative outcome. These roles can all be modified throughout the course of CPB depending on the surgical procedure and the particular stage of the operation. Roles of the perfusionist include controlling oxygenation, temperature, correcting acid-base imbalances, patient fluid status, anticoagulation, electrocardiogram (ECG) monitoring, delivering anesthetic gases such as isoflurane, and maintaining adequate mean arterial pressure (MAP) of the blood. Also, the administration of pharmacological agents and blood products, delivering cardioplegia to maintain cardiac arrest, charting, and communicating with the surgical team. The perfusionist makes decisions about how to maintain appropriate MAP during CPB based on instructions from the cardiac surgeon. Providing feedback to the cardiac surgeon and anesthesiologist on patient status is very important to a successful patient outcome. Information gained from microvascular monitoring could provide essential feedback to various

interventions during the course of the surgical procedure, and help to guide crucial decisions made amongst the team.

### **1.5.1 Normal Circulatory Homeostasis**

Normally, the maintenance of adequate cardiac output and oxygen delivery is driven by the metabolic needs of the body. Heart rate, ventricular filling pressures, myocardial contractility, and systemic vascular resistance (SVR) are balanced by autonomic nervous system tone and circulating catecholamine levels. Autonomic nervous system activity is modulated by the various baroreceptors and chemoreceptors in the central nervous system and periphery. Stimulation of the carotid sinus baroreceptors by local stretch evokes the reflexive changes in blood pressure and heart rate (Zeng et al., 2018). Chemoreflexes involve sensory information about pH, partial pressure of oxygen (PO<sub>2</sub>), and PCO<sub>2</sub> at carotid body chemoreceptors and at sensors in the brainstem (Guyenet & Bayliss, 2015). These changes are in turn directly related to tissue metabolism. As metabolic requirements increase, sympathetic tone is increased. Consequently, cardiac output and oxygen delivery are increased.

### **1.5.2 Circulatory Control During CPB**

However, the maintenance of circulatory control during CPB is no longer dependent on normal homeostatic mechanisms. Cardiac output on CPB is now controlled by the pump flow rate (FR), which can be set at any level desired by the perfusionist. Systemic and venous blood pressures are partially dependent on the patients autonomic tone but are easily manipulated by increasing or decreasing venous drainage to the HL machine. This can be done by altering pump FR, and the administration of vasopressors or vasodilators. Thus the circulation during CPB is controlled in large part by the perfusionist and the anesthesiologist. During general anesthesia, high dose opioids are generally used to attenuate the physiological response to surgical pain and blunt the sympathetic response to surgery (Caruso et al., 2019). However, opioids are associated

with prolonged intubation, increased risk of ventilator associated pneumonia, and longer ICU and hospital stays (Liu et al., 2019). Bi-spectral index monitoring during cardiac surgery provides an electroencephalogram parameter that can be used to guide the titration of general anesthesia and minimize the administration of opioids. During CPB the depth of anesthesia is monitored and maintained with use of an inline isoflurane vaporizer within the bypass circuit.

### **1.5.3 Heparinization**

The only non-thrombogenic surface in the vascular system is the normal human endothelial cell. The endothelial cell is unlikely to be duplicated because of its active metabolic processes, therefore current efforts to produce a complete non-thrombogenic surface during CPB are improbable. For that reason, inhibition of coagulation is essential prior to initiating CPB to avoid circuitry blood clotting and thromboembolic complications. Heparin is the drug administered for this purpose as it has very few side effects, a rapid onset, no limits to its duration of use, and is easily reversed with an antidote drug called protamine. The only contraindication to heparin use for CPB are in patients diagnosed with heparin-induced thrombocytopenia, an immune response that can lead to thrombosis and potentially fatal thromboembolic events (Arepally & Cines, 2020). These patients can be successfully managed with alternative available anticoagulants.

The commonly used heparin dose to initiate anticoagulation for CPB is 300-400 units/kg body weight. Determination of the effect of heparin intraoperatively is accomplished by measuring the activated clotting time (ACT) using a bedside point of care device. The optimal range for ACT values during CPB has not been firmly established, however maintenance of the ACT at 480 seconds and above is the recommended or safe value (Hanedan et al., 2020). However, it has been shown that a full dose heparinization protocol can expose patients to unnecessary excessive blood loss (Øvrum et al., 2011). It has been shown that a heparin coated circuit used in conjunction with decreased dosages of heparin may allow for a lower ACT target, and result in less blood

transfusions and post-operative bleeding (Hanedan et al., 2020).

#### **1.5.4 Cannulation**

The primary function of CPB is to divert blood away from the heart and return it to the systemic arterial circulation. Typically blood is drained by gravity to the HL machine by a venous cannula prior to entering the heart, and returned to the patient via an arterial cannula placed upon exiting the heart. A wide range of venous and arterial vessels can be cannulated specific to patient pathology and the type of surgery required. The venous cannulas can be placed in the inferior and superior vena cava, the right atrium, the right internal jugular vein, and the femoral vein. The arterial inflow cannula can be placed in the ascending aorta, the femoral artery, or the left subclavian artery.

Conventional CPB usually requires central cannulation of the chest involving the right atrium and aorta, whereas minimally invasive cardiac surgery procedures typically cannulate peripherally, using the femoral and right internal jugular veins and femoral artery. Therefore arterial inflow in MIS is in retrograde fashion to the aorta, whereas normal antegrade perfusion to the aorta is delivered during conventional CPB.

#### **1.5.5 Hemodilution**

Hemodilution is the standard of care in patients undergoing CPB, as pumps are no longer primed with donor blood given the risks associated with blood transfusions, as the risk of mortality increases with each unit of blood transfused to a patient (Khan et al., 2017). However, in cases of excessive hemodilution, maintaining normal hemostasis during cardiac surgery can be very challenging as this can lead to decreased levels of coagulation and fibrinolytic proteins during CPB (Abdel Aal et al., 2011). The perfusionist works diligently to avoid blood transfusion at all costs, as this has been shown to increase the frequency of organ dysfunction or failure, neurological dysfunction, wound infection, increased risk of transfusion related communicable diseases, and

increased morbidity and mortality after cardiac surgery (Koch et al., 2006; Rimpiläinen et al., 2008). However, there are cases where transfusion of blood cannot be avoided, and has been shown to be beneficial in improving the systemic circulation and oxygen carrying capacity, and improved functional capillary density (FCD) and oxygen saturation of the microcirculation (Yuruk et al., 2011). Reducing the pump priming solution can be done with the use of a miniaturized extracorporeal circuit, intended to reduce the negative effects associated with CPB such as hemodilution and the transfusion of donated blood products. The use of mini circuits is reported to decrease transfusion levels by 33% (Remadi et al., 2006), and has been documented to reduce hemodilution and improve microvascular perfusion when compared to conventional CPB circuits (Yuruk et al., 2012).

Upon initiation of conventional CPB, the pump is primed with approximately 1500 ml of crystalloid solution, sodium bicarbonate as a buffering agent, osmotic agents such as mannitol to improve renal function, and heparin to prevent further dilution of pre bypass heparin administration. Hemodilution during CPB contributes to inadequate oxygen delivery, triggering anaerobic metabolism and leading to increased levels of lactate (Ranucci, Carboni, et al., 2015). It has been demonstrated the lowest level of hematocrit (Hct) on CPB is independently correlated with moderate and severe hyperlactatemia, strengthening the hypothesis that poor oxygen delivery with consequent organ ischemia is the mechanism leading to hemodilution associated adverse outcomes (Ranucci, Carboni, et al., 2015). The lowest Hb level on bypass has also been identified as a risk factor for postoperative acute kidney injury (Karkouti, Beattie, et al., 2005; Ranucci et al., 1994), stroke (Habib et al., 2003; Karkouti, Djaiani, et al., 2005), and mortality (Habib et al., 2003). Hemoglobin oxygen saturation of arterial blood must be kept near 100% during hemodilution to prevent a further decline in blood oxygen transport. Excessive hemodilution occurs when blood flow cannot increase further to compensate for the reduction in blood oxygen content, therefore

ischemia of critical organs, and signs of anaerobic metabolism may develop. Blood oxygen transport should at least match or exceed the normal body oxygen consumption rate. If Hb is reduced by 50% below normal, blood FR must double if oxygen transport is to remain unchanged. Such a marked rise in CPB pump flow is not feasible, therefore it is important to combine hemodilution with hypothermia.

### **1.5.6 Hypothermia**

With hypothermia a decrease in metabolism occurs and oxygen consumption is reduced (Ho & Tan, 2011). This means that less oxygen is needed to match body oxygen supply to demand. Hypothermia during CPB permits the use of lower pump flows, thereby decreasing trauma to the blood and permitting a longer safe bypass period. With decreased flows less blood is returned to the heart and decreases blood pooling in the operative field, and improves visualization for the surgeon. However, the microcirculation can also be affected by hypothermia, due to an increase in blood viscosity and sludging of RBC's that can lead to reduced microcirculatory flow and organ ischemia (Sakai et al., 1988). Flow resistance in the capillary system is especially influenced by the deformability of the RBC's (Pop et al., 2002). Cardiopulmonary bypass and hypothermia have been documented to affect the deformability of RBC's (Kameneva et al., 1999). This causes aggregation and increased flow resistance in post capillary venules that result in sludging of the blood (Koenig & Ernst, 1992). This may also contribute to a diffuse cerebral microcirculatory disorder that leads to neurocognitive dysfunction seen commonly following CPB (Newman et al., 2001). Conducting CPB with a whole blood prime under hypothermic conditions has also reported an increase in systemic hypertension due to increased viscosity (Cook, 1999).

As temperature decreases, the affinity or strength of binding between oxygen and Hb is increased. A lower partial pressure of oxygen is needed to force a given amount of oxygen onto the Hb molecule. The oxygen-hemoglobin dissociation curve is shifted to the left, therefore the

release of oxygen from Hb is less efficient. Hemodilution can lower blood viscosity, counteracting the deleterious viscosity changes seen with hypothermia that are commonly employed during CPB. Oxygen carrying capacity would be decreased with hemodilution, but microcirculatory flow and oxygen delivery is improved by decreasing blood viscosity. Maintaining hypothermia during CPB may reduce cerebral oxygen demand and protect the central nervous system during times of cerebral ischemia (Cook, 1999). However, there are reports that indicate hypothermia may be associated with adverse outcomes, including impaired drug metabolism, diaphragmatic dysfunction, prolonged recovery from anesthesia, cardiac morbidity, coagulopathy, wound infections, and postoperative shivering (Insler & Sessler, 2006; Mills et al., 1997). Therefore the debate remains as to whether normothermic bypass may avoid these systemic complications, but may carry a potential risk of inadequate cerebral protection.

On the contrary, as patients are rewarmed oxygen transport must also increase, but this may be difficult to achieve when Hb levels are low, and the capacitance vessels within the systemic circulation begin to vasodilate causing mixed venous oxygen saturations to decrease. It has been documented that passive body heating resulted in an increase in FCD and total vessel density of small vessels upon interrogation of the sublingual microcirculation (Pranskunas et al., 2015). Upon rewarming there may be an increased need for fluid requirements. Often a decision has to be made whether to administer additional fluid with packed RBC's or a blood-free solution. Therefore, hemodilution may be advantageous during hypothermic conditions but undesirable during the later normothermic phases of bypass or after termination of CPB as patients become warm.

### **1.5.7 Blood Flow and Blood Pressure**

Cardiac output changes markedly with body size, therefore is frequently stated in terms of cardiac index, which is the cardiac output per square meter of body surface area. The normal cardiac index for adults is approximately  $3\text{L}/\text{m}^2/\text{min}$ , which is regulated based on metabolic needs.

However, when patients are placed on CPB this metabolic feedback system is disturbed as pump flow rates are under complete control of the perfusionist and manipulated as needed (De Somer, 2007). Since flow requirements of the patient can be impacted by non-physiologic conditions such as hemodilution and hypothermia, many perfusionists use a standard blood flow calculation based on a cardiac index of 2.2-2.8 L/m<sup>2</sup>/min. With increasing degrees of hypothermia, the patients oxygen demand also decreases, and consequently pump flow rates may be reduced significantly, which in turn can reduce the risk of blood damage from higher shear stress during CPB.

Several approaches can be used to assess the adequacy of tissue perfusion and oxygenation during CPB, such as in-line measurements of venous oxygen saturation and venous partial oxygen tension obtained from blood gas analysis. However, normal oxygenation values do not always ensure optimal perfusion to all organs, and can also be a poor predictor of anaerobic metabolism (De Somer, 2007). A study by Ranucci found that augmenting blood flow could reduce the negative relationship between hemodilution and renal failure. Their conclusion was that renal failure was more dependent on critical oxygen delivery of 272 ml/min/m<sup>2</sup> rather than the hematocrit value (Ranucci et al., 2005). Their feeling is perhaps that derived calculated parameters of blood flow can be replaced with critical oxygen delivery values to ensure optimal perfusion during CPB. However, oxygen delivery can be significantly reduced when pump flow is decreased during surgery, which results with an increased oxygen extraction to meet tissue oxygen demands. If flow is decreased for an extended period of time, the critical oxygen delivery point is reached, and the patient enters anaerobic metabolism and the production of lactic acid (Spiess, 2011).

Although acceptable flow rates are fairly well established, there is more controversy surrounding sufficient arterial blood pressure during CPB. At any given FR, there is distinct variability in arterial pressure from patient to patient. The concern is with hypotension and the adequacy of organ perfusion, with the brain and kidney being at a higher risk (Hogue et al., 2006).

Short periods of hypotension with a MAP of 50 mmHg, and transiently lower, are certainly well tolerated without compromising cerebral autoregulation (Murkin et al., 1987; Schell et al., 1993). A MAP of less than 30 mmHg is below the critical closing pressure of some vascular beds therefore increasing the risk of hypoperfusion (Magder, 2018; Sylvester et al., 1981). For most patients MAP is kept between 50-100mmHg during CPB due to cerebral blood flow being pressure dependent. There are some disadvantages of running higher MAP's during CPB, such as the inadvertent rewarming response of the cold ischemic heart secondary to an increase in noncoronary collateral flow from surrounding arteries such as the internal thoracic artery (Picichè, 2015). There is also a greater risk of cerebral hemorrhage with increasing MAP's in anticoagulated patients, and accidental pump tubing disconnections that could potentially occur due to increased resistance and elevated pressure within the bypass circuit.

Potent inhalation anesthetics may be administered during CPB with the use of a gas vaporizer placed inline with the oxygenator. Volatile anesthetic gases such as sevoflurane, isoflurane, and desflurane not only maintain depth of anesthesia and prevent awareness during surgery, but also can be used to control episodes of hypertension since they can reduce SVR by acting as vasodilators at higher concentrations. Decreasing SVR is the primary means of lowering MAP during bypass. These inhalational agents induce transient alterations in microvascular perfusion. Investigation of the effect of these gases on the sublingual microcirculation using orthogonal polarization spectral (OPS) imaging demonstrated that sevoflurane had a negative impact on the microcirculation with a decrease in perfused vessel density, proportion of perfused vessels (PPV) and microvascular flow index (MFI), whereas isoflurane decreased vascular density but increased the flow index. In the desflurane group there were no changes in vessel density but the PPV and flow index increased and returned to baseline values twenty four hours postoperatively (Özarslan et al., 2012). Volatile anesthetics possess cardioprotective properties, but it is unknown

if these effects are equal among them. The Randomized Isoflurane and Sevoflurane Comparison in Cardiac Surgery (RISCCS) trial showed that sevoflurane is non-inferior to isoflurane in prolonged ICU stay, and 30 day mortality (Jones et al., 2016). Isoflurane is the agent typically used because of its low blood solubility and prominent vasodilating effects at settings of 0.5-2.0 volume % during CPB.

### **1.5.8 Pulsatile vs Non-pulsatile Flow**

One of the major physiological derangements introduced by CPB is loss of P flow. Intuitively, it would be desirable to reproduce normal flow patterns as closely as possible while patients are undergoing CPB. Non-pulsatile flow is widely accepted as the perfusion mode of choice, however controversy exists regarding the effects of this perfusion modality on the microcirculation, especially in high risk cardiac patients undergoing prolonged cross clamp and pump times (Ji & Undar, 2006, 2007). The drawbacks to NP flow consist of lower mechanical energy transmission from the roller pump to the vascular wall that results in decreased endothelial shear stress. This in turn can diminish arterial baroreceptor activity leading to an increase in sympathetic nervous system activity, progressive vasoconstriction and worsening of peripheral blood flow (Markham et al., 2013). This decrease in mechanical energy linked to NP can also reduce shear responsive endothelial derived vasodilators such as NO (Baskurt et al., 2004), and also contribute to progressive capillary collapse, microcirculatory shunting and tissue hypoperfusion (Markham et al., 2013). Non-pulsatile perfusion has been associated with increased heterogeneity of flow, with the presence of microcirculatory shunting (O'Neil et al., 2012), and results in reduced oxygen offloading due to hyperdynamic capillary flow and tissue acidosis (Koning et al., 2014). The alteration in arterial blood flow patterns in the form of NP flow is also one of the proposed mechanisms of the SIRS. Non-pulsatile perfusion is related to organ and microvascular perfusion insufficiency, SIRS, and other complications during CPB (Gray et al.,

2015; Nasr & Rabinstein, 2015; O'Neil et al., 2012; Wang et al., 2015).

Several methods are commonly employed to produce arterial pulsations during CPB. If partial CPB is being used, venous drainage can be altered to produce some ejection of the heart, if an intra-aortic balloon pump is in place this could be used to impart P flow, and pulsations may also be produced by roller pumps themselves designed to rotate at varying speeds. The first two methods of producing pulsations are much more effective because the pulse is generated within the aorta, whereas P flow generated by the roller pump is limited by the dampening effects on the pulse within the CPB circuit, and the increased resistance to flow at the narrow opening of the aortic cannula (Undar, 2002). Although roller pumps do create effective pulsations, approximately twice as much energy is required by the pump to produce P flow.

Generation of pulsatile flow depends on the energy gradient rather than a pressure gradient. Instead of using arterial pulse pressure as a measure of pulsatility, precise quantification of pressure-flow waveforms in terms of hemodynamic energy is recommended. The energy equivalent pressure (EEP) formula of Shepard and colleagues is the best tool to quantify P and NP waveforms (Shepard et al., 1966; Undar, Frazier, et al., 1999; Undar, Masai, et al., 1999). This is based on the ratio between the area below the hemodynamic power curve and the area below the pump flow curve during each pulse cycle. It has been confirmed that energy created by P blood flow is significantly greater than that of NP blood flow (Undar, 2003). The surplus hemodynamic energy (SHE) is calculated by multiplying the difference between the EEP and MAP by 1332 (Undar et al., 2006). In a study by Undar, it was shown that SHE was significantly higher in a simulated pediatric model when using the Jostra HL 20 roller pump combined with a Capiiox membrane oxygenator at various P flow rates compared to NP flow (Undar et al., 2006). Increased blood flow energy created by P flow can affect vessel diameter, peripheral resistance, and endothelial function through shear force of varying amplitudes and frequencies, which can all play

a role in the improvement of microcirculation (Aletti et al., 2009; Ji & Undar, 2006; Shimoda et al., 1998).

The P perfusion method attempts to mimic the pulsatile nature of blood flow in the human body and deliver additional energy to the vascular endothelium (Undar, 2004) that can be transferred to the microvasculature where it may hold open capillary beds, enhance diffusion of oxygen and other substrates (Undar et al., 2006). This results in higher endothelial shear stress, augments the release of NO (Lanzarone et al., 2009; Nakano et al., 2000), lowers SVR and improves microvascular perfusion and oxygen consumption during and after CPB (Koning et al., 2012; Murkin, 2019; O'Neil et al., 2018; O'Neil et al., 2012). A recent study found that P flow resulted in increased vasodilatory endothelial NO synthase production when compared to NP flow, that also resulted in having a significant positive correlation with the amount of SHE measured during CPB (Dodonov et al., 2021). They also found reduced systemic and pulmonary vascular resistance under P flow conditions which resulted in a preserved renal glomerular filtration rate post CPB. Pulsatile flow has also been reported by others to deliver hemodynamic energy levels that improve the patency of vascular beds (Poswal et al., 2004), and increase blood flow to vital organs (Ji & Undar, 2006), including the brain (Undar et al., 2000), and kidney (H. K. Kim et al., 2005), along with attenuation of the systemic inflammatory response (Neuhof et al., 2001), and decreased incidences of negative post-operative outcome and death (Murkin et al., 1995). One mechanism for the improved outcomes associated with P perfusion may be a reduction in microcirculatory blood flow alterations, microvascular inflammation and I/R injury. However, this can only be ascertained from direct visualization and quantification of microvascular function.

### **1.5.9 Arterial Blood Gases**

The adequacy of oxygenation during CPB may be assessed by serial measurements of arterial blood gases. Blood gas and acid-base status should be checked soon after initiation of CPB

to calibrate all inline monitors, and every 30-60 minutes thereafter. Arterial oxygen tension is usually maintained between 100-300 mmHg by adjusting the fraction of inspired oxygen to the membrane oxygenator. Desired mixed venous oxygen tension values are greater than 30-40mmHg by adjusting pump flow and hemoglobin concentration. Keeping pH and CO<sub>2</sub> levels within normal limits allows for adequate oxygen transfer to the tissues and improves the efficacy of drug pharmacokinetics.

#### **1.5.10 Venous Oxygen Saturation**

Most CPB pumps include an in-line monitor of venous oxygen saturation. However, these values may be a deceptive measurement as abnormally high values could represent either excess cardiac output, shunting of blood flow among organs and within the microvasculature. Therefore, the use of in-line measurements of venous saturation is only an indirect reflection of tissue oxygenation globally, and does not inform us whether one or more organs are less than optimally perfused (De Somer, 2007). Such oxygen-derived parameters are a poor predictor of lactate accumulation during CPB (Mekontso-Dessap et al., 2002). Despite the lack of isolated regional information these monitors remain common place during cardiac surgery. As SvO<sub>2</sub> reflects a global assessment of tissue perfusion, the resuscitation of mean arterial pressure and cardiac output alone may fail to improve microvascular function, therefore detection and assessment of early derangements in microcirculatory blood flow is an important therapeutic goal (Bateman & Walley, 2005).

#### **1.5.11 Lactate**

If tissue hypoperfusion exists, serum lactate levels should increase secondary to decreased oxygen delivery and increased glycolysis. Tissues receiving little perfusion may accumulate large quantities of lactic acid that is not washed out into the circulation until regional perfusion improves at a later stage during CPB. Maintaining adequate tissue perfusion during CPB is a priority since

20% of all cardiac procedures show evidence of hyperlactatemia (Demers et al., 2000; Ranucci, Isgrò, et al., 2006). It has been shown that lactate concentration increases with prolonged duration of CPB and aortic cross clamping, as well as an increase in hemodilution (Boldt et al., 1999; Duke et al., 1997; Ranucci, Isgrò, et al., 2006). Hyperlactatemia is a recognized indicator of non-optimal tissue perfusion and is a risk factor for adverse outcomes (Boldt et al., 1999; Maillet et al., 2003; Takala et al., 1996). However, it is impossible to draw conclusion to the onset of hyperlactatemia from one blood measurement. Lactate production occurs very rapidly and under low oxygen concentration within organs, but its clearance by the liver takes much longer, especially if hampered by low hepatic blood flow during CPB (Takala et al., 1996). Therefore lactate levels drawn on CPB are a reflection of what was happening much earlier in the pump run. Unfortunately, serum lactate levels have not proven to be useful in the regular monitoring of tissue perfusion due to their variable nature, and hence lactate levels do not permit timely intervention.

## **1.6 PHASES OF CPB**

### **1.6.1 Initiation**

At commencement of CPB there is usually a decrease in systemic blood pressure due to several factors. Blood pressure is determined by cardiac output and SVR, therefore the decreased BP must be due to a fall in one or both of these parameters. Usually pump flow rates are comparable at commencement of CPB to the cardiac output prior to bypass, therefore any decreases in blood pressure due to a decreased cardiac output are unusual. The major cause of decreased blood pressure at initiation of CPB is a dramatic decrease in SVR. This phenomenon is due to a decrease in blood viscosity secondary to hemodilution instilled by the pump prime. It is also due to a decrease in vascular tone secondary to hemodilution of circulating catecholamines and

temporary hypoxemia. A lower colloid oncotic pressure due to hemodilution of plasma proteins also leads to tissue edema and hence increased fluid administration throughout the course of CPB.

### **1.6.2 Cross Clamp Application**

The aortic cross-clamp is placed on the ascending aorta to separate the systemic circulation from the outflow of the heart and its coronary vasculature. As the cardiac surgeon applies the aortic clamp, the perfusionist reduces the pump FR dramatically to decrease MAP and prevent dissection of the ascending aorta during clamping. Pump flow is then increased slowly to ensure proper clamp position and unobstructed blood flow to the patient.

### **1.6.3 Cross Clamp Removal**

As the surgical repair is complete and patient rewarmed, the aortic cross clamp is removed and blood flow to the coronary arteries is now unobstructed and is continuously infused via the arterial cannula. Often times a warm dose of blood cardioplegia is delivered to rewarm the heart prior to cross clamp removal resulting in an I/R effect. Once the clamp has been removed a period of reperfusion occurs and termination of CPB can occur once the patient becomes hemodynamically stable.

### **1.6.4 Termination**

Once the patient has completely rewarmed, acid base status is normalized, and the surgeon is confident with the surgery, the patient is then weaned from CPB. This is achieved by placing a clamp on the venous return line and slowly filling the heart while decreasing pump flow. Once the heart is full and native cardiac output is stable the pump is turned off. As the patient becomes more hemodynamically stable heparin is then reversed with protamine to control postoperative bleeding.

## **1.7 DETRIMENTAL EFFECTS OF CPB**

Despite being one of the marvels of modern medicine, CPB carries with it a price. The overall goal of returning the patient as close as possible to a normal physiological state following CPB can be challenging. Patients undergoing CPB usually experience some form of injurious insult when placed on CPB. These injuries are unavoidable, but can be minimized with the implementation of key circuit components and the proper conduct of perfusion. The following is a summary of some of the detrimental effects of CPB.

### **1.7.1 Pulmonary**

In the absence of severe cardiac dysfunction, approximately 25% of patients experience some form of pulmonary dysfunction following CPB due to poor lung mechanics or impaired gas exchange (Taggart et al., 1993). It is well documented that CPB is associated with complement and neutrophil activation (Westaby, 1983). As the lungs are removed from circulation, a common occurrence during CPB is the sequestration of neutrophils and mononuclear phagocytes in the pulmonary capillaries (Utoh et al., 1988). This can result in neutrophil-mediated pulmonary endothelial cell injury leading to increase capillary permeability, the release of oxygen free radicals, complete or partial lung collapse, and acute respiratory distress syndrome (ARDS) (Hernandez et al., 1987). Cardiac surgery patients usually are affected by several comorbidities, and the development of ARDS significantly affects their prognosis. Acute respiratory distress syndrome after cardiac surgery has a reported variable incidence of 0.4%-8.1% (Sanfilippo et al., 2022), and is also associated with multiple organ failure, which carries a mortality of 50 – 92% (Asimakopoulos et al., 1999). Capillary leakage due to changes in vascular permeability can lead to non-cardiogenic shock and result in increased intra-alveolar pressure, pulmonary vascular resistance, intrapulmonary shunt, and hypoxemia (Apostolakis et al., 2010).

### **1.7.2 Renal**

Renal dysfunction after cardiac surgery remains a common complication and an independent predictor of post-operative morbidity and mortality (Bove et al., 2009). Depending on the definition of acute kidney injury, the incidence of renal complication following CPB range between 7% to 28%, while post-operative renal replacement therapy is required in 1.4% of patients, with a much higher percentage of 5.1% for combined procedures (Mehta et al., 2006).

In most patients, renal complications arise from the direct result of CPB or hypo-perfusion post-operatively (Slogoff et al., 1990). Acute kidney injury after cardiac surgery may be triggered by the release of pro-inflammatory agents, hemodynamic changes during bypass, and predisposing patient related factors (Mariscalco et al., 2011). Cardiopulmonary bypass results in complement and platelet activation, cytokine production, and the release of oxygen free radical that cause leukocyte migration and fluid overload of the kidneys (Haddad et al., 2001). Kidneys are also sensitive to hemodynamic changes that occur during cardiac surgery. Specifically the renal medulla is prone to ischemia, due to its distinct blood circulation characterized by low oxygen tension with limited reserve (Di Tomasso et al., 2015). High-risk patients with predisposing comorbidities such as diabetes mellitus, impaired left ventricular function and advanced age are more susceptible to renal complications following CPB (Abu-Omar & Ratnatunga, 2006). Renal impairment during CPB may also result from embolic events, and red blood cell hemolysis that results in increased levels of plasma free hemoglobin.

### **1.7.3 Neurological**

The incidence of stroke occurs in approximately 1.5% of patients undergoing CPB (Borger et al., 2001). Most neurological deficits following CPB are thought to be caused by embolic events, hypoperfusion, hypoxia, and inflammation. Micro-embolisms are thought to be the most common cause of cerebral injury in adults. Large amounts of small capillary and arteriolar dilations due to

micro-emboli have been observed following CPB, and the amount seems to be proportional to bypass time (Moody et al., 1995). The ascending aorta has been identified as the main source of particulate micro-emboli (Borger et al., 2001), and surgical manipulation of the aorta and heart coincide with the appearance of emboli monitored by doppler ultrasound of the middle cerebral artery (Stump et al., 1996). The most common cause of neurological damage due to embolization is from manipulation of the aorta during arterial cannulation and application of the aortic cross clamp (Kapetanakis et al., 2004). Impaired cerebral blood flow autoregulation occurs in 20% of patients during CPB. Patients with impaired autoregulation are more likely than those with functional autoregulation to have perioperative stroke (Ono et al., 2012). Other causes of injury include extreme hypertensive episodes resulting in cerebral hemorrhage, excessively low arterial PCO<sub>2</sub> levels and vasoconstriction leading to hypo-perfusion, and extreme cooling and re-warming gradients resulting in the formation of micro-bubbles in the blood.

#### **1.7.4 Ischemia Reperfusion (I/R) Injury**

Following an ischemic insult, re-establishment of blood flow can lead to a series of functional, structural and metabolic alterations. This is called ischemia-reperfusion injury. During ischemia, a decrease in arterial blood flow to a tissue or organ can result in a reduction of oxygen and the supply of energy to its cells (Granger, 1988). In this case, stored levels of ATP within the mitochondria are utilized which are ultimately reduced to hypoxanthine. Coincidental with the degradation of ATP, xanthine dehydrogenase is converted to xanthine oxidase during periods of ischemia. With reperfusion and the reintroduction of oxygen, this enzyme catalyzes the conversion of hypoxanthine into xanthine, which can be recycled to form ATP. A by-product of this reaction is the formation of an oxygen free radical called superoxide (O<sub>2</sub><sup>-</sup>) (Granger, 1988). Superoxide is a highly reactive oxidant that causes damage to cells and can also activate local inflammatory mediators and cytokines (Badhwar et al., 2004).

Ischemia reperfusion injury is another aspect of CPB that contributes to the SIRS and postoperative morbidity and mortality. The reintroduction of oxygen into an ischemic tissue stimulates the release of additional proinflammatory mediators thereby increasing serum cytokine levels in the systemic circulation. During CPB the lungs are excluded from circulation once the aortic cross clamp is applied. Following removal of the clamp, the lungs are perfused after an extended period of ischemia. Although the systemic inflammatory response contributes to lung injury, the primary mechanism responsible for the development of lung impairment is pulmonary reperfusion injury due to a decrease in bronchial blood flow during CPB (Schlensak et al., 2002) (Dodd-o et al., 2004). The heart is also isolated from the circulation during CPB, rendering the myocardium ischemic for extended time intervals, which can result in post-ischemic myocardial dysfunction upon reperfusion (De Hert & Moerman, 2015). Central in the pathogenesis of ischemic myocardial injury is the depletion of high energy phosphates and the disturbance of normal intracellular calcium homeostasis (Elahi & Matata, 2006). Intermittent doses of cardioplegia solution are given throughout surgery to maintain arrest, provide nourishment to the cardiac muscle, and protect the myocardium from intraoperative ischemic damage and reperfusion injury.

### **1.7.5 Systemic Inflammatory Response Syndrome (SIRS)**

The SIRS is also one of the most widely studied complications of cardiac surgery, arising by the combined insult of the surgical procedure (Gu et al., 1999), and the effects of CPB (Strüber et al., 1999). The systemic inflammatory response to CPB has multiple causal factors that can lead to organ injury and post-operative morbidity (Butler et al., 1993). The response is triggered by exposure of blood to foreign surfaces of CPB, surgical trauma, the release of endotoxin and I/R injury (Wan et al., 1997). This can result in complement activation, coagulation, fibrinolysis, kallikrein and neutrophil activation (Butler et al., 1993). Following CPB, whole body systemic inflammatory response, also known as post-pump syndrome, causes disturbances in vascular tone,

fluid balance, and vascular permeability that is responsible for end organ damage following CPB (Wan et al., 1997). An excessive inflammatory response during CPB can affect organs and tissues beyond the surgical site, leading to multiple organ dysfunction, increased length of hospital stay, and death (Bennett-Guerrero et al., 1997).

Compared to SIRS due to sepsis, the importance of SIRS following CPB is less well recognized, although their mechanisms are very similar (Casey, 1993). Complement derived anaphylatoxins can stimulate the release of inflammatory mediators, such as proinflammatory cytokines interleukin (IL) 6 and tumor necrosis factor (TNF-alpha), along with activation of monocytes, neutrophils and tissue macrophages (McGuinness et al., 2008; Wan et al., 1997). This leads to endothelial cell activation and the adhesion of white blood cells to the endothelium (Eikemo et al., 2004). Subsequently, an increase in endothelial cell permeability and microvascular dysfunction occurs due to endothelial cell swelling, narrowing of capillaries, and a decrease in FCD (Hála, 2007), along with the generation of oxygen free radicals (Elahi et al., 2008). This inflammatory response to CPB represents a very moderate and manageable challenge for most patients, but can be very serious and life threatening to others. Due to the deleterious effects on the microcirculation, moderating and controlling the SIRS during CPB remains a significant concern for the perfusionist.

## **1.8 MACROHEMODYNAMIC MONITORING OF THE CARDIAC SURGICAL PATIENT**

Patients presenting for cardiac surgery require extensive monitoring because of severe and often unstable cardiovascular disease, coexisting multisystemic diseases, and the non-physiological conditions associated with CPB. Therefore cardiovascular monitors used intraoperatively are absolutely essential to the surgical team for a successful clinical outcome.

### **1.8.1 Electrocardiogram (ECG)**

The ECG not only measures heart rate but is also useful for the detection and diagnosis of dysrhythmias, conduction defects, and the diagnosis of electrolyte disturbances. In cardiac surgical patients, the detection of ischemia by ECG becomes even more important because the usual symptoms of angina cannot be communicated under general anesthesia. The ECG is monitored closely during CPB to ensure asystole to allow for an optimal surgical repair.

### **1.8.2 Non-invasive Blood Pressure Monitoring**

Non-invasive blood pressure cuff measurements are not adequate for monitoring hemodynamic parameters during a cardiac surgical procedure utilizing CPB. The reasons being that blood pressure measurements can only be captured intermittently, the potential for inaccuracies during extreme instances of hypertension and hypotension, and the loss of P flow during CPB. Non pulsatile CPB is common place in operating room theatres while the benefits of P flow during CPB is still up for debate. Because of these limitations, non-invasive monitoring are often used as adjuncts to invasive blood pressure monitoring. For example, from a research perspective, the Hutchinson Technology's InSpectra is a NIRS device that incorporates a non-invasive blood pressure cuff. This device uniquely measures StO<sub>2</sub> in the microcirculation following an I/R challenge induced by a VOT using a blood pressure cuff (O'Neil et al., 2018).

### **1.8.3 Intravascular Pressure Measurements**

The continuous direct measurement of pressures within blood vessels has become the standard of care for the cardiac surgical patient. Arterial blood pressure is often measured by placing a catheter in radial or femoral arteries, while other catheters are placed within the central circulation via the right internal jugular vein to measure the central venous or intracardiac pressures. Most cardiac surgical patients are hemodynamically unstable in the perioperative period and require close monitoring with continuous invasive measurements. Also arterial catheters

provide access and close surveillance of arterial blood gases and other blood chemistries when needed. Lastly, direct arterial pressure measurement is made possible during NP CPB as pressure exerted from continuous flow can be detected and recorded as the MAP. The central venous pressure measures right atrial pressure and is affected by circulating blood volume, venous tone, and right ventricular function. Monitoring of ventricular preload, and at least central venous pressure, is indicated during CPB surgery in which large blood losses and large volume shifts are expected. These catheters can also be used to infuse fluid or blood products, as well as vasoactive drugs. Pulmonary artery catheters can be inserted to reflect right ventricular function, pulmonary vascular resistance, left atrial filling pressures, and measurement of cardiac output pre and postoperatively.

#### **1.8.4 Transesophageal Echocardiography (TEE)**

TEE is based on the use of ultrasonic waves to obtain information on the structure and function of the heart from a probe that is placed directly into the esophagus. TEE is used intraoperatively to interrogate valve replacements and repairs, assessment of ventricular function and wall motion abnormalities, and for the evaluation of retained intracardiac air following open heart procedures.

### **1.9 MICROVASCULAR MONITORING OF THE CARDIAC SURGICAL PATIENT**

#### **1.9.1 Changes in Microcirculation and Tissue Perfusion during CPB**

Cardiac output and arterial pressure can be easily maintained at normal values during CPB. Maintenance of cardiac stability during CPB requires the obvious interplay between pump flow, and patient factors such as SVR and venous compliance. Autoregulation of blood flow to various organ beds during CPB obviously depends on blood flow and pressure requirements. Physiologically, autoregulation of blood flow refers to the ability of organ vasculature, through

neural, chemical, and direct smooth muscle effects, to regulate local resistance in order to maintain relatively constant flow despite significant changes in perfusion pressure.

The best indicator of adequate microvascular perfusion during CPB would be the measurement of partial pressure of oxygen within tissues, but this obviously cannot be done in the clinical setting. Therefore, other less precise measures of perfusion adequacy are employed. Unfortunately, increases in serum lactate levels are variable and have not proven to be a useful moment to moment indicators of tissue perfusion. Monitoring specific organ function during CPB such as the kidney can be a means of ensuring adequate tissue perfusion. Urine output is the simplest measure of renal function, and the only physical indicator of microvascular perfusion available to the perfusionist. Therefore, direct visualization of the microcirculation would be a useful monitoring tool.

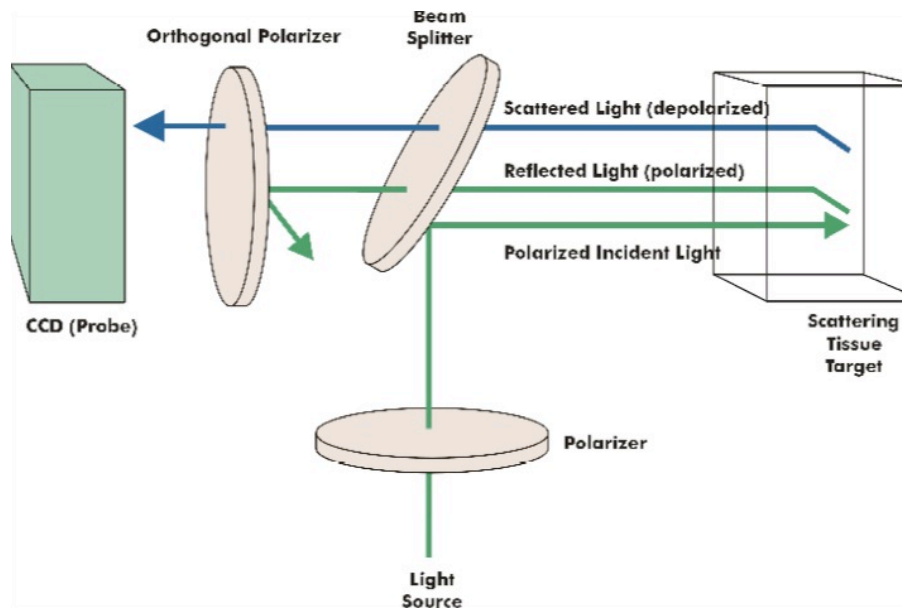
### **1.9.2 Orthogonal Polarization Spectral (OPS) Imaging**

With SIRS playing an important role in post-operative vital organ injury and dysfunction due to impaired microvascular blood flow (den Uil et al., 2008), direct visualization of perfusion abnormalities could be very useful. Investigation of the microcirculation can be performed clinically with the introduction of a bedside version of intravital video microscopy (IVVM) called Orthogonal Polarization Spectral (OPS) imaging (Fig.1.5). This device is portable, requires no image enhancing dyes, and can be applied to mucous membranes and surfaces of solid organs with the sublingual mucosal approach being the easiest and most accessible (Groner et al., 1999; Hamilton et al., 2008).



**Figure 1.5:** Orthogonal polarization spectral imaging with the use of Cytoscan A/R (Cytometrics Inc, Philadelphia, PA). <http://www.cytometrics.com>.

OPS imaging creates a virtual light source within tissues being examined. This technology applies a polarized light source and captures the depolarized reflected light (Fig.1.6) (Cerný et al., 2007; Groner et al., 1999). A high intensity lamp emits a linearly polarized light at a wavelength of 548nm after it has passed through a spectral filter and a polarizer. A beam splitter is placed inline to reflect the polarized light toward the target area where it becomes focused onto the tissue. The light is reflected back toward the source either polarized or depolarized. An orthogonal polarizer is positioned to block the reflected polarized light, and allows the deep layer of depolarized scattered light to pass through for projection onto a charged coupled device video camera. The blood becomes visible as Hb in the RBC absorbs the depolarized light and white blood cells can be seen as refringent bodies (Boerma et al., 2005). The image is passed from the camera to the OPS imaging processor unit for display onto the monitor. The final image on the screen represents an area of tissue approximately 0.5mm in diameter.



**Figure 1.6:** OPS imaging technology. Incident polarized light is reflected toward the target tissue by a beam splitter. Depolarized scattered light passes through an orthogonal-polarizer analyzer and is projected into a CCD video camera. The orthogonal polarizer eliminates reflected polarized light. <https://www.semanticscholar.org/paper/Orthogonal-polarization-spectral-imaging.-Černý-Turek/edb5a4b4d64f6e13dc3313c8c08c938be6641e9b>

OPS imaging has been validated as an effective method of investigating and obtaining quantitative measurements of the microcirculation in both animal and human studies. Contrast quality was validated in a study of images obtained from the hamster dorsal skinfold microcirculation (Gröner et al., 1999). In a comparison study with intravital fluorescence video-microscopy on striated muscle in the dorsal skinfold chamber of the awake Syrian hamster, Harris concluded that OPS imaging produced similar measurements in microvascular diameter and red blood cell velocity (Harris et al., 2000). Another study involving ten healthy male subjects reported

similar results comparing OPS images of the nailfold skin with those obtained from conventional capillary microscopy for capillary diameter and red blood cell velocity (Mathura et al., 2001). The sublingual mucosa is the preferred site for obtaining OPS images as it is readily accessible and has been documented to correlate with that of vital internal organs. In patients with septic shock, sublingual carbon dioxide levels monitored with a microelectrode sensor matched levels within the gastric mucosa using tonometry, and changes in these pressures correlated with alterations in sublingual microcirculatory blood flow (Creteur et al., 2006).

Clinical use was reported by De Backer on microvascular blood flow alterations in patients with sepsis using OPS imaging. Results showed that alterations were more severe in non-survivors and the topical application of acetylcholine reversed these alterations (De Backer et al., 2002). In another study, very similar results were reported in patients with severe heart failure and cardiogenic shock (De Backer et al., 2004). The general conclusion in both studies is that microvascular blood flow alterations may impair tissue oxygenation and participate in the development of multiple organ failure in patients with sepsis and severe heart failure. In 2004, a study by Sakr using OPS imaging reported an improvement in microcirculatory alterations in patients surviving from septic shock as opposed to non-survivors. Further microvascular impairment was documented in patients dying from multiple organ failure regardless of whether shock had resolved or not (Sakr et al., 2004).

Its application in cardiac surgery has gained popularity with research focusing mainly on the effects of CPB on the sublingual microcirculation (Bauer et al., 2007; De Backer et al., 2009; den Uil et al., 2008; O'Neil et al., 2018; O'Neil et al., 2012). Bauer et al successfully used sublingual mucosal microscopy to study changes in the microcirculation during CPB. They found no difference comparing NP perfusion velocity to baseline values, but documented a moderate reduction in FCD during the late stages of CPB (Bauer et al., 2007). Another study by De Backer

et al compared the effects of cardiac surgery with and without CPB, and the role of general anesthesia on the sublingual microcirculation. They found significant microcirculatory alterations in the proportion of perfused small vessels ( $< 20\mu\text{m}$  diameter) in both groups, with CPB further inducing a transient decrease in perfusion (De Backer et al., 2009). An alternative technology adapted from OPS imaging called Sidestream Dark Field (SDF) imaging has also been utilized within the cardiac surgery domain. Den Uil et al used SDF imaging to investigate the sublingual microcirculation during CPB assisted cardiac surgery (den Uil et al., 2008). A significant decrease in the microvascular flow index was detected in medium sized blood vessels during the early stage of CPB with subsequent recovery to baseline values post-operatively. Another study by O'Neil addressed a critical issue in the controversy surrounding the benefits of P flow during CPB. At the core of the debate is the lack of evidence supporting the benefits of P perfusion on the microcirculation. Using OPS imaging, their group presented the first direct visual evidence of the benefits of P versus NP flow on microvascular perfusion during CPB. Results indicate a reduction in the inflammatory response and the maintenance of normal microvascular perfusion in the P group during and after CPB (O'Neil et al., 2012).

### **1.9.3 Near Infrared Spectroscopy (NIRS)**

The introduction of pulse oximetry in the early 1980's revolutionized management of monitored patients by providing a real time, non-invasive and continuous measurement of blood oxygen saturation. By using specific wavelengths of near infrared light preferentially absorbed by oxygenated hemoglobin and gated to arterial pulsation, it provides early warning of hypoxemia such that pulse oximetry is now the single most widely applied form of clinical monitoring. The introduction of cerebral oximetry in mid 1990's utilized similar near infrared spectroscopy (NIRS) principles, however, it is not gated to arterial pulsation thus enables measurement of brain tissue hemoglobin oxygen saturation (StO<sub>2</sub>) in pulseless situations like CPB (Murkin et al., 2007). The

NIRS monitor was an important development for assessing the adequacy of regional tissue perfusion in a variety of disease states (Fig.1.7).

NIRS provides a non-invasive measurement of chromophores in tissues by way of light absorption within the NIR range. A deeper photon penetration can be achieved in the range of 650-950 nm where chromophores such as oxy-hemoglobin, deoxy-hemoglobin, and water are most common (Jo Bsis-Vandervliet, 1999). Using different wavelengths, the proportion of light absorbed by each chromophore can be determined and related to their various concentrations, thus providing a measurement such as tissue oxygen saturation (StO<sub>2</sub>). The incident light delivered to tissue can be affected by both absorption and scatter before it returns to the spectrometer.

This technology is based on the Beer-Lambert law, which states that a proportion of light transmitted through a solution containing a colored compound (chromophore) is absorbed by the compound. As a result, the intensity of the emerging light is reduced. The relationship between the absorption and concentration of a chromophore is described by the Beer-Lambert equation:

$$A = \log (I_0/I) = e * c * d$$

Where A is the absorption of light, expressed as optical density (log of the ratio of the intensities of incident ( $I_0$ ) and transmitted (I) light), c the chromophore concentration, e its extinction coefficient, and d the width (optical path length) through the solution or medium. The Beer-Lambert law assumes that tissue scatter does not change despite the most dominant interaction in tissue. To assess the impact of scatter, a modified Beer-Lambert law was developed. The original equation was adapted to describe intensity lost due to light scattering by accounting for the inverse distance that a photon must travel before it is scattered as opposed to being absorbed (Delpy et al., 1988). The scatter term also depends on the concentration of RBC's, and therefore will change

due to hemodilution upon initiation of CPB. However, the magnitude of change in light incidence is greater due to the absorption of hemoglobin (Hb) rather than that of scattering. Tissue oxygenation is the result of a complex interaction of perfusion, arterial oxygen tension, Hb level and dissociation conditions, and local oxygen consumption. Tissue oximetry with near-infrared light combines measurements of arterial, venous, and capillary blood, depending on the absorbance of different wavelengths of near infrared light. Unlike pulse oximetry, NIRS does not involve detection of pulsatile flow but relies entirely on the Beer-Lambert law that relates the concentration of a substance to its light absorption (Murkin & Arango, 2009).

Due to lung dysfunction following CPB, a decreased ratio between the arterial oxygen tension ( $\text{PaO}_2$ ) and the inspired oxygen fraction ( $\text{FiO}_2$ ) less than 200 mmHg can result in tissue hypoxia in 30.6% of cardiac patients (Ranucci et al., 2014). The rate of tissue hypoxia has also been reported to be as high as 49.5% in patients following an aortic dissection (Wang et al., 2013). These concerns have led to a growing interest in non-invasive monitoring of regional perfusion as an adjunct to standard hemodynamic parameters. The use of NIRS in the operating room can be utilized to better understand the complex pathophysiology of acute circulatory failure and aid in earlier detection of tissue hypoperfusion.

Studies have evolved in addressing tissues susceptible to hypoperfusion, with cerebral oxygenation being the most common application of NIRS in cardiac surgery patients. During cardiac surgery with CPB, undetected episodes of cerebral hypoperfusion predisposes the patient to an increased risk of postoperative neurological complications. NIRS is a promising technique for preventing these complications following surgical procedures (Murkin & Arango, 2009; Taillefer & Denault, 2005), however its routine application is still not part of standard clinical practice. This is partly due to the fact that clear evidence for a defined desaturation threshold requiring intervention during CPB is still lacking. Neuromonitoring with newer technology

combining diffuse correlation spectroscopy (DCS) and broadband NIRS can provide real-time assessment of cerebral perfusion and metabolism, alerting clinicians to relevant hemodynamic events before brain injury occurs (Rajaram et al., 2020). Broadband NIRS can measure the oxidative state of cytochrome c oxidase reflecting cerebral energy metabolism (Bale et al., 2016), while DCS provides continuous cerebral blood flow monitoring by tracking RBC's in the microvasculature (He et al., 2018; Milej et al., 2020).

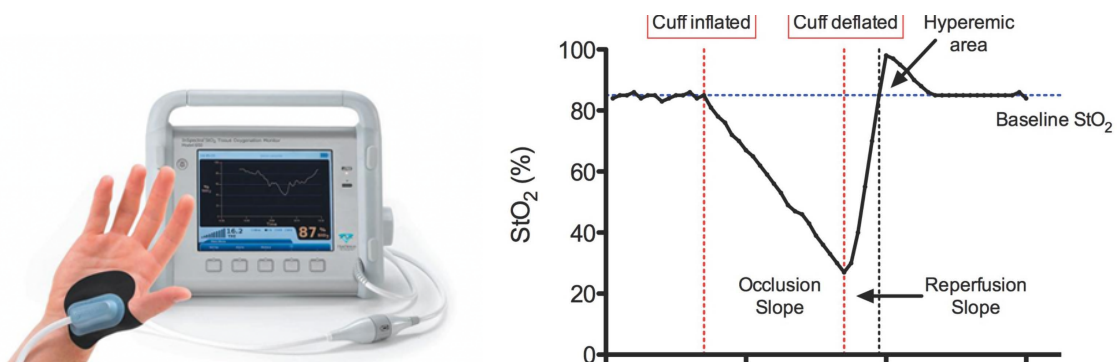
Some studies have failed to prove that intraoperative interventions to correct cerebral oxygen saturations lead to improved neurological outcome (Zheng et al., 2013). Part of the explanation can be linked to the fact that cerebral oximetry does not take into account the cerebral autoregulatory activity. Cerebral autoregulation is the intrinsic system that maintains adequate cerebral blood flow at a wide range of different perfusion pressures. Patients can be at increased risk of cerebral edema and hemorrhage in cases where oximetry readings can be close to baseline values while the cerebral autoregulation system is severely disturbed. Maintaining arterial blood pressure and avoiding large fluctuations in hemodynamic parameters during CPB will preserve autoregulation. Therefore, monitoring autoregulation rather than focusing solely on cerebral oxygen saturation measurements may optimize patient care during CPB.



**Figure 1.7:** INVOS 5100C Cerebral/Somatic Oximeter. <https://www.medtronic.com/covidien/en-ca/products/cerebral-somatic-oximetry/invos-5100c-cerebral-somatic-oximeter.html>

### 1.9.4 Vascular Occlusion Test (VOT) and NIRS

NIRS uses specific, calibrated wavelengths of near-infrared light to noninvasively illuminate the tissue below a sensor placed on the skin. These wavelengths of light scatter in the tissue and are absorbed differently dependent on the amount of oxygen bound to hemoglobin in the microcirculation. Light that is not absorbed is returned as an optical signal and analyzed to produce a ratio of oxygenated hemoglobin to total hemoglobin, expressed as percent  $\text{StO}_2$ . The InSpectra  $\text{StO}_2$  VOT research system (Fig.1.8: left panel) is used to invoke a quantifiable measure of microvascular responsiveness to an I/R intervention using NIRS. Following baseline  $\text{StO}_2$  recording, a pneumatic blood pressure cuff placed over the brachial artery is inflated to 50mmHg above patient systolic blood pressure. Once  $\text{StO}_2$  reaches 40% the cuff is rapidly deflated while  $\text{StO}_2$  measurements continue to be recorded back to baseline. Calculations derived from the VOT include: baseline  $\text{StO}_2$ ; descending occlusion slope; ascending reperfusion slope; and the hyperemic response (Fig.1.8: right panel).



**Figure 1.8:** (Left) Hutchinson InSpectra oxygen saturation ( $\text{StO}_2$ ) monitor. (Right) Vascular occlusion testing graph. Occlusion/ischemic slope is indicative of metabolic rate. Reperfusion slope is characteristic of microvascular responsiveness / vasoreactivity.

<https://pubmed.ncbi.nlm.nih.gov/29391150/>

The fundamental concept underlying the VOT resides in its ability to challenge the distal microcirculation by means of a calibrated I/R stimulus to assess and quantify the integrity of microcirculatory responses. In patients with microcirculatory dysfunction, reactive hyperemia is impaired, and the slope of StO<sub>2</sub> recovery during VOT can be used to quantify such an impairment (Doerschug et al., 2007). The application of NIRS technology in conjunction with VOT to measure peripheral StO<sub>2</sub> of the thenar muscle has been examined in a variety of critical illness settings, including septic and hypovolemic shock (Creteur, 2008; Nanas et al., 2009; Pareznik et al., 2006; Skarda et al., 2007). In critically ill patients with severe sepsis, the VOT reperfusion slope was significantly impaired in comparison with non-septic patients, and is also more pronounced in the presence of shock, as well as being a predictor of organ dysfunction and mortality (Creteur et al., 2007; Doerschug et al., 2007). For patients in the early phase of multiple organ failure, it was also shown that impaired values of VOT such as a decrease in reperfusion slope was associated with an increase in 28 day mortality (Haertel et al., 2019).

During cardiac surgery, prolonged exposure to CPB is associated with abnormal vasomotor responses and subsequent end organ dysfunction (Ruel et al., 2004). The use of NIRS/VOT was used in pilot study to determine the impact of NP CPB on vasoreactivity of the microcirculation. Results showed a significant decrease in reperfusion slope during CPB when compared to pre and post bypass, as well as a progressive decline with duration of CPB (Smith & Murkin, 2014). Whether differences in reperfusion slopes have clinical significance similar to sepsis is unknown, the continuous decline may reflect post-operative organ dysfunction known to occur in proportion to increased bypass time (Salis et al., 2008). Another study also reported the effects of general anesthesia in cardiac surgery patients using the VOT. Compared to baseline they observed a 30% decrease in metabolic rate (occlusion slope) and 40% reduction in microvascular responsiveness (reperfusion slope) (Bernet et al., 2011). In a study using VOT

comparing P versus NP flow during CPB, there were no differences found between groups in baseline StO<sub>2</sub> and consumption slope, however reperfusion slope was substantially different at 24 hours following CPB indicating improved microvascular responsiveness which may reflect attenuation of the SIRS and I/R (O'Neil et al., 2018).

## **1.10 MOTIVATION FOR THE STUDY**

The microcirculation is an elaborate network of microvessels that function to deliver adequate amounts of oxygen to tissues that are essential for proper organ function. When these vessels become dysfunctional a mismatch occurs between oxygen delivery and tissue demand. Early onset of microcirculatory dysfunction during the course of sepsis and heart failure has been linked to an increased rate of mortality (De Backer et al., 2013; De Backer et al., 2010; den Uil et al., 2010). In certain disease states the microcirculation may not improve despite successful therapeutic interventions aimed at improving blood pressure or macrohemodynamics (De Backer et al., 2013; De Backer et al., 2010). This is called a loss of hemodynamic coherence, which can result in progressive tissue hypoperfusion despite optimization of macrohemodynamics (Ince, 2015). These microvascular observations were made possible with use of real time analysis of the microcirculation at the bedside using devices such as OPS and SDF imaging, which has allowed researchers the ability to recognize and quantify microcirculatory dysfunction (Groner et al., 1999). Having this ability to interrogate the microcirculation during certain disease states is extremely beneficial considering the disconnect between micro and macro hemodynamics.

Although CPB makes it possible to repair damage to the heart, at the same time it exposes the patient to other organ dysfunction due to I/R injury and the SIRS. Cardiopulmonary bypass can result in microvascular injury similar to that associated with sepsis including vascular tone, fluid balance and vascular permeability that is responsible for end organ damage (Wan et al., 1997).

Compared to SIRS due to sepsis, the importance of SIRS following CPB is less well recognized, although their mechanisms are very similar (Casey, 1993). Impaired microvascular perfusion resulting from SIRS during CPB may lead to the development of post-operative complications such as multiple organ failure (Murphy & Angelini, 2004). Following cardiac surgery, the prolonged treatment of multiple organ failure in the intensive care unit may lead to excess mortality and cardiovascular morbidity after discharge (Mazzoni et al., 2006). This inflammatory response to CPB represents a very moderate and manageable challenge for most patients, but can be very serious and life threatening to others. Due to the deleterious effects on the microcirculation, moderating and controlling the SIRS during CPB remains a significant concern for the perfusionist.

To help guide us during CPB we rely heavily on cardiac monitors that reflect global perfusion status or macro-hemodynamics. However, given the effects of the SIRS, monitoring the microcirculation is of utmost importance to help identify and alleviate the deleterious effects of bypass. Bedside evaluation of the microcirculation during CPB has evolved over the years, using hand held IVVM imaging similar to that used in sepsis and critical illness. Studies linked to CPB have documented microvascular dysfunction despite the presence of normal macrohemodynamic similar to other critical illness disease states (Bauer et al., 2007; De Backer et al., 2009; den Uil et al., 2008; Koning et al., 2012; O'Neil et al., 2012). Since microvascular alterations can exist in the presence of normal macrovascular hemodynamics, it can become very difficult to identify and treat during cardiac surgery without proper monitoring of the microcirculation.

The use of handheld sublingual videomicroscopy devices, along with NIRS and StO<sub>2</sub> derived vascular occlusion tests, provide only a functional snapshot in time of the microcirculation, therefore a need exists for improved microvascular monitors that can continuously track dynamic changes in the microvasculature over time. A real time monitor that can reflect the time-dependent variability of microvascular perfusion and provide detailed information of oxygen supply and

demand. Such a custom monitor was designed and built in our lab with the assistance of members of the bioptics group in our department using NIRS technology that reflected the time-dependent variability of microvascular hemoglobin content (MHC). This monitor was subsequently used in a pilot study of ICU patients (Mendelson et al., 2021) which successfully demonstrated that MHC monitoring in the ICU can provide continuous physiological data for assessing peripheral perfusion in skeletal muscle. The ability to detect microvascular perfusion with this device was very encouraging, which lead us to conduct a simultaneous pilot study in cardiac surgery patients undergoing CPB.

Considering the mechanisms of I/R injury and the SIRS linked to CPB and their effects on the microcirculation, the objective of this thesis was to investigate the microvasculature using real time monitoring techniques.

In our first study, OPS imaging, NIRS and the VOT are being evaluated as reliable, non-invasive measures of poor tissue perfusion and microcirculatory dysfunction in cardiac surgery patients undergoing P and NP perfusion. The purpose of the study is to determine whether pulsatility generated by the roller pump during CPB improves microcirculatory blood flow and tissue oxygen saturation compared to NP flow in high risk patients. We aim to validate whether changes in sublingual mucosal microcirculation using OPS imaging correlate with indices of microvascular function as measured by NIRS following a VOT under both perfusion modalities.

Our subsequent study involved a less cumbersome technology using isosbestic NIRS to measure the temporal changes in MH) of skeletal muscle during NP CPB, which can be used as a surrogate for RBC flow within the microcirculation.

In our third study we applied a continuous wavelet transform (CWT) analysis to our isosbestic NIRS time series data to detect power within various frequency bands over the course of the surgical procedure. The goal of CWT analysis was to reflect the time-dependent variability

in MHC which may provide important autoregulatory information due to oxygen supply and demand.

The clinical implications of real time monitoring of the microcirculation would permit earlier, non-invasive detection of significant physiological derangements, and allow for more accurate and timely therapeutic interventions in the attenuation of I/R injury and the SIR response linked to CPB. Therefore, a resuscitation strategy of the microcirculation could be performed based on a bedside monitoring device, potentially reducing the risk of multiple organ failure following cardiac surgery, length of hospital stay and associated health care costs.

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## **CHAPTER 2: MICROVASCULAR RESPONSIVENESS TO PULSATILE AND NON-PULSATILE FLOW DURING CARDIOPULMONARY BYPASS**

### **2.1 INTRODUCTION**

Macrohemodynamics is very limited in predicting the state of the microcirculation (Shoemaker & Czer, 1979). Despite optimization in global hemodynamics, an inverse relationship may exist with microcirculatory blood flow. This is well documented in septic patients, as microvascular perfusion abnormalities have been linked to a systemic inflammatory response (SIRS) (De Backer et al., 2002). Microcirculatory dysfunction has also been reported in cardiac surgical patients undergoing CPB (Bauer et al., 2007; De Backer et al., 2009; O'Neil et al., 2012). The switch to a non-physiological pulse may potentiate the SIRS leading to impaired microvascular blood flow, organ injury and postoperative morbidity (Ji & Undar, 2006).

The significance of the pulse during CPB continues to be debated. Adding to the controversy is whether perfusion mode during CPB has any beneficial effect on the microcirculation (Ji & Undar, 2007). This can be ascertained from direct visualization and quantification of microvascular function with technologies such as orthogonal polarization spectral (OPS) imaging, near-infrared spectroscopy (NIRS) and vascular occlusion testing (VOT). OPS imaging is a portable version of intra-vital videomicroscopy that can be applied to mucous membranes and surfaces of solid organs with the sublingual mucosal approach being the easiest and most accessible. OPS imaging ultimately reflects the capacity of oxygen delivery to the tissues by determination of red blood cell velocity and functional capillary density in the microcirculation (Groner et al., 1999). Hutchinson Technology's InSpectra is a NIRS device that uniquely measures tissue hemoglobin oxygen saturation (StO<sub>2</sub>) in the microcirculation following an ischemia reperfusion (I/R) challenge induced by VOT.

Based on our current knowledge of pulsatile flow and microcirculatory changes under certain pathological conditions, the purpose of this study was to determine whether pulsatility generated by the roller pump during CPB improves microcirculatory blood flow and StO<sub>2</sub> compared to non-pulsatile flow in high-risk cardiac surgical patients. We investigated whether changes in sublingual mucosal microcirculation using OPS imaging correlate with indices of thenar muscle StO<sub>2</sub> and its recovery during VOT using NIRS under pulsatile and non-pulsatile conditions.

## **2.2 METHODS**

### **Study Design**

The local Research Ethics Board approved this prospective, randomized cohort study of twenty high-risk cardiac surgical patients undergoing CPB at University Hospital, London Health Sciences Centre, London, Ontario. All adult patients admitted for elective or urgent cardiac surgery including: requirement for concomitant coronary artery bypass grafting with any valve repair/replacement, combined valves, and aortic replacement procedures were invited in this study. Exclusion criteria included patients requiring pre-operative intra-aortic balloon pump insertion, need for axillary or femoral arterial cannulation, and cases where pre-operative consent was unattainable. Once informed written consent was obtained, an intra-operative sealed opaque envelope randomization protocol was utilized to assign study participants to pulsatile or non-pulsatile group.

### **Data Collection**

Patient demographics including age, sex, height, weight, body surface area, and pre-operative risk factors were obtained for all participants. Hemodynamic monitoring for heart rate, mean arterial pressure, temperature, and cardiac index were recorded throughout the study. Arterial

pulse pressure during CPB was noted for all patients randomized to the pulsatile group (note: pulse pressure for patients in the non-pulsatile group was 0). Arterial blood samples were drawn at various time intervals for analysis of acid/base and oxygenation status, hemoglobin, lactate, and serum creatinine levels.

### **Extra-Corporeal Circuit**

CPB was performed using the Jostra HL 20 heart-lung machine (Maquet-Dynamed Inc.). The extra-corporeal circuit was composed of: x-coating custom tubing pack (Terumo Cardiovascular Systems), capiox SX25 membrane oxygenator (Terumo Cardiovascular Systems), quart arterial line filter (Maquet-Dynamed Inc.), and microplegia using the Myocardial Protection System (Quest Medical Inc.). Arterial inflow was achieved using a Sarns 24Fr. metal-tip aortic cannula. The circuit length was kept to a minimum to reduce pressure fluctuations and minimize energy loss during CPB. A standard priming solution of Lactated Ringers was used in all cases.

### **Conduct of Cardiopulmonary Bypass**

Patients received a bolus of heparin (400u/kg) pre-bypass to achieve an activated clotting time greater than 480 seconds. During CPB, patients were cooled to a systemic temperature of 32-34°C with active re-warming to 36-37°C prior to termination of bypass. A minimum cardiac index of 2.2-2.4 L/min/m<sup>2</sup>, and mean arterial pressure maintained greater than 60mmHg was standard for both groups during the course of CPB. Following aortic cross-clamping the systemic perfusion pattern was converted to pulsatile flow at a rate of 70 beats/min for patients randomized into the pulsatile group at the following set parameters: base flow=20, start time=20, and stop time=60. These settings were chosen to closely approximate the normal physiological cardiac cycle. A pulse pressure greater than 25 mmHg was maintained for the duration of CPB. In contrast, the

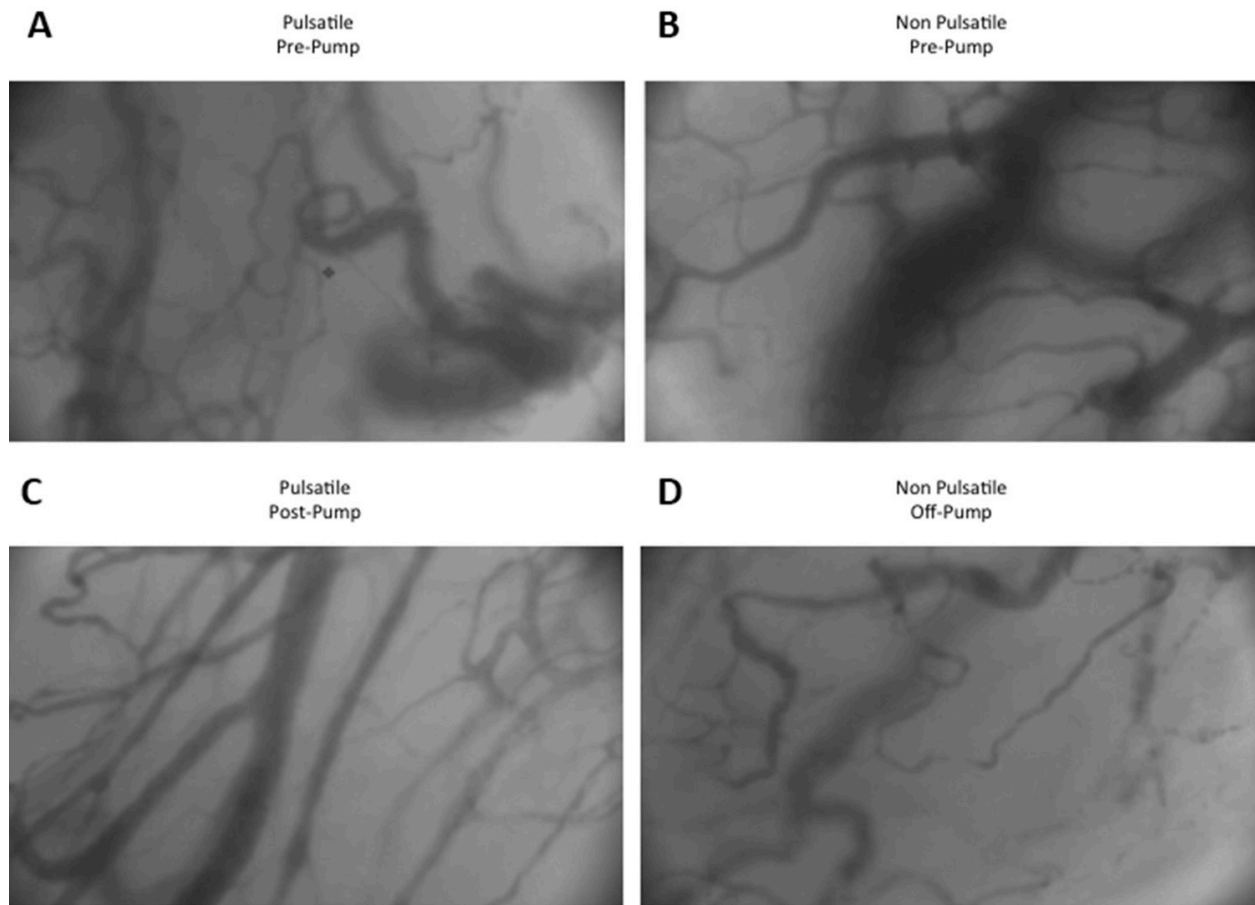
non-pulsatile group received continuous linear blood flow. Anesthetic maintenance during CPB was managed with inline sevoflurane. Weaning from CPB included the administration of vasopressors and inotropic support medications considered necessary by the anesthesiologist.

### OPS Imaging

OPS imaging of the sublingual microcirculation was performed using the Cytoscan-A/R (Cytometrics Inc., Philadelphia, PA) (Fig2.1, Figs 2.2A-D)). Intra-vital videomicroscopy of the microcirculation was achieved using 10x objective (final magnification=450X). Linearly polarized light is emitted from the objective probe and deflected toward the target tissue by a beam splitter. The deep layer of depolarized scattered light is then reflected, and passed through the orthogonal polarizing analyzer for projection into a charged coupled device video camera. Simultaneously, light that is not depolarized is reflected and removed by the orthogonal polarizer. The visualized field of view represents an area of 0.5mm.



**Fig 2.1.** (Left) Orthogonal polarization spectral imaging with the use of Cytoscan A/R (Cytometrics Inc, Philadelphia, PA). (Right) Hand held probe for imaging of the sublingual vascular network. <http://www.cytometrics.com>.

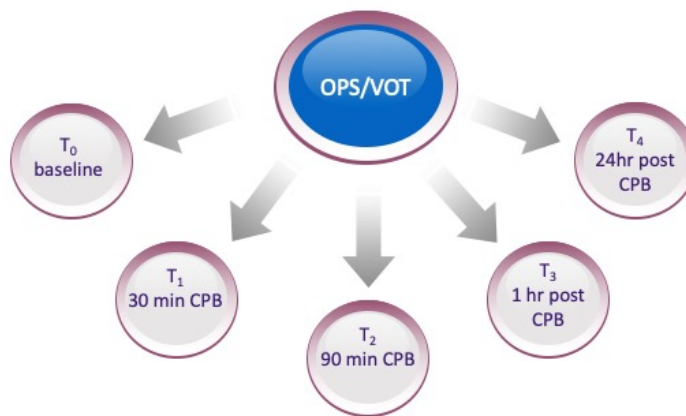


**Fig 2.2.** Sublingual microcirculatory images. (A, B) Baseline images similar in both groups. (C, D) Preservation of normal microvascular perfusion in the pulsatile group versus the non-pulsatile group after cardiopulmonary bypass.

### Microvideoscopic Measurements and Analysis

The sublingual microvascular network was imaged by gently applying the probe on the lateral side of tongue with minimal force to avoid both pressure and movement artifacts. Thirty-second recordings from 3 adjacent areas were captured at various time points (Fig2.3). Three equidistant horizontal and three equidistant vertical lines are drawn on the screen. Vessel density was calculated as the number of vessels crossing the lines divided by the total length of the lines.

All discernible microvessels  $<20\mu\text{m}$  in diameter were scored based on blood flow characteristics: 0=absent flow, 1=slow continuous/intermittent ( $<250\mu\text{m}/\text{sec}$ ), 2=normal ( $250\text{--}350\ \mu\text{m}/\text{sec}$ ), 3=hyperdynamic (immeasurably fast). At each time point, the number of vessels with the same score was divided by the total number of vessels and reported as the PPV%. All images were digitally stored and analyzed off-line semi-quantitatively.



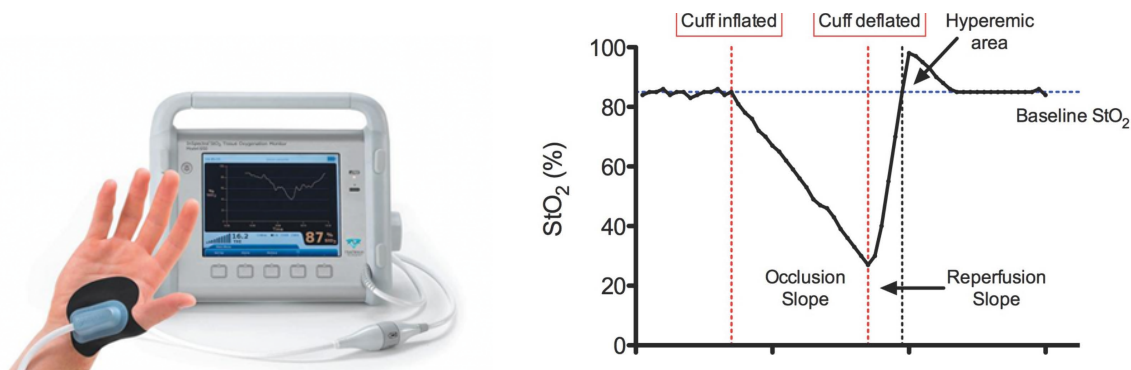
**Fig 2.3.** Orthogonal polarization spectral (OPS) imaging and vascular occlusion testing (VOT) were done at the following time points: baseline after anesthesia (T0), 30 minutes on cardiopulmonary bypass (CPB) (T1), 90 minutes on CPB (T2), 1 hour after CPB (T3), and 24 hours after CPB (T4).

### Near Infrared Spectroscopy

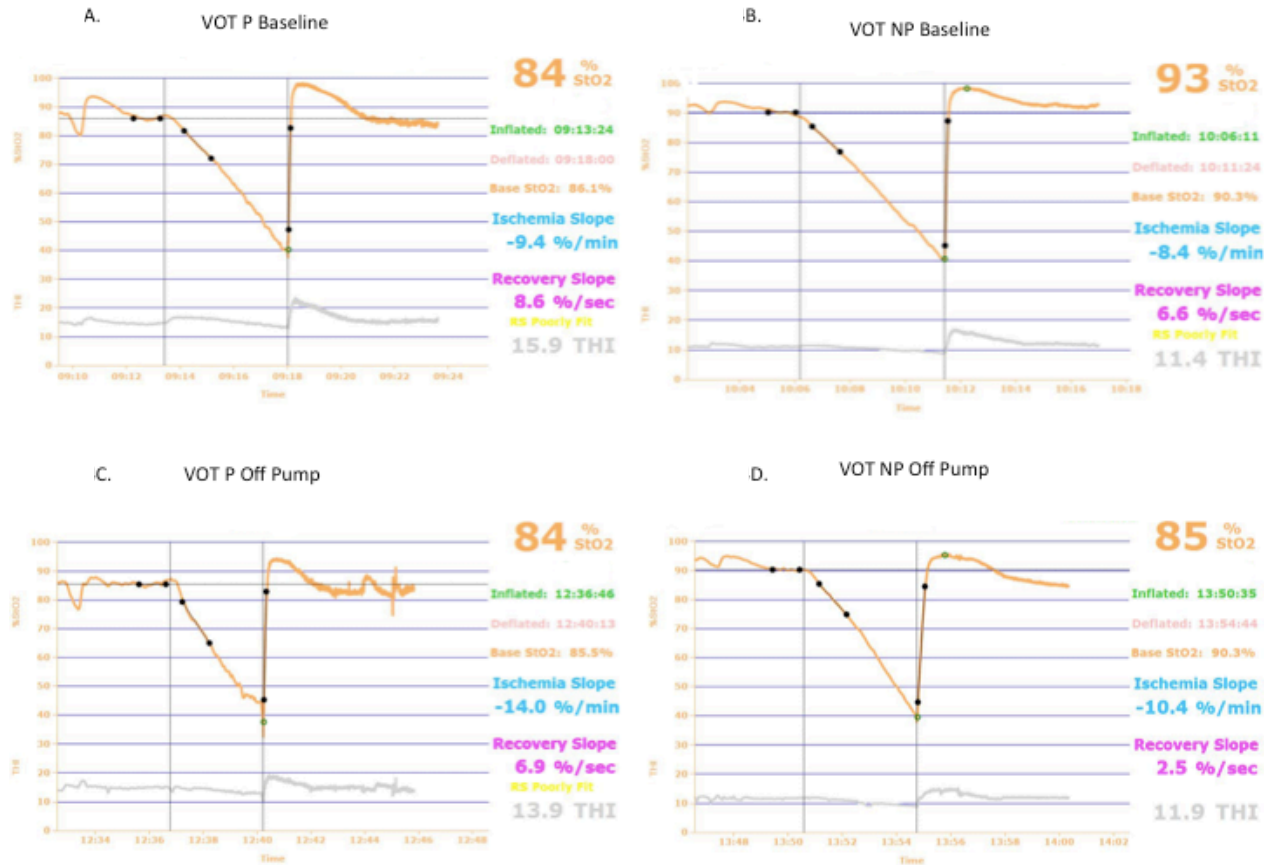
NIRS uses specific, calibrated wavelengths of near-infrared light to noninvasively illuminate the tissue below a sensor placed on the skin. These wavelengths of light scatter in the tissue and are absorbed differently dependent on the amount of oxygen attached to hemoglobin in the microcirculation. Light that is not absorbed is returned as an optical signal and analyzed to produce a ratio of oxygenated hemoglobin to total hemoglobin, expressed as percent StO<sub>2</sub>.

## Vascular Occlusion Test

The InSpectra StO<sub>2</sub> VOT research system (Fig 2.4: left panel) was used to invoke a quantifiable measure of microvascular responsiveness to an I/R intervention using NIRS at each time point. Following baseline StO<sub>2</sub> recording, a pneumatic blood pressure cuff placed over the brachial artery is inflated to 50mmHg above patient systolic blood pressure. Once StO<sub>2</sub> reaches 40% the cuff is rapidly deflated while StO<sub>2</sub> measurements continue to be recorded back to baseline. Calculations derived from the VOT include: baseline StO<sub>2</sub>; descending occlusion slope; ascending reperfusion slope; and the hyperemic response (Fig 2.4: right panel, Figs 2.5A-D).



**Fig 2.4.** (Left) Hutchinson InSpectra oxygen saturation monitor. (Right) Vascular occlusion testing graph. Occlusion/ischemic slope is indicative of metabolic rate. Reperfusion slope is characteristic of microvascular responsiveness/vasoreactivity. <https://pubmed.ncbi.nlm.nih.gov/29391150/>



**Fig 2.5.** Microvascular responsiveness to an ischemia–reperfusion intervention with the use of near-infrared spectroscopy. (A, B) Baseline measurements similar in both groups. (C, D) Improved reperfusion/vasoreactivity after cardiopulmonary bypass in the pulsatile (P) group versus the non-pulsatile (NP) group. (StO2 . tissue oxygen saturation; THI . total hemoglobin index; VOT . vascular occlusion testing.)

### Statistical Analysis

In all tables, the data are presented as mean±standard deviation, median {25<sup>th</sup>-75<sup>th</sup> percentile}, or absolute number percentages as specified. Group means were compared using Two-way ANOVA *post-hoc* test when comparing multiple variables. One-way ANOVA with Kruskal-

Wallis multiple comparison *post-hoc* tests was used when changes with time and perfusion mode did not interact. When two values were compared, a two-tailed Students *t*-test or Fishers exact test was used as specified. Results are considered significant when corresponding *P*-value was  $< 0.05$ . All statistical tests were performed using GraphPad prism6 (GraphPad Software, San Diego, CA, USA).

## **2.3 RESULTS**

### **Demographic and Intra-operative Data**

There were no significant differences in patient demographics and intra-operative risk factors between groups ( $p < 0.05$ ) (Tables 2.1,2.2). The predictive operative mortality (Euroscore II) was similar between groups. The duration of CPB and aortic cross-clamp times were similar between groups. There were no differences in the adequacy of myocardial protection during the aortic cross-clamp period between groups. The artificial pulse generated resulted in a significant pulse pressure well above the minimum requirement set by our protocol. There were no significant differences in phenylephrine, hemoconcentration requirements, and blood transfusion between groups.

**Table 2.1: Demographic Data**

<b>Characteristic</b>	<b>Pulsatile (n=10)</b>	<b>Non-Pulsatile (n=10)</b>	<b><i>p</i>-value</b>
<b>Pre-operative Data</b>			
Age(y)	72.4±14.5	74.6±9.9	0.70
Males(%)	5(50)	7(70%)	0.65
Body Surface Area(m <sup>2</sup> )	2.03±0.19	1.91±0.26	0.23
<b>Risk Factors</b>			
Hypertension	7(70%)	8(80%)	1.0
Hypercholesterolemia	3(30%)	7(70%)	0.18
Diabetes Mellitus	2(20%)	2(20%)	1.0
Left Ventricular Ejection Fraction < 40%	0(0%)	1(10%)	1.0
Atrial Fibrillation	2(20%)	2(20%)	1.0
Euroscore II	3.40±2.0	4.8±1.85	0.14

No statistical differences between group patient demographics and risk factors. Data are presented as mean±SD, or absolute numbers (percentage); *p*-value <0.05 using students t-test and Two-tailed Fishers Exact test.

**Table 2.2: Intra-operative Data**

Characteristic	Pulsatile (n=10)	Non-Pulsatile (n=10)	<i>p</i> -value
<b>Operative Procedure</b>			
Aortic Valve Replacement	10(100%)	10(100%)	1.0
Mitral Valve Replacement	1(10%)	0(0%)	1.0
Coronary Artery Bypass	8(80%)	10(100%)	0.47
Ascending Aorta Replacement	1(10%)	1(10%)	1.0
<b>Intra-operative Data</b>			
CPB Time(min)	128.6±30.6	146.7±26.1	0.17
Cross-Clamp Time(min)	97.7±24.0	109.3±16.6	0.22
Cardioplegia Volume(ml)	8792±4192	7557±2381	0.43
Phenylephrine(ug)	4860±1504	3630±902	0.49

No statistical differences between group types of procedures and intra-operative data. Data are presented as mean±SD, or absolute numbers (percentage); *p*-value <0.05 using students *t*-test and Two-tailed Fishers Exact test.

**Hemodynamic Data**

There were no statistical differences in heart rate (excluding T<sub>1</sub>-T<sub>2</sub>), cardiac index, mean arterial pressure, temperature and hemoglobin parameters between groups at any time point (Table 2.3). The effects of hemodilution resulted in a significant decrease ( $p < 0.05$ ) in hemoglobin levels compared to baseline values at all time points within groups. Core temperature decreased in both groups during surgery according to the standard protocol.

**Table 2.3: Hemodynamic Data**

		<b>T0</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>
<b>Cardiac Index (L/min/m<sup>2</sup>)</b>	P	1.9(1.7-2.6)	2.2-2.4	2.2-2.4	2.8(2.5-3.1)	2.3(1.8-2.8)
	NP	2.2(2.1-2.3)	2.2-2.4	2.2-2.4	2.6(2.4-3.2)	2.8(2.2-3.3)
<b>Heart Rate (bpm)</b>	P	63(53-69)	70(0)	70(0)	78(74-86)	79(74-86)
	NP	64(58-71)	0(0)	0(0)	77(73-85)	89(77-97)
<b>Mean Arterial Pressure (mmHg)</b>	P	76(66-78)	65(61-75)	64(61-74)	68(58-86)	71(67-78)
	NP	73(66-79)	71(67-78)	74(67-82)	77(66-83)	74(67-84)
<b>Temperature (°C)</b>	P	35.6 (35.3-36.1)	33.1 (32.5-34.0)	35.1 (33.1-35.5)	36.6 (36.3-36.7)	36.8 (36.6-37.0)
	NP	35.8 (35.6-35.9)	33.3 (32.5-34.2)	34.3 (32.9-35.0)	36.7 (36.4-36.9)	36.9 (36.7-37.2)
<b>Hemoglobin(g/L)</b>	P	121(109-145)	87(81-96) <sup>a</sup>	84(78-96) <sup>a</sup>	85(80-92) <sup>a</sup>	97(89-105) <sup>a</sup>
	NP	119(106-123)	80(75-88) <sup>a</sup>	80(77-86) <sup>a</sup>	85(82-91) <sup>a</sup>	89(79-103) <sup>a</sup>

No statistical differences were found between group hemodynamic data. The effects of hemodilution resulted in a significant decrease ( $p < 0.05$ ) in hemoglobin levels compared to baseline values at all time points within groups. Post-hoc comparisons: <sup>a</sup> $p < 0.05$  vs. T<sub>0</sub>; NP=non-pulsatile; P=pulsatile

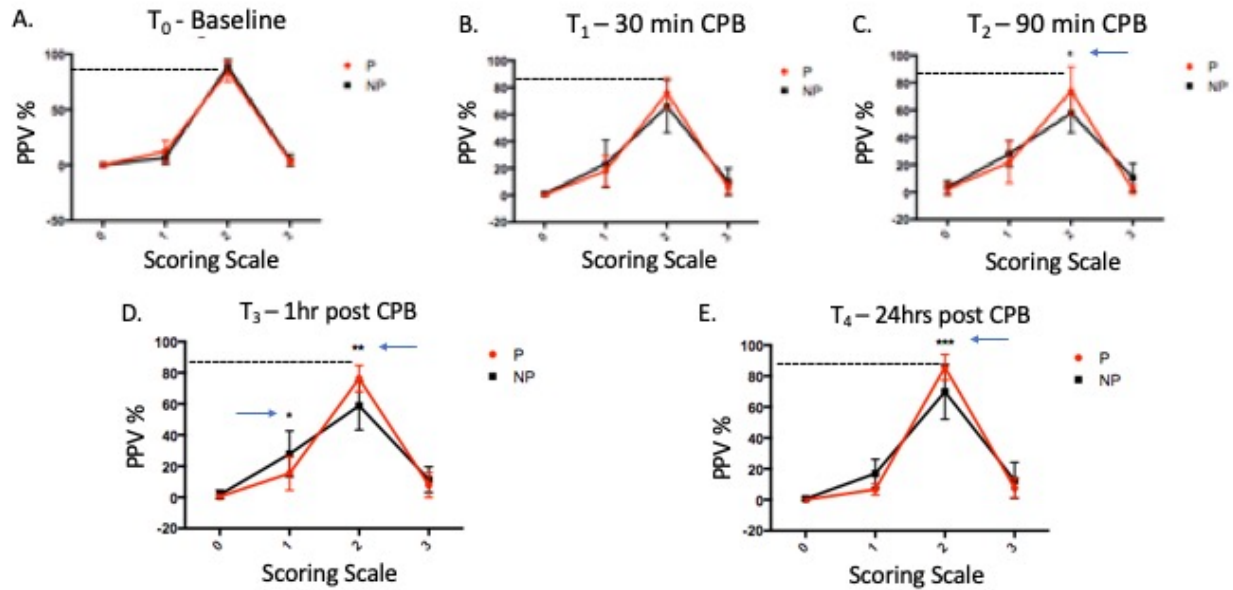
### **Microvideoscopic Measurements and Analysis**

There was no significant differences in blood flow characteristics between groups at T<sub>0</sub>-T<sub>1</sub>. At T<sub>2</sub>, the non-pulsatile group had 17% fewer normally perfused vessels than those in the pulsatile group at the expense of an increase in hyperdynamically flowing vessels. During the post CPB period (T<sub>3</sub>-T<sub>4</sub>), the non-pulsatile group continued to have significantly fewer normally perfused vessels in addition to an increase in slow continuous/intermittent blood flow characteristics compared to the pulsatile group (Table 2.4 and Figure 2.6). There were no significant differences in vessel density between groups at all time points ( $p < 0.05$ ) (Table 2.5).

**Table 2.4: Proportion of Perfused Vessels (PPV%)**

Scoring Scale		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
<b>0</b>	P	0.00±0.00	0.32±0.22	2.20±1.50	0.53±0.28	0.00±0.00
	NP	0.00±0.00	0.82±0.46	3.55±1.70	1.79±0.94	0.63±0.36
<b>1</b>	P	12.41±3.10	18.11±3.66	21.94±4.88	15.32±3.41	6.61±1.13
	NP	6.59±1.83	23.27±5.88	28.19±3.35	27.90±4.93 <sup>a</sup>	16.98±3.10 <sup>a</sup>
<b>2</b>	P	84.44±2.90	75.97±3.59	74.00±5.61	76.22±2.68	85.72±2.60
	NP	88.90±1.93	65.92±6.50	57.61±5.00 <sup>a</sup>	58.89±5.21 <sup>a</sup>	69.82±5.87 <sup>a</sup>
<b>3</b>	P	3.15±0.98	5.55±1.35	1.86±1.00	7.93±2.52	7.67±1.96
	NP	4.51±1.74	9.99±3.37	10.66±3.63 <sup>a</sup>	11.41±2.76	12.56±3.86

Sublingual microvascular perfusion expressed as the proportion of perfused vessels (PPV %) revealed an increase in the number of normally perfused vessels in the pulsatile (P) group compared to non-pulsatile (NP) at T<sub>2-4</sub>. PPV scoring scale: 0=absent flow, 1=slow continuous/intermittent (<250µm/sec), 2=normal (250-350µm/sec), and 3=hyperdynamic (immeasurably fast). Post-hoc comparisons: <sup>a</sup>p<0.05 vs. pulsatile group.



**Fig 2.6** The proportion of perfused vessel (PPV%) analysis for each score is seen here across all time points. Compared to baseline, deviations from the proportion of normally perfused vessels (score of 2) is represented by the black dotted line at subsequent time points for both P group (red line) and NP group (black line). \*  $p < 0.05$  compared to NP group.

**Table 2.5: Vessel Density and NIRS / VOT Data**

Characteristic		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Vessel Density	P	13.18±0.67	12.67±0.63	11.79±0.54	12.79±0.41	13.46±0.56
	NP	12.47±0.51	12.75±0.60	11.08±0.72	12.67±0.41	13.79±0.48
StO <sub>2</sub> baseline	P	85.4±2.40	83.43±3.06	84.00±3.47	84.99±2.35	77.87±2.94
	NP	83.21±1.06	84.49±3.52	86.13±1.60	84.33±1.95	81.51±1.66
Occlusion Slope	P	-8.35±0.46	-9.41±0.81	-10.95±1.01	-9.94±0.35	-13.80±1.47 <sup>b</sup>
	NP	-8.15±0.32	-8.01±0.78	-9.23±0.87	-10.42±0.83	-13.99±1.96 <sup>b</sup>
Reperfusion Slope	P	4.66±0.91	2.35±0.44	2.28±0.51	2.90±0.51	6.10±0.58
	NP	4.42±0.38	1.88±0.38	2.35±0.45	2.52±0.38	3.65±0.48 <sup>a</sup>
THI	P	13.51±1.57	13.16±2.31	13.33±2.14	13.35±2.24	10.91±3.0
	NP	13.35±2.80	11.43±2.43	11.45±2.79	12.23±1.94	11.18±2.13

Reperfusion slope was significantly different between groups at T<sub>4</sub> indicating improved microvascular responsiveness in pulsatile group Mean±/ SEM, Kruskal-Wallis multiple comparisons, Post-hoc comparisons: <sup>a</sup>p<0.05 vs. pulsatile group; <sup>b</sup>p<0.05 vs. T<sub>0</sub>. THI = total hemoglobin index.

**Near-Infrared Spectroscopy and Vascular Occlusion Testing**

No significant differences between groups in baseline StO<sub>2</sub> and consumption slope at all time points. Reperfusion slope was significantly different between groups at T<sub>4</sub> (24hrs post CPB) indicating improved microvascular responsiveness in pulsatile vs. non-pulsatile group (Table 2.5).

**Post-operative Data**

Compared to baseline, plasma lactate levels increased with a similar trend in both groups. Despite no significant differences between groups at all time points, lactate levels remained elevated in the non-pulsatile group at T<sub>4</sub>. Conversely, serum creatinine levels increased significantly post-operatively in the non-pulsatile group at T<sub>4</sub> compared to the pulsatile group ( $p < 0.05$ ) (Table 2.6).

**Table 2.6: Lactate and Creatinine Data**

		<b>T0</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>
<b>Lactate (meq/L)</b>	P	1.4(0.8-1.9)	1.6(1.2-2.4)	1.8(1.6-2.4) <sup>b</sup>	2.4(1.7-3.5) <sup>b</sup>	1.2(1.1-1.3)
	NP	1.0(0.9-1.1)	1.3(1.1-2.1) <sup>b</sup>	1.4(1.2-2.1) <sup>b</sup>	2.6(2.0-4.4) <sup>b</sup>	1.9(1.3-2.5) <sup>b</sup>
<b>Creatinine (umol/L)</b>	P	80(61-96)	N/A	N/A	76(62-104)	76(58-110)
	NP	89(78-119)	N/A	N/A	92(78-121)	117(90-182) <sup>a</sup>

Lactate levels increased with a similar trend in both groups and remained elevated in the non-pulsatile group at T<sub>4</sub>. Conversely, serum creatinine levels increased significantly post-operatively in the non-pulsatile group at T<sub>4</sub> compared to the pulsatile group. Post-hoc comparisons: <sup>a</sup>p<0.05 vs. pulsatile group; <sup>b</sup>p<0.05 vs. T<sub>0</sub>; N/A=not applicable.

## 2.4 DISCUSSION

SIRS has been linked to microvascular blood flow alterations found in various pathological conditions such as sepsis, shock, and I/R injury. In cardiac surgical patients, I/R injury and SIRS are the main culprits responsible for many of the detrimental effects to CPB. The switch to a non-physiological pulse may potentiate this. Non-pulsatile flow during CPB remains popular despite theoretical advantages of pulsatile flow published in the literature. At the core of the debate is lack of evidence supporting the benefits of pulsatile perfusion on the microcirculation (Ji & Undar, 2007). Using OPS imaging, we present direct visual evidence of the benefits of pulsatile perfusion, specifically preservation of normal microvascular perfusion characteristics during and after CPB. Utilizing NIRS measurements of StO<sub>2</sub> and the VOT, we also report improved microcirculatory vasoreactivity in patients undergoing pulsatile CPB. The decrease in reperfusion slopes in non-pulsatile group is likely due to vasomotor dysfunction similar to those measured in previous studies (Kim et al., 2015; Smith & Murkin, 2014). Our study indicates that non-pulsatile flow is associated with significant microvascular alterations, impaired vasoreactivity and increased post-operative serum creatinine levels, while pulsatile flow resulted in a relative maintenance of homeostasis.

Strategies toward containing the inflammatory response during CPB should include combined therapies due to the multi-factorial nature of the insult. One of the proposed mechanisms of SIRS is alteration in arterial blood flow patterns in the form of non-pulsatile flow. This deviation from the normal physiological pulse generates less shear stress on the vessel wall resulting in less endogenous nitric oxide (NO) production (Baskurt et al., 2004). Preservation of NO levels during pulsatile CPB has been reported to attenuate the inflammatory response (Lanzarone et al., 2009), and vasodilation produced by shear-stress-induced NO is important in the regulation of systemic vascular resistance and tissue perfusion (Balligand et al., 2009). Since

the endogenous production of NO is impaired during CPB, many studies have reported advantages of NO administration as a compensatory mechanism to attenuate I/R injury and the SIR (Freyholdt et al., 2003; Massoudy et al., 2000). This interaction between increased shear stress and endothelial NO production during pulsatile flow may have contributed to our results. Maintenance of normal levels of NO, or at least higher levels relative to non-pulsatile, may have attenuated the inflammatory response, microcirculatory deficits and I/R injury during pulsatile flow, resulting in preservation of a normal perfusion profile and improved microcirculatory vasoreactivity following VOT.

Hyperlactatemia may occur in up to 20% of patients undergoing cardiac surgery with CPB (Ranucci, De Toffol, et al., 2006). Lactic acidosis present during CPB and the early phase of recovery is associated with increased risk of morbidity and mortality (Demers et al., 2000). Although not a marker for organ specific perfusion, lactate concentration is considered to be a good indicator of non-optimal tissue perfusion. In the present study we found increased levels in both groups compared to baseline values. Postoperatively, lactate remained elevated in the non-pulsatile group whereas the pulsatile group returned to baseline.

Transient renal dysfunction following CPB is also very common, but can lead to acute renal failure requiring dialysis in 1-5% of patients (Fortescue et al., 2000). In most patients, renal complications arise from a direct result of CPB and post-operative hypoperfusion (Slogoff et al., 1990). In forty high-risk patients undergoing CPB, pulsatility improved microcirculation and renal function with decreases in urea and creatinine concentration compared to non-pulsatile flow (Kocakulak et al., 2005). In the present study, we report microvascular perfusion deficits in conjunction with increased serum creatinine levels 24-hours post-operatively following non-pulsatile CPB.

We found no significant differences in hemodynamic variables such as heart rate, mean arterial pressure, and cardiac index between groups. However, the literature suggests that macrohemodynamics is limited in predicting the state of the microcirculation (Shoemaker & Czer, 1979). We report that microvascular perfusion alterations exist despite optimization in global hemodynamics. A similar inverse relationship between the microcirculation and global hemodynamics was reported in septic patients (De Backer et al., 2002). In fact, microvascular perfusion deficits and impaired vasoreactivity persisted for 24-hours post-operatively with normal hemodynamics in the non-pulsatile flow group. Despite normal hemodynamics, microcirculatory alterations found in early post-operative period is associated with an increased risk of complications (Jhanji et al., 2009). Persistent microcirculatory alterations are also associated with poor outcomes (Sakr et al., 2004). Microvascular alterations seen early during our study may be linked to impaired blood distribution within the kidneys resulting elevated creatinine levels and renal dysfunction in the non-pulsatile group.

Restoration of perfusion pressure using vasoconstrictors may also mask hypoperfusion at the capillary level (O'Dwyer et al., 1997). It has been suggested that an increase in perfusion pressure using phenylephrine may result in arteriovenous shunting within capillaries (Maier et al., 2009). Although we report no differences in phenylephrine usage between groups, perhaps an increase in systemic vascular resistance commonly linked to non-pulsatile perfusion may result in arteriovenous capillary shunting. Reports indicate that non-pulsatile flow has been associated with microcirculatory shunting and capillary collapse (Ji & Undar, 2007). This would also be inline with our results, as we document an increase in hyperdynamic perfusion in non-pulsatile group that may be representative of capillary shunting. During pulsatile flow we found no significant differences in microcirculatory blood flow under similar macrohemodynamic conditions. This

could be explained by previous reports that pulsatile flow results in less severe microvascular alterations (O'Neil et al., 2012), and a reduction in the SIRS (Lanzarone et al., 2009).

In the present study, microvascular perfusion and vasoreactivity alterations found at T<sub>3-4</sub> were not affected by inotropic and vasopressor support postoperatively. There were no statistical differences found in the number of patients and mean doses between groups. Limitations to this study include a very small patient cohort leading to an underpowered statistical analysis. Also, the clinician acquiring intraoperative data was not blinded to patient specific group; however, data analysis was performed in a blinded fashion.

In conclusion, we have addressed a critical issue in the controversy surrounding the benefits of pulsatile flow during CPB. At the core of the debate is the lack of evidence supporting the benefits of pulsatile perfusion on the microcirculation. Using OPS imaging, we present direct visual evidence of the benefits of pulsatile versus non-pulsatile perfusion, specifically the maintenance of normal microvascular perfusion and vasoreactivity during and following CPB. All indices of microcirculation (VOT reperfusion, OPS measurements, lactate) remained abnormal at 24-hours in the nonpulsatile group and were associated with higher levels of creatinine compared to the pulsatile group. Taken together, this may lead to a reduction in post-operative vital organ injury and dysfunction. The evidence here suggests that implementation of pulsatile flow should be considered as one of the many strategies aimed at reducing the systemic inflammatory response in high-risk cardiac surgical patients undergoing prolonged CPB. The clinical implications are that NIRS may permit earlier, non-invasive detection of significant physiological derangements and allow for more accurate and timely therapeutic interventions in the attenuation of I/R injury and the SIR response linked to CPB. Therefore, a resuscitation strategy of the microcirculation

could be performed based on a bedside monitoring device, potentially reducing the risk of multiple organ failure following cardiac surgery, length of hospital stay and associated health care costs.

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## **CHAPTER 3: VARIABILITY IN MICROVASCULAR HEMOGLOBIN LEVELS DURING CARDIOPULMONARY BYPASS**

### **3.1 INTRODUCTION**

Physiological dysfunction in patients following cardiac surgery is typically linked to cardiopulmonary bypass (CPB) with two of the main culprits being the systemic inflammatory response (SIR) and ischemia reperfusion (I-R) injury. Both can lead to an increased risk of end organ complications such as acute kidney injury, heart failure, and neurological dysfunction (Paparella et al., 2002). To minimize these effects, the goal of CPB is to approximate a normal physiological environment in order to improve patient outcomes. Due to technological advancements and changes in the conduct of perfusion, several strategies have been put into place over the years to achieve these goals. In an effort to provide effective extracorporeal support for our patients it is also important to improve our ability to evaluate, diagnose and guide therapeutic interventions with updated monitoring capabilities.

When caring for cardiac surgical patients in the operating room we monitor global perfusion status or macrohemodynamics. This would include parameters such as heart rate (HR), mean arterial blood pressure (MAP), central venous pressure, and pulse oximetry. Even blood values such as lactate and venous oxygen saturations are still representative of central perfusion markers. However, normalization of these variables do not ensure that sufficient oxygenation occurs at the microvascular level. Hemodynamic coherence occurs when resuscitation efforts aimed at correcting macrohemodynamics effectively improve microcirculatory perfusion and oxygen delivery to the cells of vital organs. However, conditions such as shock, reperfusion injury and inflammation can disrupt hemodynamic coherence by affecting hormonal, neural,

biochemical, and vascular regulatory control systems needed to regulate microvascular blood flow (Ince, 2015). The loss of hemodynamic coherence resulting in the normalization of macrohemodynamics without improved microvascular perfusion and oxygenation in sepsis have been published in the literature (De Backer et al., 2013; Trzeciak et al., 2008). Physiological dysfunction as it relates to the systemic inflammatory response syndrome (SIRS) originates at the microvascular level, precedes end organ failure and is distinct from any macrovascular hemodynamics (De Backer et al., 2010). This means that microvascular alterations can exist in the presence of normal macrovascular hemodynamics. Therefore, a need exists for improved microvascular monitors in the operating room that can continuously track dynamic changes in the microvasculature over time .

Tools for quantifying peripheral perfusion at the bedside have emerged in recent decades. Current clinical microvascular observation can be performed using bedside versions of Intra-vital video microscopy technology such as Orthogonal Polarization Spectral (OPS) imaging and Sidestream Dark Field (SDF) imaging. OPS imaging ultimately reflects the capacity of oxygen delivery to the tissues by determination of red blood cell (RBC) velocity and functional capillary density (FCD) in the microcirculation (Groner et al., 1999). The sublingual mucosa is the preferred site for obtaining OPS images as it is readily accessible (Hamilton et al., 2008) and has been documented to correlate with that of vital internal organs (Creteur et al., 2006; Verdant et al., 2009). Its application in cardiac surgery has gained popularity with research focusing mainly on the effects of CPB on the sublingual microcirculation (Bauer et al., 2007; De Backer et al., 2009; den Uil et al., 2008; O'Neil et al., 2012). However, these types of modalities provide only a snapshot of the microcirculation, and do not consider the time-dependent variability and heterogeneity in microvascular blood flow that occurs over the entire duration of CPB.

A heterogeneous flow pattern has been reported to be less tolerable for tissue oxygenation than a homogenous decrease in perfusion in skeletal muscle during sepsis (Goldman et al., 2006). Heterogeneity in microvascular perfusion may occur at different times and for different reasons during the course of CPB due to various interventions. For example, heterogeneity and loss of hemodynamic coherence can exist due to inflammation with obstructed capillaries next to capillaries with flowing RBC's, as well as hemodilution in which a loss of RBC filled capillaries results in increased diffusion distances between RBC's and tissue cells, the vasoconstriction of arterial vessels with phenylephrine may also result in microvascular shunting and ischemia, and finally tissue edema caused by capillary leakage can result in increased diffusion distances between the RBCs and tissue cells (Ince, 2015). Therefore processing the continuous distribution of RBC's over time during CPB would allow us to better understand the microvasculature, providing detailed information unavailable during short time interval images captured using OPS and SDF imaging.

Current devices such as OPS imaging also carry many limitations including the application of too much pressure with the probe underestimating blood flow in the area under investigation (den Uil et al., 2008) and repeated measurements from the same vascular bed are nearly impossible due to movement artifacts between the tongue and the probe (Lindert et al., 2002). In cardiac surgical patients this can be very challenging for the investigator to gain access to the tongue while having both an endotracheal tube and transesophageal echo probe in place, as well as having to coordinate video capture with the anesthesiologist. Capturing timely video may also depend on the progression of surgery as to not disrupt the normal standard of care. Although development of these monitoring technologies have helped us to better understand the pathophysiology of acute circulatory failure in surgical patients, the challenge to accurately monitor the state of tissue oxygenation still remains.

The introduction of near-infrared spectroscopy (NIRS) for assessing the adequacy of regional tissue perfusion was an important development in tissue monitoring. However, tissue oxygen saturation (StO<sub>2</sub>) values are limited as they combine measurements of arterial, venous, and capillary blood. Since venules contain the majority of blood in the microcirculation, StO<sub>2</sub> can be viewed primarily as a measure of regional venous oxygen saturation which is still not a true indicator of microvascular blood flow (Mesquida et al., 2013).

During cardiac surgery with CPB the brain is most commonly assessed using NIRS, as undetected episodes of hypoperfusion predispose the patient to an increased risk of postoperative neurological complications. Studies have suggested that optimization of cerebral oximetry values aid in preventing neurological complications and that maintaining adequate StO<sub>2</sub> levels is beneficial to all vital organs (Murkin & Arango, 2009). It has been proposed that cerebral oximetry can be used as an organ index, meaning that adequate StO<sub>2</sub> levels is beneficial for all vital organs (Murkin & Arango, 2009). It has also been concluded in systematic reviews that tissue oximetry is a promising technique for preventing postoperative neurological complications (Taillefer & Denault, 2005). Neuromonitoring with combined diffuse correlation spectroscopy and broadband NIRS can provide real-time assessment of cerebral perfusion and metabolism, alerting clinicians to relevant hemodynamic events before brain injury occurs (Rajaram et al., 2020). Other studies have failed to prove that intraoperative interventions correcting StO<sub>2</sub> values lead to an improvement of neurological outcomes because the cerebral autoregulation (CA) is not taken into account (Zheng et al., 2013). Cerebral autoregulation is the intrinsic system that maintains adequate cerebral blood flow at a wide range of perfusion pressures. A disturbed CA may lead to hyper-perfusion and predispose the patient to an increased risk of cerebral edema and hemorrhage (Henriksen, 1986). The neuroprotective autoregulatory system prevents both hypo-perfusion and

hyper-perfusion by reactive vasodilation and constriction following changes in MAP and arterial carbon dioxide tension (Paulson et al., 1990). Therefore it is important to target a MAP within autoregulatory range (50-150mmHg), and to maintain normocapnia (35-45 mmHg) in preserving cerebral autoregulation during CPB and minimize neurological complications (Ševerdija et al., 2015).

NIRS can also be applied non-invasively to continuously measure StO<sub>2</sub> in skeletal muscle and overlying tissue (Barstow, 2019). NIRS probes have been used to monitor distal limb calf perfusion during minimally invasive cardiac surgical (MIS) procedures that require arterial cannulation of the femoral artery as opposed to standard cannulation of the ascending aorta (Tarui et al., 2018). StO<sub>2</sub> values act as a trending tool that aid in troubleshooting measures to ensure adequate systemic and leg perfusion during these types of surgeries.

The microcirculation is the final destination of the cardiovascular system that is responsible for oxygen transfer from RBC's in the capillaries to parenchymal cells. Therefore, these cells rely on a functional microvascular unit comprised of arterioles, capillaries and venules to maintain their viability and effectively support organ function. The goal of this study is to apply a custom monitor using NIRS technology previously tested on patients in the intensive care unit (ICU) (Mendelson et al., 2021) into the cardiac operating room to reflect changes in microvascular hemoglobin content (MHC). We hypothesize that changes in MHC can provides us with more detailed information of oxygen supply and demand in response to various interventions in cardiac surgical patients undergoing CPB.

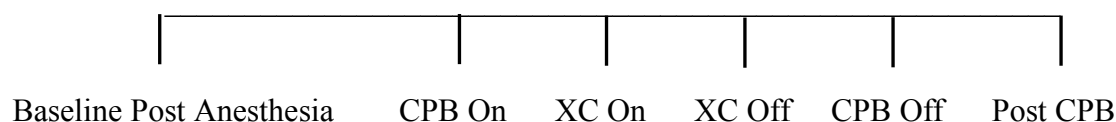
## 3.2 METHODS

### Study Design

This is a prospective cohort study approved by the local Research Ethics Board of twenty cardiac surgical patients undergoing non-pulsatile (NP) CPB at University Hospital, London Health Sciences Centre with the intent to determine the feasibility of MHC monitoring of the peripheral perfusion. Using high-resolution near-infrared spectroscopy, we assess the feasibility of signal acquisition, subsequent algorithmic analysis, and its characterization during the course of CPB. All adult patients admitted for elective cardiac surgery involving any valve repair/replacement, and aortic replacement procedures were invited in this study. Exclusion criteria included patients requiring coronary artery bypass grafting due to the prepping of both legs, and cases where pre-operative consent was unattainable.

### Data Collection

Patient demographics including age, sex, body surface area, and pre-operative risk factors were obtained for all participants. Hemodynamic monitoring for heart rate, mean arterial pressure, temperature, and pump flow rate were recorded continuously throughout the study. Arterial blood samples were drawn at various time intervals for analysis of acid/base and oxygenation status, Hb, and lactate levels. Continuous NIRS sampling was initiated following general anesthesia and endotracheal intubation, and concluded beyond termination of CPB just prior to chest closure. NIRS data was correlated with physiologic variables and particular events of interest during bypass: CPB On; Cross Clamp On (XC On); Cross Clamp Off (XC Off); CPB Off.



## **Cardiopulmonary Bypass**

CPB was performed using the Jostra HL 20 heart-lung machine (Maquet-Dynamed Inc.). The extra-corporeal circuit was composed of: x-coating custom tubing pack (Terumo Cardiovascular Systems), capiox FX25 integrated membrane oxygenator (Terumo Cardiovascular Systems), and microplegia using the Myocardial Protection System (Quest Medical Inc.). Arterial inflow was delivered into the ascending aorta for conventional CPB, and femoral artery for minimally invasive procedures. A standard priming solution of Lactated Ringers was used in all cases.

Upon arrival to the OR, the anesthesiologist inserted a right radial arterial pressure line to monitor MAP and acid base balance. This was followed by general anesthesia and placement of a central venous line into the right internal jugular vein for right heart pressure monitoring and drug administration. Patients were heparinized (400u/kg) pre-bypass to achieve an activated clotting time greater than 480 seconds. During CPB, patients were cooled to a systemic temperature of 34-35°C with active re-warming to 36-37°C prior to termination of bypass. A minimum cardiac index of 2.2-2.4 L/min/m<sup>2</sup>, and mean arterial pressure maintained greater than 60mmHg was targeted during the course of non-pulsatile CPB. Anesthetic maintenance during CPB was managed with inline sevoflurane. Weaning from CPB included the administration of vasopressors and inotropic support medications considered necessary by the anesthesiologist.

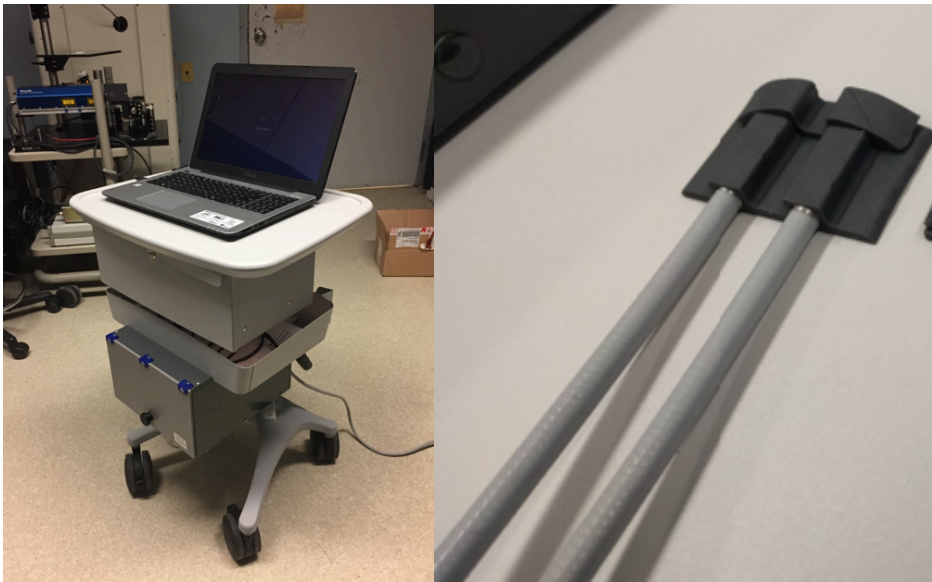
## **Near Infrared Spectroscopy**

Near-infrared spectroscopy can be applied continuously and non-invasively to measure StO<sub>2</sub> of hemoglobin in skeletal muscle and overlying tissue. All patients were managed routinely except for the intra-operative application of a NIRS probe placed on the right vastus medialis muscle for continuous microvascular monitoring. Probe placement was applied following general

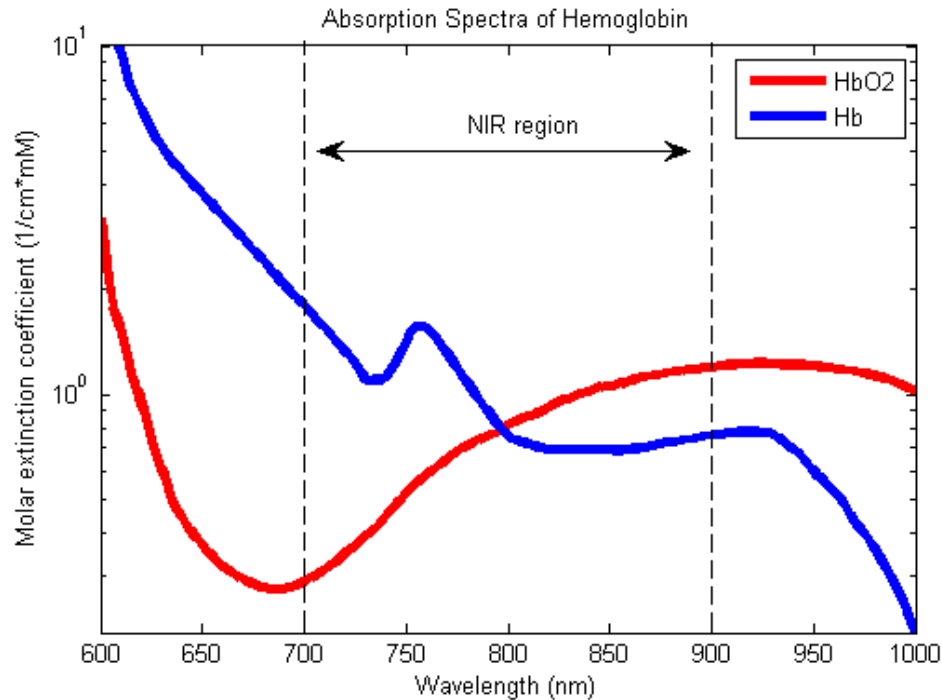
anesthesia and steady state baseline imaging was captured. NIRS data was collected and time synched to pump flow rate (FR) and MAP. Assessment of microcirculatory blood flow was ascertained and correlated with physiologic variables and particular events of interest at various time points.

A broadband continuous wave-NIRS device was previously designed by our lab used for this study (Fig 3.1). The device consists of a modified QE65000 spectrometer (Ocean Optics, Dunedin, Florida), a broadband light source (Ocean Optics HL-2000-HP), custom detection fiber bundles (Fiberoptics Technology, Inc., Pomfret, Connecticut) and prisms (Thorlabs, Newton, NJ) (Diop et al., 2014). A 3D printed probe holder was used to secure the fiber bundles and allow for proper positioning on the patient leg. Similar probe placement was previously used in ICU patients to interrogate the peripheral microcirculation using this same technology. The skeletal muscle of the thigh was considered an appropriate acquisition site for cardiac surgical patients given similar probe placements on the calf muscle used to measure StO<sub>2</sub> values during minimally invasive surgeries. It is the largest organ in the body, main determinant of systemic vascular resistance, and the main site of action for vasoactive medications. For the purpose of this study skeletal muscle of the right thigh was chosen over the left thigh in the event a saphenous vein was needed from the left leg to perform an urgent coronary artery bypass graft. As the light source travels down the fiber bundles and illuminates the tissue, a small fraction of light returns to the spectrometer via the detection fibers after being scattered within the tissue and absorbed by Hb and other chromophores. The returning light intensities are recorded using Spectra Suite™ software (Ocean Optics, Inc.). The spectral analysis of light collected yields quantitative data on Hb and oxygen saturation levels over a wide range of wavelengths from 600 to 900 nm at a continuous sampling frequency of 10 Hz (10 samples per second).

Both Hb concentration and oxygen saturation affect light absorption, therefore our analysis focuses on light intensities recorded at the isosbestic wavelength 798 nm (Fig 3.2). This means that the amount of light absorbed by Hb is independent of whether its oxygenated or not since the isosbestic wavelength for Hb have identical extinction coefficients for both oxy and deoxy hemoglobin (J. G. Kim et al., 2005). We don't measure Hb directly but we measure changes in the optical density ( $\Delta OD$ ) at the isosbestic wavelength to give us changes in Hb over time. A custom MATLAB program was used to convert isosbestic wavelength intensities captured by Spectra Suite to MATLAB files, and subsequently converted to  $\Delta OD$  vs time. Subsequent analysis of  $\Delta OD$  tracked changes in MHC in response to CPB as a potential measure of autoregulation.



**Figure 3.1:** Commercial NIRS devices typically measure StO<sub>2</sub> only. Here we have designed and built a NIRS device reflecting changes in MHC required to match oxygen supply and demand. The device is contained within a cart as the skeletal muscle of the thigh can be interrogated with proper probe placement. Photos by Asher Mendelson.



**Figure 3.2:** Our analysis consists of changes in OD at the Isosbestic wavelength of hemoglobin in the NIR range (798nm). The amount of light absorbed by Hb is independent of whether its oxygenated or not, therefore changes in OD represent changes in MHC.  
[https://commons.wikimedia.org/wiki/File:Oxy\\_and\\_Deoxy\\_Hemoglobin\\_Near-Infrared\\_absorption\\_spectra.png](https://commons.wikimedia.org/wiki/File:Oxy_and_Deoxy_Hemoglobin_Near-Infrared_absorption_spectra.png)

### Statistical Analysis

In all tables, the data are presented as mean  $\pm$  standard error of the mean, median {25<sup>th</sup>-75<sup>th</sup> percentile}, or absolute number percentages as specified. Results are considered significant when corresponding *P*-value was  $< 0.05$ . A One-way ANOVA using non parametric Kruskal-Wallis test was used to compare macrohemodynamics and  $\Delta OD$  at four major interventional time points. Univariate linear regression analysis was used to calculate the coefficient of determination ( $R^2$ )

between several dependent variables versus independent variables. Statistical tests were performed using GraphPad prism9 (GraphPad Software, San Diego, CA, USA).

To adjust for possible confounders, multivariable linear regression models were constructed. In the first model, the outcome examined was the area below baseline. We first examined through univariate analyses the association between the dependent variable (area below baseline) versus several independent variables that were selected based on clinical knowledge (lowest temperature on pump; the pump duration; pre-pump hemoglobin level; and phenylephrine usage). Variables that were significantly associated with the outcome (i.e., those with a p-value  $<0.05$ ) were entered into the multivariable linear regression model, which ultimately included the lowest temperature on pump and the pump duration. This was also in concordance with the parsimony principle of multivariable linear regression allowing us to enter a total of 1 independent variable for each 10 outcomes, therefore preventing model overfitting.

In the second model, the outcome examined was the post-pump lactate. This outcome was positively skewed as assessed visually by histogram and also by skewness and kurtosis tests for normality. Therefore, lactate was log-transformed. We then examined through univariate analyses the association between the dependent variable (log post-pump lactate) versus several independent variables that were selected based on clinical knowledge (area below baseline; lowest temperature on pump; pre-pump hemoglobin level; and phenylephrine usage). Variables that were significantly associated with the outcome (i.e., those with a p-value  $<0.05$ ) were entered into the multivariable linear regression model, which ultimately included the area below baseline and lowest temperature on pump. This was also in concordance with the parsimony principle of multivariable linear regression allowing us to enter a total of 1 independent variable for each 10 outcomes, therefore preventing model overfitting. The estimated coefficient of the independent variables was

transformed back to the linear scale and was interpreted as the percentage change in the outcome (i.e., post pump lactate) for one-unit change in the independent variable, holding the other independent variables constant.

In the third model, the outcome examined was phenylephrine on pump. This outcome was positively skewed as assessed visually by histogram and also by skewness and kurtosis tests for normality. Therefore, phenylephrine was log-transformed. We then examined through univariate analyses the association between the dependent variable (i.e., log phenylephrine) versus several independent variables that were selected based on clinical knowledge (these variables were the area below baseline; lowest temperature on pump; the pre-pump hemoglobin level; the pump duration, and pre-pump lactate). Variables that were significantly associated with the outcome (i.e., those with a p-value  $<0.05$ ) were entered into the multivariable linear regression model, which ultimately included the lowest temperature on pump and the pre-pump lactate. This was also in concordance with the parsimony principle of multivariable linear regression allowing us to enter a total of 1 independent variable for each 10 outcome measurements, therefore preventing model overfitting. There was a strong trend for pre-pump hemoglobin, with a p-value of 0.056, and this variable was only included in a subsequent sensitivity model. The estimated coefficient of the independent variables was transformed back to the linear scale and was interpreted as the percentage change in the outcome (i.e., phenylephrine on pump) for one-unit change in the independent variable, holding the other independent variables constant.

Statistical analysis for multivariable linear regression models was performed using STATA (StataCorp. 2021, Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.).

### 3.3 RESULTS

#### Demographic Data

Twenty two patients were approached for consent to participate in the study. Consent was unattainable for one patient. Of the remaining 21 patients enrolled one patient exhibited a poor NIRS signal, and macrohemodynamic data was not detected in another patient. Therefore, the final data set comprised 19 patients. In total there was 88:55:22 (88 hours, 55 minutes, 22 seconds) of continuous NIRS data collected from the right thigh muscle with a mean recording time of  $4.7 \pm .61$  hours (range: 1.7 – 13.02). The average sample rate was  $9.272 \pm 0.128$ . A subset of patients (n=4), underwent MIS requiring peripheral cannulation strategies, while the remaining patients (n=15) underwent conventional CPB with central cannulation. The majority of patients presented with aortic stenosis pathology therefore retrograde autologous priming (RAP) of the extracorporeal circuit was avoided. No differences were found between MIS and conventional CPB groups with respect to the data collected. A full list of operative procedures are listed in Table 3.1. Demographics and notable risk factors for the final study cohort are provided in Table 3.2.

**Table 3.1: Type of Procedure**

<b>Conventional CPB (n=15)</b>	<b>Minimally Invasive CPB (n=4)</b>
AVR, n=11	MV Repair, n=2
AVR/Aortic Root Enlargement	MV Repair/TR Repair
AVR/ECMO	Left Atrial Myxoma
AVR/MVR/TR Repair	
MVR/Ablation	

AVR = Aortic Valve Replacement; ECMO; extracorporeal membrane oxygenation;

MVR = Mitral Valve Replacement; MV = Mitral Valve; TR = Tricuspid Valve.

**Table 3.2: Demographic Data**

Characteristic	Cardiopulmonary Bypass (n =19)		
Patient Demographics	Minimum	Median	Maximum
Age (y)	50	69	82
BSA (m <sup>2</sup> )	1.58	2.0	2.63
<b>Risk Factors</b>			
NYHA II-III		13 (68%)	
Hypertension		11 (58%)	
Diabetes Mellitus		7 (37%)	
Hyperlipidemia		11 (58%)	

Data are presented as minimum, median and maximum values, or absolute numbers (percentage);

BSA = body surface area; NYHA = New York Heart Association classification.

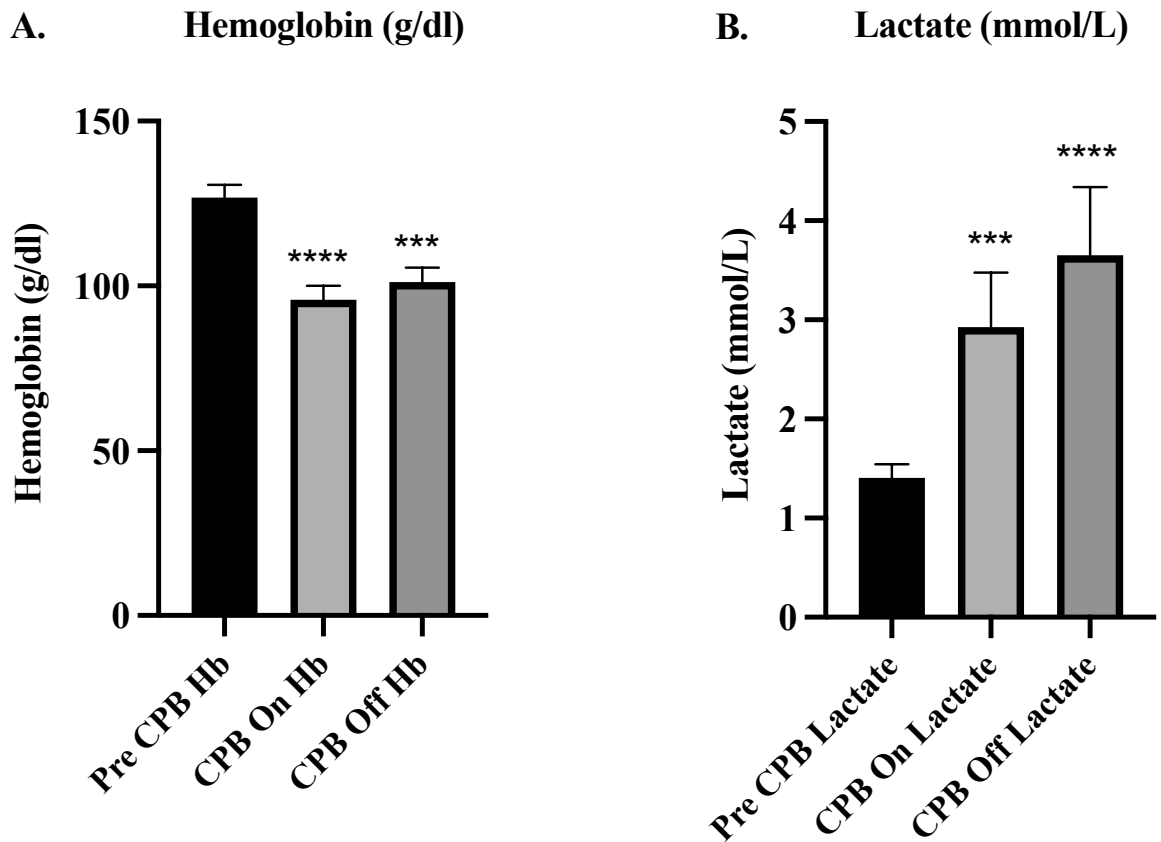
### **Intraoperative Data**

Intraoperative data and important blood values are also recorded in Table 3.3. A significant difference was found between pre CPB Hb levels versus lowest Hb on CPB, as well as pre CPB Hb level compared to post CPB values (Fig 3.3A). Similarly, peak serum lactate levels on CPB were significantly higher than pre CPB lactate levels, as well as post CPB lactate levels were significantly elevated from pre CPB lactate levels (Fig 3.3B). Delta lactate refers to the difference in pre CPB and post CPB lactate. Linear regression shows an increase in delta lactate over the course of CPB that was due to increased CPB time, hemodilution as seen by increasing delta Hb, and lowest temperature on CPB (Fig 3.4A-C). Variability in phenylephrine usage was also affected by temperature (Fig 3.4D). Less phenylephrine was used as patients were cooled and increased amounts of phenylephrine were used as patient temperature increased.

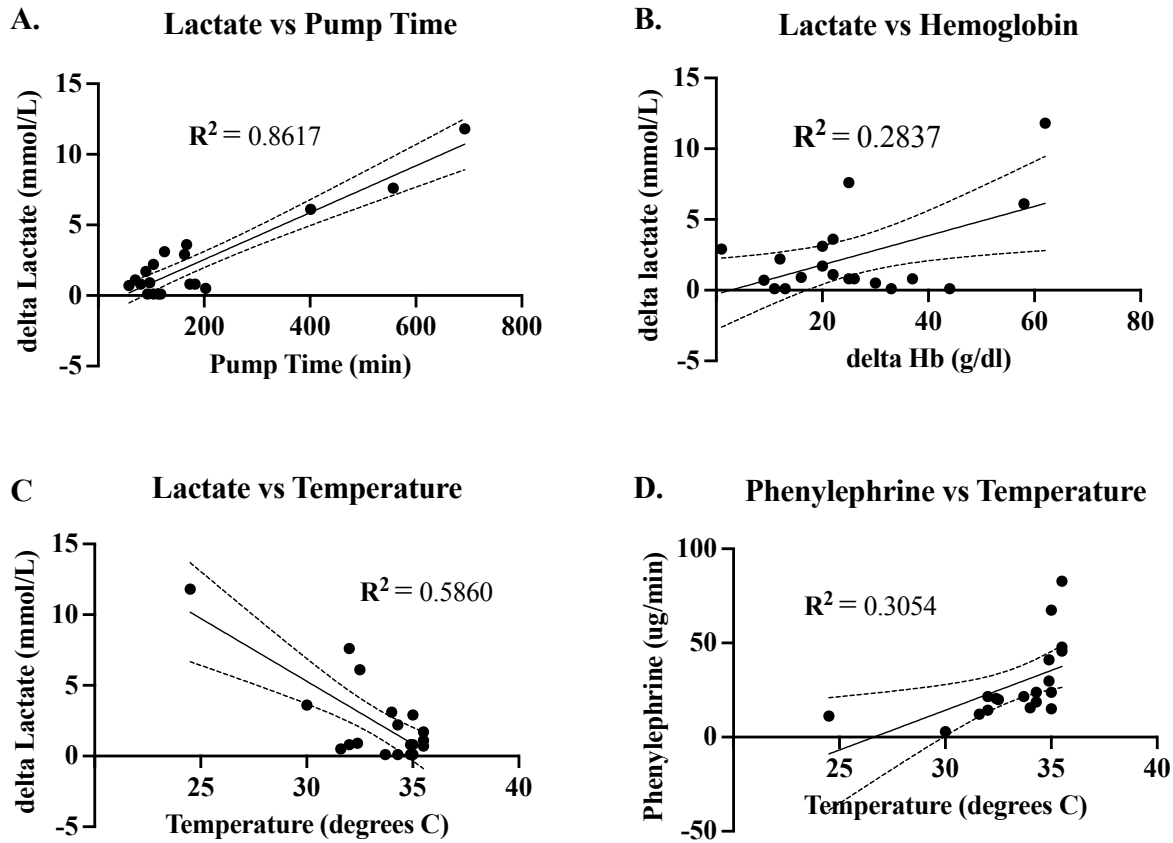
**Table 3.3: Intra-operative Data**

<b>Characteristic</b>	<b>Cardiopulmonary Bypass (n = 19)</b>		
	<b>Minimum</b>	<b>Median</b>	<b>Maximum</b>
CPB time (min.)	58	118	692
XC time (min.)	47	87	319
Lowest Temp Celsius (NP)	24.5	34.3	35.5
Phenylephrine usage (ug/min)	3.0	20.6	82.8
Pre CPB Hb	80	130	154
On CPB Hb (lowest)	61	97	128
Off CPB Hb	60	107	122
Pre CPB lactate	0.5	1.3	3.0
On CPB lactate (peak)	1.3	2.2	11.3
Off CPB lactate	1.5	2.5	13.4

Data are presented as minimum, median, and maximum values. CPB = cardiopulmonary bypass; Hb = hemoglobin; NP = nasopharyngeal; XC = cross clamp.



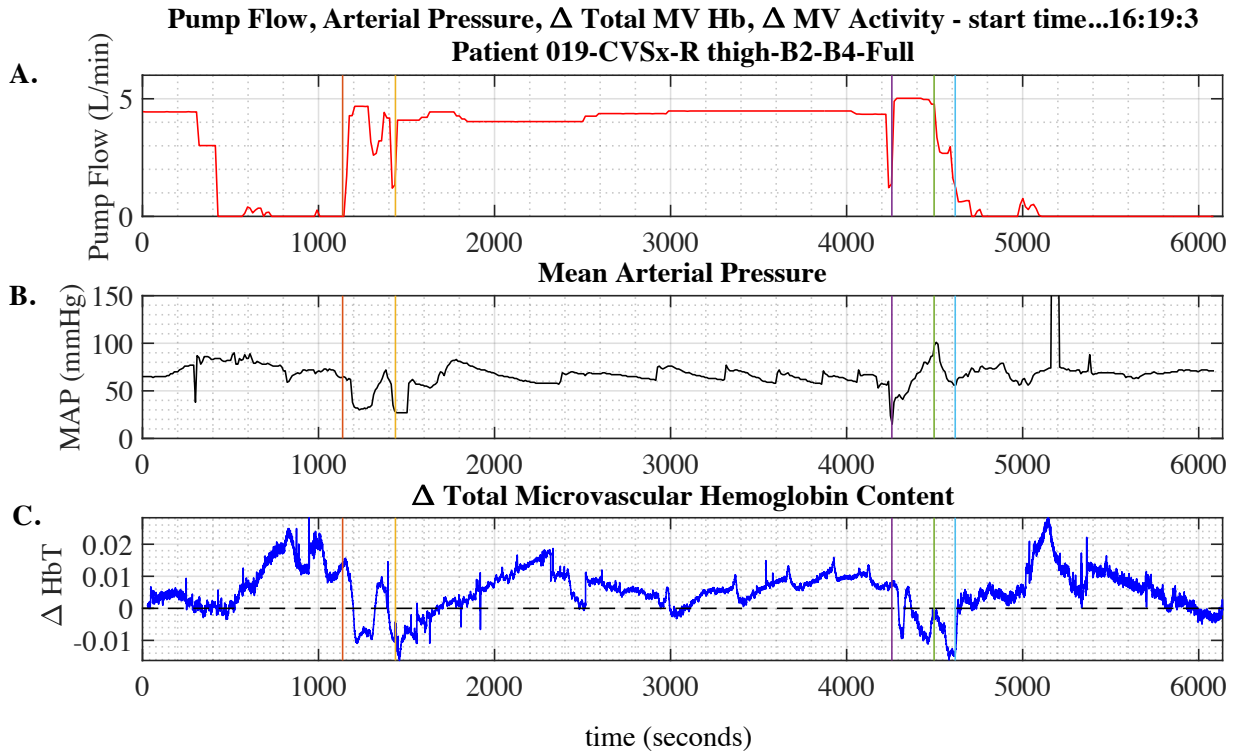
**Figure 3.3:** One way ANOVA with multiple comparisons using Kruskal Wallis test. Fig 3A: Pre CPB Hb vs lowest Hb on CPB, significant decrease, \*\*\*\* p value < 0.0001. Pre CPB Hb vs post CPB Hb, significant decrease, \*\*\* p value = 0.0004. Fig 3B: Pre CPB lactate vs peak lactate on CPB, significant increase, \*\*\* p value = 0.0006. Pre CPB lactate vs post CPB lactate, significant increase, \*\*\*\* p value < 0.0001.



**Figure 3.4:** Univariate linear regression shows significant correlation with solid line. Dotted line represents 95% confidence interval. Fig. 4A: Lactate vs Pump Time shows significant correlation,  $R^2$  .8617, slope = 0.001613, Y-intercept 0.4076,  $p = <0.0001$ ; Fig. 4B: Lactate vs Hb shows significant correlation,  $R^2$  .2837, slope = 0.03986, Y-intercept 1.191,  $p = 0.0189$ ; Fig. 4C:  $R^2$  .5860, slope = 0.1811, Y-intercept 6.048,  $p = 0.0001$ ; Fig. 4D:  $R^2$  .3054, slope = 1.546, Y-intercept 51.62,  $p = 0.0141$ .

## **NIRS and Macrohemodynamics**

NIRS data was collected in 19 patients, all of which were time synched to pump FR and MAP. Changes in microvascular activity have been identified in association with particular events that occur during the cardiac surgical operation. Interventions such as hemodilution, vasopressor usage, and IR events can be identified. Along with changes in CPB pump FR and patient MAP can be observed. Figure 5 illustrates 6000 seconds (100 minutes) of continuous data collection for one patient in the cohort. The top panel (Fig 3.5A) represents pump FR, the independent variable adjustable by the perfusionist using the HL machine. The middle panel (Fig 3.5B) shows the resultant MAP, the dependent variable resulting from changes in pump FR and administration of pharmacological agents. The bottom panel (Fig 3.5C), also a dependent variable, corresponds to  $\Delta OD$  at the isosbestic wavelength to give us changes in Hb over time. The dotted line represents baseline values obtained from steady state capture for 2 minutes following initial probe application. All three panels (Fig 3.5A, Fig 3.5B representative of macrohemodynamic data, Fig 3.5C serving as microvascular perfusion data) depict 4 main interventional time points: CPB On – red bar, XC On – yellow bar, XC Off – purple bar, CPB Off – green bar.

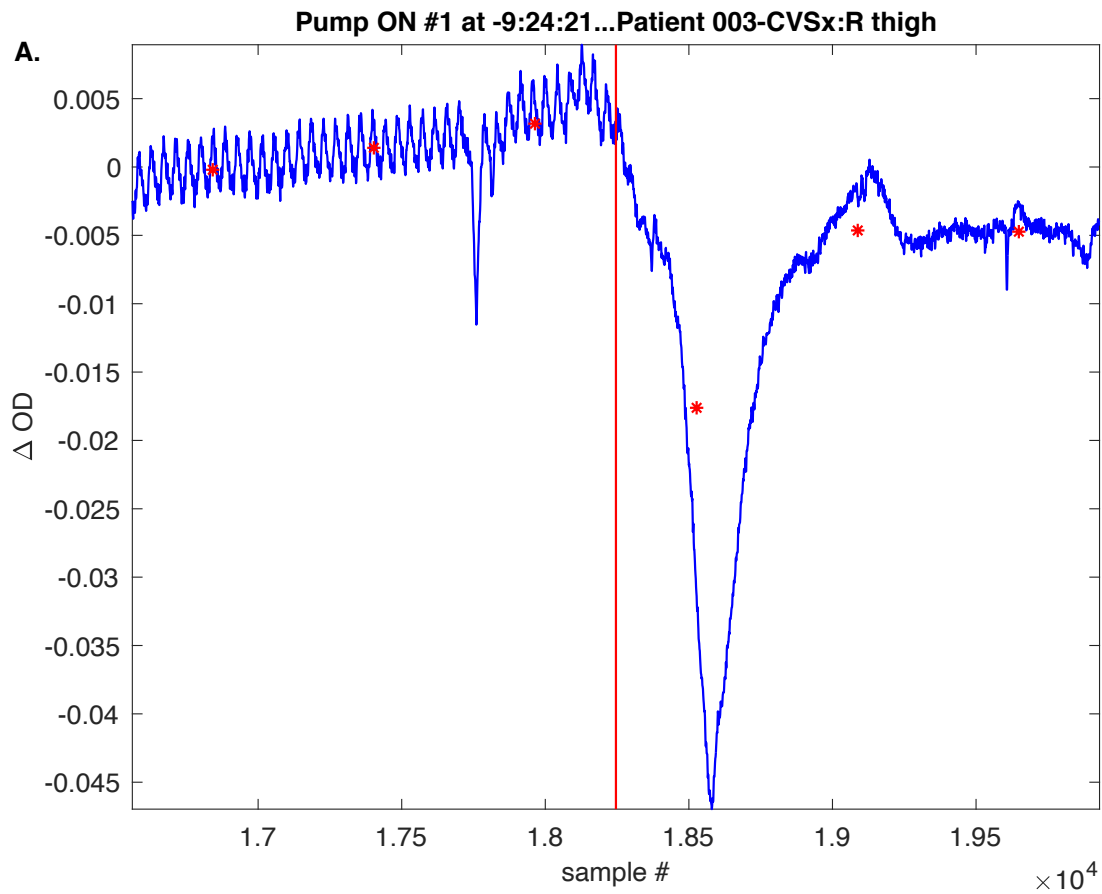


**Figure 3.5:** Single patient data representing macrohemodynamic and microvascular values. Fig 5A: Pump FR vs time; Fig 5B: MAP vs time; Fig 5C:  $\Delta$ OD vs time. Dotted line represents baseline OD.

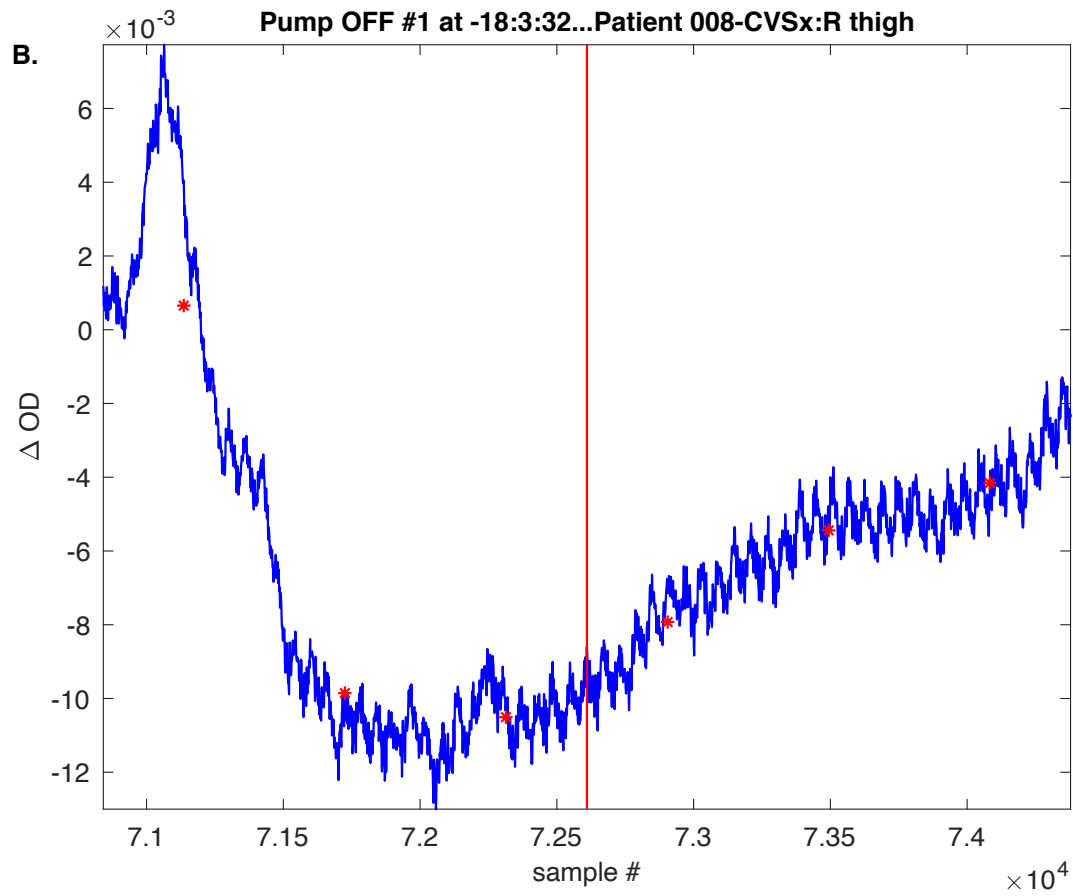
## Effects of Mechanical Ventilation and CPB Transitioning

Changes in OD versus sample number are presented at CPB on and CPB off time points (Fig 3.6A, Fig 3.6B). The solid red line indicates the occurrence of CPB on and CPB off respectively. Solid red dots demonstrate the average  $\Delta OD$  value for each three minute time period prior to and following each event. Of particular interest is the effect of mechanical ventilation on the NIRS signal with changes in  $\Delta OD$ . Respiratory frequencies were readily detectable with NIRS monitoring in this cohort. Figure 3.6A highlights changes noted as a result of mechanical ventilation prior to the commencement of CPB and the signal lost once CPB was established and the ventilator was turned off. In Figure 3.6B the respiratory signal reappears as the ventilator is turned on just prior to discontinuing CPB. Figure 3.6A also shows the dramatic decrease in  $\Delta OD$  that occurs when CPB is initiated due to the hemodilution effect of the crystalloid pump prime. Once CPB has been terminated  $\Delta OD$  trends in an upward direction as the patient is transfused further volume the HL machine thereby increasing blood volume and microvascular perfusion.

The effect of hemodilution upon  $\Delta OD$  at initiation of CPB for 19 patients is also shown in Figure 3.6C. The dotted line shows baseline  $\Delta OD$  value pre CPB. The y-axis represents  $\Delta OD$  calculated as the difference between the value of  $\Delta OD$  at CPB on and the lowest  $\Delta OD$  value achieved following CPB on, with a greater difference reflected as a more negative  $\Delta OD$  value (downward slope). The x-axis is viewed as delta Hb calculated as the difference between the patient pre CPB Hb and CPB on Hb representing the amount of hemodilution upon initiation of CPB. An increase in delta Hb is reflected in a more positive value and greater hemodilution. Linear regression shows here that  $\Delta OD$  is significantly reduced as a result of less circulating Hb in the patient. This translates into less MHC captured with NIRS device during a period of massive hemodilution.

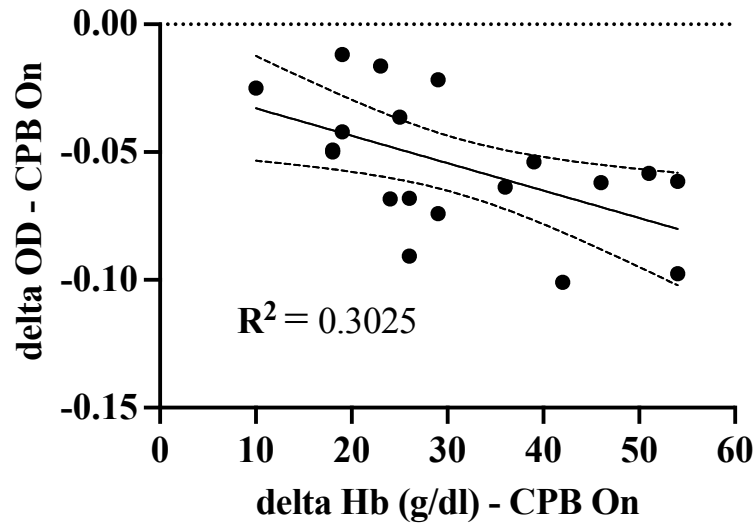


**Figure 3.6A:** represents  $\Delta OD$  at CPB On versus sample number. The effects of mechanical ventilation on  $\Delta OD$  prior to CPB on (solid red line), and effects of hemodilution on  $\Delta OD$  following CPB on.



**Figure 3.6B:** represents changes in OD versus sample number at CPB off. The return of mechanical ventilation is seen prior to CPB off (solid red line).

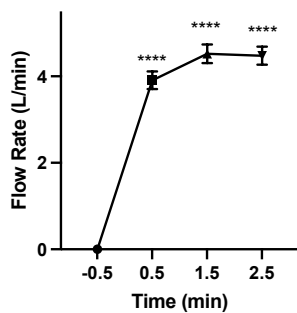
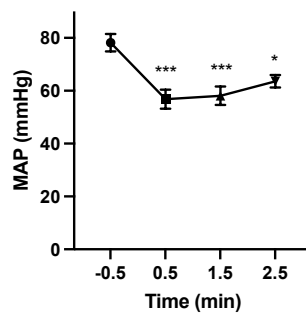
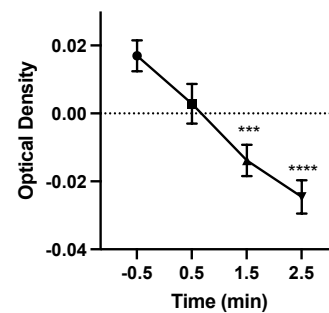
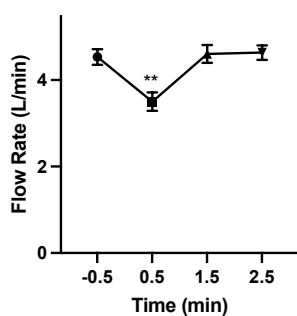
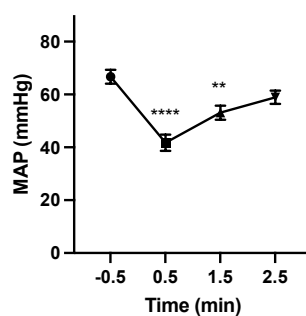
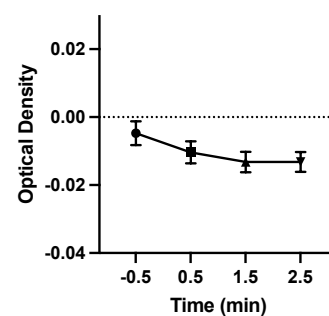
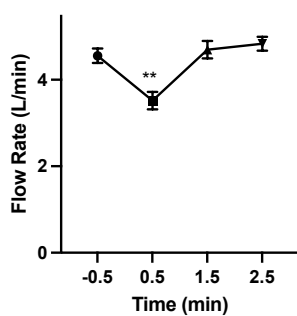
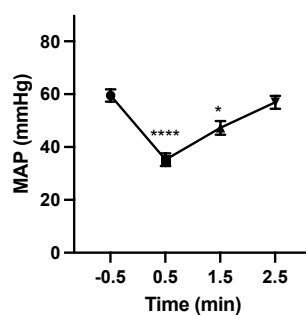
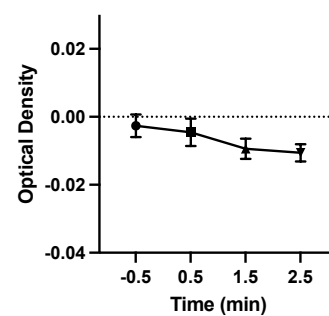
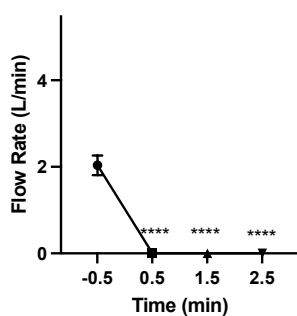
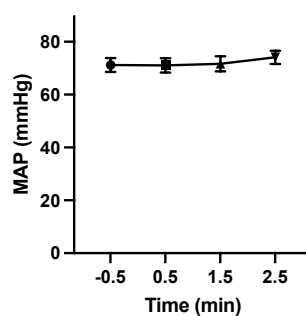
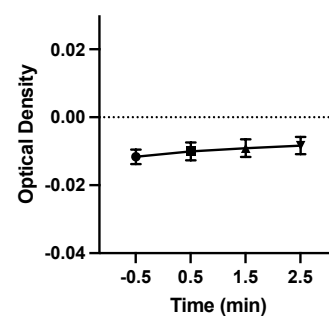
### C. Optical Density vs Hemoglobin - CPB On



**Figure 3.6C:** Univariate linear regression shows significant correlation with solid line. Dotted line represents 95% confidence interval.  $\Delta OD$  vs delta Hb at CPB on shows significant correlation, R square .3025, slope = 0.03950, Y-intercept 0.01325,  $p = 0.0147$ .

### Macrohemodynamics versus Microvascular Perfusion

Changes in  $\Delta OD$  affected by MAP and pump FR at four primary interventions (CPB on, XC on, XC off, and CPB off) are presented in Figure 3.7 for all patients. Time is shown on the x-axis with time = 0 representing the start of the event. The mean  $\pm$  SD is plotted for one minute prior to the event and for each three minutes following the event. A one-way ANOVA with Kruskal Wallis test was performed comparing the means of each minute following the event to the mean baseline value prior to the event. It appears that macrohemodynamics are dissociated from any changes in  $\Delta OD$ , except for initiation of CPB which is an important and dramatic event. Initiation of CPB shows a significant increase in FR resulting in a significant decrease in MAP and  $\Delta OD$  secondary to the effects of hemodilution (Fig 3.7A, Fig 3.7B, Fig 3.7C). Upon cross clamp application, FR is significantly decreased causing a significant decrease in MAP, with no significant correlation in  $\Delta OD$  (Fig 3.7D, Fig 3.7E, Fig 3.7F). As the aortic cross clamp is removed, FR is again significantly decreased resulting in a significant decrease in MAP, without significant correlation in  $\Delta OD$  (Fig 3.7G, Fig 3.7H, Fig 3.7I). Upon termination of CPB, pump FR is significantly decreased resulting in no changes in MAP and  $\Delta OD$  as the patient is weaned from CPB and transitions to native cardiac output (Fig 3.7J, Fig 3.7K, Fig 3.7L).

**A: CPB On - FR****B: CPB On - MAP****C: CPB On - OD****D: XC On - FR****E: XC On - MAP****F: XC On - OD****G: XC Off - FR****H: XC Off - MAP****I: XC Off - OD****J: CPB Off - FR****K: CPB Off - MAP****L: CPB Off - OD**

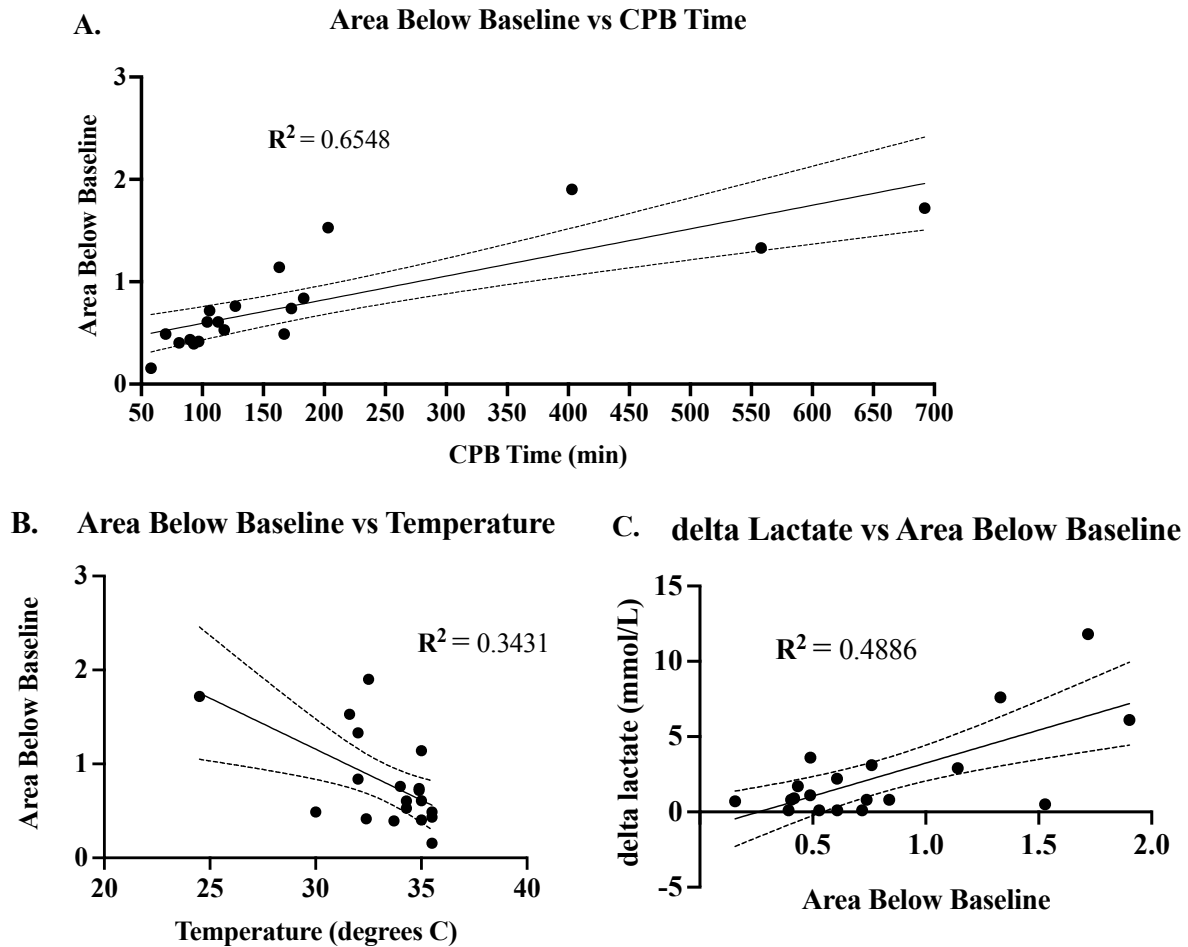
**Figure 3.7:** One-way ANOVA using Kruskal Wallis test representing rows of four primary interventions, and columns of FR, MAP, and  $\Delta OD$ . FR being the independent variable resulted in significant changes in dependent variables MAP and  $\Delta OD$ . \*\*\*\* p value < 0.0001, \*\*\* p value < 0.001, \*\* p value < 0.01, \* p value < 0.05.

### **Standardized Area Below Baseline and Average Area Below Baseline**

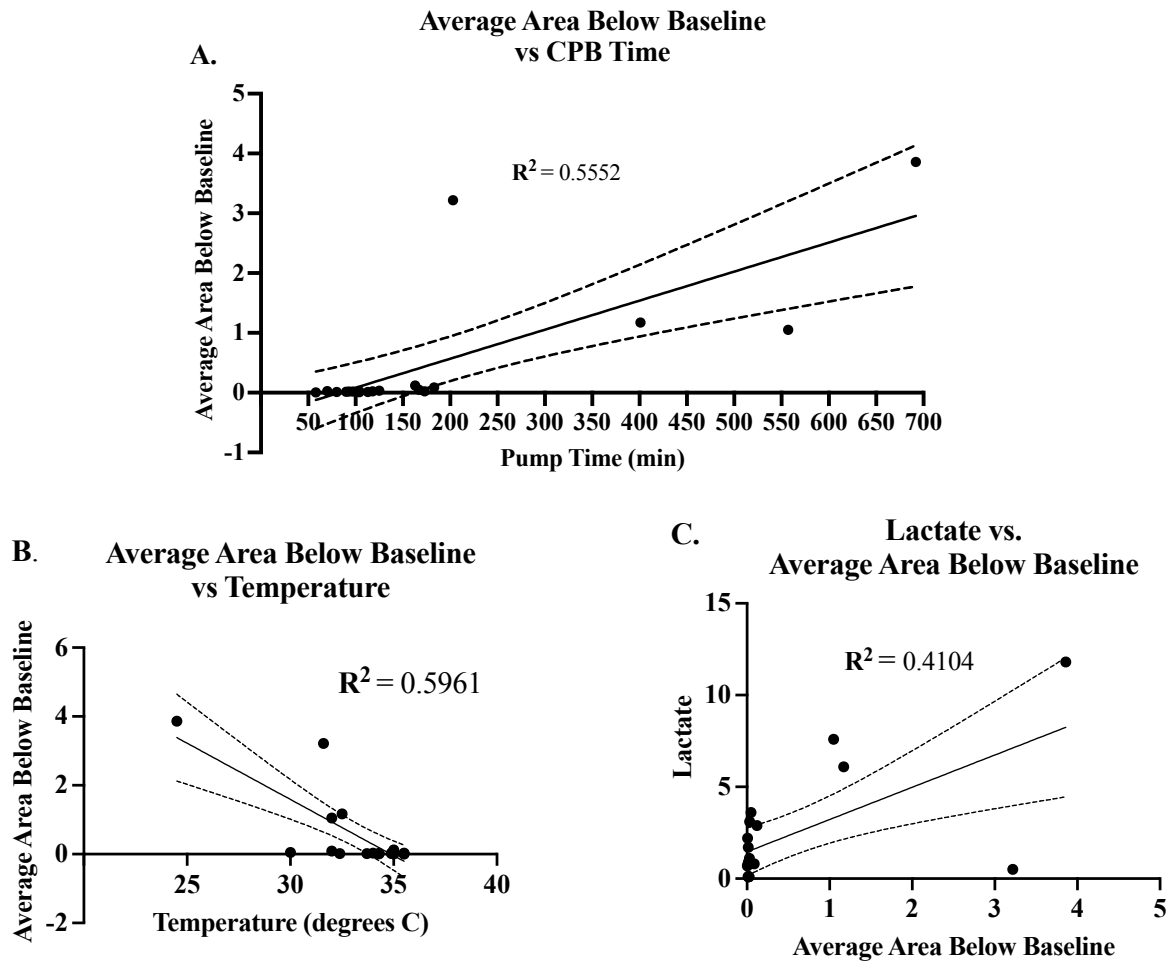
Following intubation and NIRS probe application, a mean baseline intensity was calculated for each patient over a time period of two minutes. The change in OD relative to the mean baseline intensity during this time interval was deemed as the patient's own baseline  $\Delta OD$  (value of zero). This value was calculated for all 19 patients. The area below baseline is  $\Delta OD$  (difference between absolute OD value and baseline) x time. We standardized these values for each patient by the amount of time spent on CPB through simply dividing by time. Therefore the units of standardized area below baseline is simply units of  $\Delta OD$  which in itself is unitless. Using univariate linear regression models the standardized area below baseline was affected by length of CPB time, and temperature (Fig 3.8A, Fig 3.8B). The area below baseline increased with increasing length of CPB time and a decrease in patient temperature. Subsequently, the greater amount of area below baseline resulted in elevated lactate levels, likely mediated through increased CPB time (Fig 3.8C). Results show that lactate accumulation occurs as a result of impaired microvascular perfusion secondary to increased bypass time and decrease temperature.

The area below baseline increasing with CPB time seems like a statement that is true by virtue of logic. Therefore another metric termed the average area below baseline was calculated to represent the magnitude, or how far below baseline was each patient. Here we show that both the length of CPB time and temperature also had an effect on the average area below baseline (Fig

3.9A, Fig 3.9B). As CPB time progresses beyond 200 minutes the average area below baseline significantly increases compared to values closer to zero under shorter pump runs. A very similar correlation occurred in those patients that were cooled to a nasopharyngeal temperature of less than 30-32 degrees Celsius. Also, with a significant increase in average area below baseline there was a significant rise in postoperative lactate levels. Alternatively, lactate levels remained much lower in patients with only a minimal increase in the average area below baseline (Fig 3.9C).



**Figure 3.8:** Univariate linear regression shows significant correlation with solid line. Dotted line represents 95% confidence interval. **Fig 3.8A:** Area below baseline vs CPB time shows significant correlation,  $R^2$  .6548, slope = 0.0004064, Y-intercept 0.1029,  $p < 0.0001$ ; **Fig 3.8B:** Area below baseline versus temperature shows significant correlation,  $R^2$  .3431, slope = 0.03642, Y-intercept 1.216,  $p = 0.0084$ ; **Fig 3.8C:** Delta lactate versus area below below baseline shows significant correlation  $R^2$  .4886, slope = 1.087, Y-intercept 1.013,  $p = 0.0009$ .



**Figure 3.9:** Univariate linear regression shows significant correlation with solid line. Dotted line represents 95% confidence interval. **Fig 3.9A:** Average area below baseline vs CPB time shows significant correlation,  $R^2$  0.5552, slope = 0.001054, Y-intercept 0.2665,  $p = 0.0003$ ; **Fig 3.9B:** Average area below baseline versus temperature shows significant correlation,  $R^2$  0.5961, slope = 0.06521, Y-intercept 2.178,  $p = 0.0001$ ; **Fig 3.9C:** Delta lactate versus average area below baseline shows significant correlation,  $R^2$  0.4104, slope = 0.5108, Y-intercept 0.6176,  $p = 0.0031$ .

## Results Multivariate Linear Regression

The first multivariable linear regression model revealed that the area below baseline was significantly dependent on the pump duration, but not on the lowest temperature on pump. In fact, for every 10-minutes increase in the pump time, the area below baseline was increased by 0.026 unit (95% Confidence Interval (CI) 0.012 to 0.040,  $p=0.001$ ). This was not the case for the lowest temperature on pump, with a non-significant coefficient of 0.023 (95%CI -0.069 to 0.115,  $p=0.60$ ).

The second multivariable linear regression model revealed that the post-pump lactate was significantly dependent on the area below baseline, but not the lowest temperature on pump. In fact, for every one-unit increase in the area below baseline, the post-pump lactate was increased by 87.6% (95%CI 9.3% to 221.7%,  $p=0.025$ ). This was not the case for the lowest temperature on pump, with a non-significant coefficient of -7.0% (95%CI -15.9% to 2.7%,  $p=0.14$ ).

The third multivariable linear regression model revealed that phenylephrine on pump was significantly dependent on the lowest temperature on pump, but not on the pump duration. In fact, for every one-unit increase in the temperature on pump, the phenylephrine was increased by 30.5% (95%CI 9.8% to 55.0%,  $p=0.005$ ). This was not the case for the pump duration, with a non-significant coefficient of 1.7% (95%CI -1.0% to 4.4%,  $p=0.20$ ).

In the sensitivity analysis of the third model, the multivariable linear regression model which included log phenylephrine as the outcome, and lowest temperature on pump, pre-pump lactate, and pre-pump hemoglobin as independent variables, also revealed that phenylephrine on pump was significantly dependent on the lowest temperature on pump, but not on the pump duration; however, it showed that phenylephrine on pump was significantly dependent on pre-pump hemoglobin. In fact, for every 10-unit increase in pre-pump hemoglobin, the phenylephrine was decreased by 15.7% (95%CI -27.4% to -2.1%,  $p=0.028$ ).

### 3.4 DISCUSSION

To help guide us during CPB we rely heavily on cardiac monitors that reflect global perfusion status or macro-hemodynamics. With the SIRS microvascular alterations can exist in the presence of normal macrovascular hemodynamics, thus making it difficult to identify and treat (De Backer et al., 2010). The overall goal of mitigating consequences as best as possible can be very challenging following CPB given the role of the SIRS in post-operative vital organ injury and dysfunction (Laffey et al., 2002). Injuries related to the SIRS are unavoidable during CPB, but can be minimized with the proper use of equipment and conduct of perfusion. To help alleviate the deleterious effects of bypass a real-time microvascular monitor describing underlying physiology can be very useful and permit earlier detection. Here we report on a pilot project and clinical validation of the successful design of a custom real time, dynamic, continuous microvascular monitor using near infrared spectroscopy for use in the operating room theatre.

Bedside evaluation of the microcirculation during CPB has evolved over the years using videomicroscopy technology such as OPS and SDF imaging (Bauer et al., 2007; De Backer et al., 2009; den Uil et al., 2008; Maier et al., 2009; O'Neil et al., 2018; O'Neil et al., 2012), along with NIRS technology that uniquely measures StO<sub>2</sub> in the microcirculation of the thenar muscle following a VOT. These research systems are used to invoke a quantifiable measure of microvascular responsiveness to an I/R challenge induced by brachial artery occlusion (O'Neil et al., 2018). The aforementioned techniques provide only a functional snapshot of the microcirculation, whereas technology in the current study applies a continuous measure of microcirculatory function. This new approach is also advantageous because it is non-invasive, operator independent, offers deep tissue measurements, and is not subject to semi-quantitative analysis.

The primary advantage of constructing a personal NIRS device was to gain access to the raw light intensity data returning to the spectrometer at a high enough frequency to perform our own analysis, whereas commercial NIRS devices do not provide this data. Light intensities were recorded at the isosbestic wavelength 798 nm with a sampling frequency of 9.3 Hz (9.3 samples per second) whereas most conventional NIRS systems capture values at approximately one sample every ten seconds. The high resolution NIRS device used in this study specifically measures the temporal change in MHC, as a surrogate for RBC flow within the underlying microcirculation (Mendelson et al., 2021). Conventional NIRS devices only provide relative values of tissue oxygenation, what they don't account for is oxygen supply controlled by the microcirculation. It is that control mechanism which is just as important as the oxygen saturation values themselves. Instead of focusing solely on relative changes, adding information with functional marker of how well the microvascular is regulating can help personalize our therapies based on these markers.

As a feasibility study the changes in MHC witnessed during mechanical ventilation was encouraging and added strength to the fact that we were able to detect what we had hoped for. In patients who are ventilated, intrathoracic pressure increases during inspiration thereby causing a decrease in venous return and expansion of blood volume in the peripheral venous compartment (Leung et al., 2008; Wolf et al., 1997). Similar variations with mechanical ventilation have previously been demonstrated with cerebral NIRS to measure regional venous saturation (Wolf et al., 1997). And more recently the effect of mechanical ventilation on MHC was also documented by our lab in ICU patients using the same NIRS technology (Mendelson et al., 2021). In this study we were able to capture changes in  $\Delta OD$  also termed MHC in sync with each mechanical breath of the ventilator as venous return fluctuates prior to CPB (Fig 3.6A). The signal disappeared upon initiation of CPB when the heart and lungs were removed from circulation and taken over by the

HL machine (Fig 3.6A), and recurred just prior to termination of CPB upon reinstitution of mechanical ventilation (Fig 3.6B). The magnitude of MHC displaced by mechanical ventilation likely depends on the interaction between ventilator parameters, pulmonary mechanics, and patient hemodynamics, but is unclear whether there are direct consequences on organ function (Mendelson et al., 2021).

Along with the NIRS signal response seen with mechanical ventilation, it was also very encouraging to see changes in  $\Delta OD$  related to changes in macrohemodynamics at four chosen critical time points during CPB (Fig 3.7). This is particularly important as the literature suggests that macrohemodynamics is limited in predicting the state of the microcirculation (De Backer et al., 2010), and the imbalance between macrohemodynamics and microvascular perfusion has been well documented in patients with sepsis using OPS imaging (De Backer et al., 2002). Microcirculatory alterations despite normal hemodynamics found using SDF imaging in the early post-operative period of patients requiring major abdominal surgery has also been associated with an increased risk of complications (Jhanji et al., 2009).

In cardiac surgery patients, a study by Bauer using OPS imaging reported no significant alterations in sublingual microvascular perfusion velocity during non-pulsatile (NP) CPB while maintaining adequate macrohemodynamics. However, they do report a moderate reduction in functional capillary density (FCD) well after cross clamp removal under hypothermic conditions (Bauer et al., 2007). Similarly, a pulsatile (P) versus NP flow study also found no significant differences in the proportion of perfused small vessels during CPB, however, alterations in blood flow patterns were documented following CPB and persisted for 24 hours post-operatively (O'Neil et al., 2012). Unlike the Bauer study, they did not find any perfusion deficits during the late stages of CPB as their patients were typically normothermic prior to cross clamp removal. In an animal

study using IVVM, microvascular perfusion was shown to be markedly reduced during hypothermia, but was completely restored upon rewarming (Kamler et al., 2005), therefore reperfusion at a decreased temperature following aortic cross clamp removal may have been a contributing factor in the Bauer group results. Another study by DeBacker also investigated the sublingual microcirculation in patients undergoing cardiac surgery with NP CPB while maintaining adequate macrohemodynamics (De Backer et al., 2009). Their results parallel the O'Neil study in that the proportion of perfused vessels (PPV %) were decreased post-operatively for up to 24 hours. However, they also report significant alterations during CPB, whereas the O'Neil study did not. One of the differences between the two studies lies in their methodologies, as results from the Bauer study were compared to baseline values obtained prior to anesthesia. Recordings from the O'Neil group were captured following the induction of anesthesia, therefore results were a true reflection of the effects of CPB and any anesthesia-mediated alterations in microvascular perfusion and/or inflammation were appropriately controlled. The DeBacker study also report a significant decrease in microvascular perfusion within a group of patients subjected to off-pump cardiac surgery with native pulsatile flow. However, the O'Neil study did not show any significant deficits in their P perfusion group despite exposure to CPB and its deleterious effects. The systemic inflammatory response resulting from off pump cardiac surgery may not be much less anticipated compared to CPB, as manipulation of the heart during beating heart surgery may cause a reduction in venous return leading to systemic hypoperfusion and ischemic reperfusion injury (Fransen et al., 1998). Using OPS imaging in our previous study we also have shown that microvascular perfusion alterations exist during and after CPB despite optimization in global hemodynamics when comparing P vs NP flow (O'Neil et al., 2018). These results are also in line with the DeBacker study which showed perfusion alterations during and after CPB (De

Backer et al., 2009).

The four particular time points chosen in this study are representative of an external stimulus imposed upon the microcirculation by the perfusionist that would normally not occur without the use of CPB. In this current study it appears that macrohemodynamics are dissociated from any changes in  $\Delta OD$ , except for initiation of CPB which is an important and dramatic event (Fig 3.7C). The absence in correlation in  $\Delta OD$  we see at various time points (Fig 3.7F, Fig 3.7I) is an interesting finding, as it supports the ability of the microcirculation to regulate oxygen delivery independently from changes in MAP and FR, except for initiation of CPB due to the massive change in systemic Hb levels. Upon initiation of CPB the transition from native cardiac output to an artificial non-pulsatile output occurs, along with the introduction of extensive hemodilution. The massive effect of hemodilution upon initiation of CPB is unavoidable in most cases. Once the crystalloid and blood mixture stabilize at a reduced hematocrit level, blood viscosity and oxygen carrying capacity are decreased compared to pre CPB. In this study the extreme nature of both events cause macrovascular and microvascular values to be affected in the same direction. The effect of hemodilution resulted in a significant decrease in both patient systemic Hb concentration (Fig 3.3A) and  $\Delta OD$  (Fig 3.6A, Fig 3.7C). MAP also decreased upon initiation of CPB likely due to a dramatic decrease in systemic vascular resistance (SVR) secondary to the hemodilution (Fig 3.7B). This effect is can also be potentiated by a decrease in vascular tone secondary to hemodilution of circulating catecholamines and temporary hypoxemia at the onset of CPB. Retrograde autologous priming (RAP) is a maneuver that can be applied to minimize the effects of hemodilution prior to initiation of CPB by displacing portion of the pump prime with blood from the patient aorta. To avoid the risk of hemodynamic instability prior to

commencing CPB, RAP was avoided with the majority of patients presenting with aortic stenosis and afterload dependency.

With regards to aortic cross clamp application and its removal, MHC was unchanged under the presence of abnormal macrohemodynamics. In both instances the intervention of decreasing pump FR by the perfusionist (Fig 3.7D; Fig 3.7G) resulted in a significant decrease in MAP (Fig 3.7E; Fig 3.7H) but had no significant effect on  $\Delta OD$  values (Fig 3.7F; Fig 3.7I). In this case MAP was completely dependent on pump FR which is the goal during application and removal of the aortic XC. A decreased MAP is desired in an attempt to avoid catastrophic events such as an aortic dissection while applying clamps to the aorta. Finally at termination of CPB, a smooth transition occurred from the pump to the patient native cardiac output without changes in MHC (Fig 3.7J-L). In fact  $\Delta OD$  shows a slight increase following CPB, most likely due to reinfusion of the remaining pump blood therefore increasing total blood volume and microvascular perfusion.

Although acceptable flow rates are fairly well established during CPB, there is more controversy surrounding sufficient MAP during CPB. At any given flow rate, there is distinct variability in arterial pressure from patient to patient. Short periods of hypotension with a MAP of 30 are certainly well tolerated, however a MAP of less than 30 mmHg is below the critical closing pressure of some vascular beds increasing the risk of hypoperfusion. The concern with hypotension is the adequacy of organ perfusion, with the brain and kidney being at a higher risk. For most patients MAP is kept between 50-100mmHg during CPB with cerebral blood flow being pressure dependent, in addition to maintaining sufficient urine output.

Progressive hemodilution during CPB has been shown to cause microcirculatory dysfunction and to depress oxygen delivery via a diminished capillary density by reducing blood viscosity (Atasever et al., 2011; De Backer et al., 2009). The lowest systemic Hct or Hb level on CPB is a

recognized independent risk factor for major morbidity and mortality in cardiac surgery patients as a result of poor oxygen delivery and dysoxia to end organs (Habib et al., 2003). Compared to systemic Hct levels, the Hct in narrow vessels is reduced due to concentration of fast flowing RBC's in the center, and of slower flowing plasma along the wall of the vessel, which in combination with plasma skimming at bifurcations leads to the striking heterogeneity of local hematocrit in branching capillary networks known as the network Fahraeus effect (Reinhart et al., 2017). The use of NIRS in this study does not measure absolute microvascular Hct or Hb levels, but what it does show is how the regulatory system will try to compensate to maintain oxygen delivery seen by changes in  $\Delta OD$ . In this study, as patients are hemodiluted upon initiation of bypass, systemic Hct levels remain significantly decreased during CPB (Fig 3.3A), however MHC does not remain below baseline and has a tendency to rebound and recover in most patients (Fig 3.5C). Some patients spend time above and below the area below baseline and some have a greater magnitude of being above or below baseline. A healthy functioning microvasculature will try to regulate and respond to hemodilution, which is likely the case in those patients that recovered from hemodilution with pump times less than 200 minutes (Fig 3.8A, Fig 3.9A). If they do not recover the area below baseline increases and correlate with our increased lactate data (Fig 3.8C, Fig 3.9C). The exact regulatory mechanism behind maintaining microvascular perfusion cannot be determined in this study and is a topic for future research in the laboratory.

Hyperlactatemia is a marker of dysoxic metabolism, and is also associated with bad outcomes in cardiac surgery (Ranucci, Carboni, et al., 2015). A previous study by DeBacker also showed that CPB exhibited a decrease in the proportion of perfused microvessels using OPS imaging and a subsequent increase in lactate levels (De Backer et al., 2009). Our study shows a significant decrease in systemic Hb along with significant increase in peak lactate levels during and after CPB

(Fig 3.3A-B). A positive linear relationship between lowest level of Hb on pump and peak lactate was also shown (Fig 3.4B). Non-pulsatile perfusion has also been linked to increases in lactate during CPB due to microcirculatory shunting and widespread capillary collapse resulting in reduced oxygen consumption and tissue acidosis (Ji & Undar, 2007; O'Neil et al., 2012). If tissue hypoperfusion exists, serum lactate levels should increase secondary to decreased oxygen delivery and increased anaerobic metabolism (Ranucci, De Toffol, et al., 2006). Unfortunately, serum lactate levels have not proven to be useful in the regular monitoring of tissue perfusion due to their variable nature. With our novel device we were also able to recognize that lactate production was affected by the measured by  $\Delta OD$  area below baseline (Fig 3.8C), and also that a significant increase in average area below baseline resulted in a significant rise in postoperative lactate levels. Alternatively, lactate levels remained much lower in patients with only a minimal increase in the average area below baseline (Fig 3.9C).

Our multivariable linear regression model also revealed that post-pump lactate was significantly dependent on the area below baseline during CPB. In fact, for every one-unit increase in the area below baseline, the post-pump lactate was increased by 87.6% (95% Confidence Interval (CI) 9.3% to 221.7%,  $p=0.025$ ). Our findings regarding lactate fall in line with studies using sublingual videomicroscopy that showed low perfused vessel density and high microcirculatory heterogeneity associated with and increased intensity and duration of lactic acidosis after cardiac surgery with CPB (Greenwood et al., 2021; O'Neil et al., 2012). We also showed that as the average area below baseline significantly increases the postoperative lactate levels were also elevated.

Prolonged bypass time has also been documented to impair microvascular perfusion. In previous studies patients were considered high risk if predicted to be on pump for greater than 2

hours. Results showed alterations in microvascular perfusion post CPB with extended pump times beyond this time frame (O'Neil et al., 2012). Increased levels of plasma free hemoglobin and hemolysis have been associated with longer CPB times (Vercaemst, 2008; Vermeulen Windsant et al., 2011), and have been documented to scavenge and deplete nitric oxide (NO) (Gow & Stamler, 1998; Morris et al., 2008; Reiter et al., 2002). Along with increased plasma free hemoglobin and NO consumption, prolonged CPB times have been linked to increased pulmonary and systemic vascular resistance (Rezoagli et al., 2017). Prolonged CPB times increase the risk of hemolysis due to the excessive amount of suction blood, the use of cell salvage devices, and the increased risk of stored RBC transfusion, all of which are major sources of plasma free Hb during and after CPB (Bennett-Guerrero et al., 2007; Vermeulen Windsant et al., 2011; Wright, 2001; Yazer et al., 2008).

In this study patients were not deemed high risk with the majority of cases involving valvular procedures with shorter predicted bypass times. As complications arose in some patients the effects on the microvasculature become more apparent as pump time was extended. This study shows the standardized area below baseline was affected by length of bypass with a significant increase as length of CPB time increases (Fig 3.8A). This resulted in elevated lactate levels that were likely mediated through increased CPB time (Fig 3.8C). The magnitude of the average area below baseline was also affected by pump times greater than 200 minutes, as the average area below baseline significantly increased compared to a shorter duration of CPB (Fig 3.9A). Therefore, the longer the pump run not only do we show we spend more time below baseline we are also seeing that the magnitude becomes greater the longer you are on pump.

CPB times extending beyond two hours have been associated with an increase in complement activation resulting in C5a stimulation of neutrophil aggregation and adherence to endothelium

(Kirklin et al., 1983). Increases in C3a levels are also associated with increased ventilation times following CPB (Moore et al., 1988), and CPB times of 120-150 min. have also been reported to result in pulmonary dysfunction (Kirklin et al., 1983). Prolonged CPB times have also been linked to impaired microvascular perfusion with increased levels of lactate (Ranucci, De Toffol, et al., 2006). Here we show the effects of increased pump time on lactate (Fig 3.4A) and that longer pump times have significant effects on the microcirculation with an increase in  $\Delta OD$  area below baseline and the average area below baseline (Fig 3.8A, Fig 3.9A). Our multivariable linear regression model also revealed that area below baseline was significantly dependent on the pump duration. In fact, for every 10 minute increase in the pump time, the area below baseline was increased by 0.026 unit (95% CI 0.012 to 0.040,  $p=0.001$ ).

Hypothermia reduces cellular metabolic activity and oxygen consumption, but also increases red cell aggregation and blood viscosity (Sakai et al., 1988). The latter effect may be counteracted by the beneficial effects of hemodilution to improve cerebral, cardiac, and renal blood flow during CPB. Hypothermia also permits the use of lower pump flows, thereby decreasing blood trauma and permitting a longer safe bypass period. In this study we were able to demonstrate an increase in the standardized area below baseline and average area below baseline in those patients actively cooled to a nasopharyngeal temperature of less than 30-32 degrees Celsius (Fig. 8B,9B), along with a buildup of lactate (Fig 3.4C). However, our multivariable linear regression model revealed that the area below baseline was not significantly dependent on the lowest temperature on pump, nor was post-pump lactate dependent on the lowest temperature on pump.

Vasoconstriction due to hypothermia also correlated with the usage of less phenylephrine, and as patients were rewarmed in this study a greater amount of phenylephrine was needed likely due to the effects of vasodilation (Fig 3.4D). Once normal body temperature is restored, oxygen

transport must also rise, but this may be difficult to achieve when Hb levels are too low, causing mixed venous oxygen saturations to decrease. Rewarming usually dilates the capacitance vessels in the body, and increases fluid requirements. If attempts at maintaining oxygen delivery by increasing pump FR is unsuccessful, a decision has to be made whether to administer additional fluid with blood or a blood free solution. Hemodilution, which is advantageous during hypothermic CPB, may be undesirable during the later normothermic phases of CPB or after termination of CPB. However, despite these concerns, adult patients infrequently receive blood transfusions during CPB unless absolutely necessary to avoid the risks associated with donor blood.

In conclusion, we were able to successfully apply an optical device previously designed for ICU patients to continuously monitor microvascular function in real time in the cardiac surgical operating room. Given the slower change of StO<sub>2</sub> in skeletal muscle (i.e. regional venous oxygen saturation), StO<sub>2</sub> functions mostly as a trend monitor over minutes to hours rather than second-to-second variability as observed with MHC. Trends that we see in MHC gives us confidence in the measurements that we are making. We have shown that we can track changes in MHC during various interventions during CPB. Mechanical ventilation and hemodilution evoke microvascular OD responses, while changes in FR affect macrovascular (MAP) responses with simultaneous trends in microvascular (OD). A monitor of this nature would be best used for personalized management of CPB, identifying high-risk events, and optimization of macrohemodynamics and microhemodynamics.

This technology should continue to be worked on with the goal of verifying that MHC below baseline values could be a surrogate for low oxygen delivery, and studied to see if there is a correlation with clinical outcomes and potential therapeutic targets. Key metrics established

from this study need refinement in order to help guide the perfusionist to improve best practice. Maintaining MHC at or above baseline levels, and reducing the amount of time and magnitude spent below a certain threshold may be prognostic for adverse outcomes. This current data is a compelling pre-requisite to this goal.

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## CHAPTER 4: CONTINUOUS WAVELET TRANSFORM ANALYSIS

### 4.1 INTRODUCTION

The main function of the microcirculation is to deliver oxygen and nutrients to the tissues as well as the removal of carbon dioxide. It also serves to regulate blood flow and tissue perfusion based upon oxygen supply and demand. As oxygen supply and demand changes the arterioles respond by redistributing blood flow to the capillaries by vasoconstriction or vasodilation (Ellis et al., 2005; Segal, 2005). It is through regulation of vascular tone that arterioles control the distribution of blood flow to organs via their thick layer of muscle cells on the vessel wall (Bateman et al., 2003). The red blood cell (RBC) plays a significant role in coordinating the autoregulatory response to changes in oxygen demand or oxygen delivery. An increase in oxygen demand results in a decrease in oxygen saturation ( $SO_2$ ) within the RBC. In response to the drop in  $SO_2$ , RBC's release adenosine triphosphate (ATP) which bind to purinergic receptors on vascular endothelium. Although there is a gradient in  $SO_2$  down the arteriolar tree that likely causes vasodilation of arterioles, the greatest effect occurs in the capillary bed where every RBC experiences a drop in  $SO_2$  as they pass through the capillaries and the subsequent release of ATP. This also results in a conducted vasodilator signal by the electrically coupled endothelium along the arteriolar tree from the capillary bed (Bagher & Segal, 2011; Ellis et al., 2012; Ellsworth et al., 2016). Therefore, oxygen supply is increased by vasodilation, delivering more RBC's to areas in need of oxygen.

Microvascular hemoglobin content (MHC) is a term previously used by our lab to signify the total hemoglobin (Hb) contained in all the RBC's of the microcirculation. Therefore, variability in MHC may represent the continuous regulation of RBC's within the microcirculation

(Mendelson et al., 2021). Given the role of the RBC, we feel that MHC will respond to changes in metabolic demand and key interventions during CPB, and potentially serve as a novel hemodynamic metric for monitoring the dynamic regulation of microvascular blood flow in cardiac patients undergoing extra-corporeal circulation. Information gained from conventional near infrared spectroscopy (NIRS) devices provide only relative values of tissue oxygenation (StO<sub>2</sub>). What they don't account for is the time-dependent variability and biologically dynamic regulation of oxygen supply controlled by the microcirculation. Instead of focusing solely on relative changes in StO<sub>2</sub>, adding information with a functional marker of how well the microvasculature is regulating can help personalize our therapies during CPB.

Laser Doppler Flowmetry (LDF) is a widely accepted technology that can provide continuous non-invasive microcirculatory perfusion measurements of the skin (Holowatz et al., 2008). Mechanisms of cutaneous microcirculatory function and dysfunction that have been studied using LDF may also be representative of generalized systemic vascular dysfunction (Abularrage et al., 2005; IJzerman et al., 2003). This allows researchers to study the periodic oscillations that occur as a result of rhythmic tone changes within the microvasculature (Reynès et al., 2020). There are six frequency bands that can be derived from high resolution laser doppler studies, each associated with a proposed physiological event (Table 4.1). These events include intrinsic or local mechanisms such as B1-metabolic (0.005-0.0095 Hz), B2 endothelial (0.0095-0.02 Hz), B3 neurogenic (0.02-0.06 Hz), and B4 myogenic (0.06-0.15 Hz) regulation. As well as extrinsic or central mechanisms such as B5 respiratory (0.15-0.667 Hz) and B6 cardiac (0.667-3.16 Hz) rhythms (Kvandal et al., 2006; Stefanovska et al., 1999). Using continuous wavelet transform (CWT), a power versus frequency versus time series analysis can be representative of oscillations attributed to these components of the microvasculature (Bernjak et al., 2008). In our

previous study, we used a high-resolution continuous wave broadband NIRS device to capture real time changes in optical density ( $\Delta OD$ ) at the isosbestic wavelength of Hb in the NIR range (798nm). Feasibility of this custom monitor using NIRS technology was previously tested by our lab on critically ill patients in the intensive care unit (ICU) (Mendelson et al., 2021). The goal of this current study is to apply a CWT analysis to the  $\Delta OD$  data collected to reflect the time-dependent variability of microvascular hemoglobin content (MHC) at key time points during cardiopulmonary bypass (CPB), thus providing us with dynamic information on the microvascular regulation of oxygen supply to match O<sub>2</sub> demand.

**Table 4.1: Frequency Bands**

Frequency Band	Frequency (Hz)	Periods (seconds)	Cycles/minute
B1-Metabolic	0.005-0.0095	105.3-200	0.3-0.57
B2-Endothelial	0.0095-0.02	50-105.3	0.57-1.2
B3-Neurogenic	0.02-0.06	16.67-50	1.2-3.6
B4-Myogenic	0.06-0.16	6.25-16.67	3.6-9.6
B5-Respiratory	0.15-0.667	1.5-6.25	9.6-40
B6-Cardiac	0.667-3.16	0.32-1.5	40-186

There are six frequency bands that can be derived from high resolution laser doppler studies, each associated with a proposed physiological event. These events include intrinsic or local mechanisms (frequency bands B1-B4), as well as extrinsic or central mechanisms (respiratory-B5; cardiac- B6) (Mendelson et al., 2021).

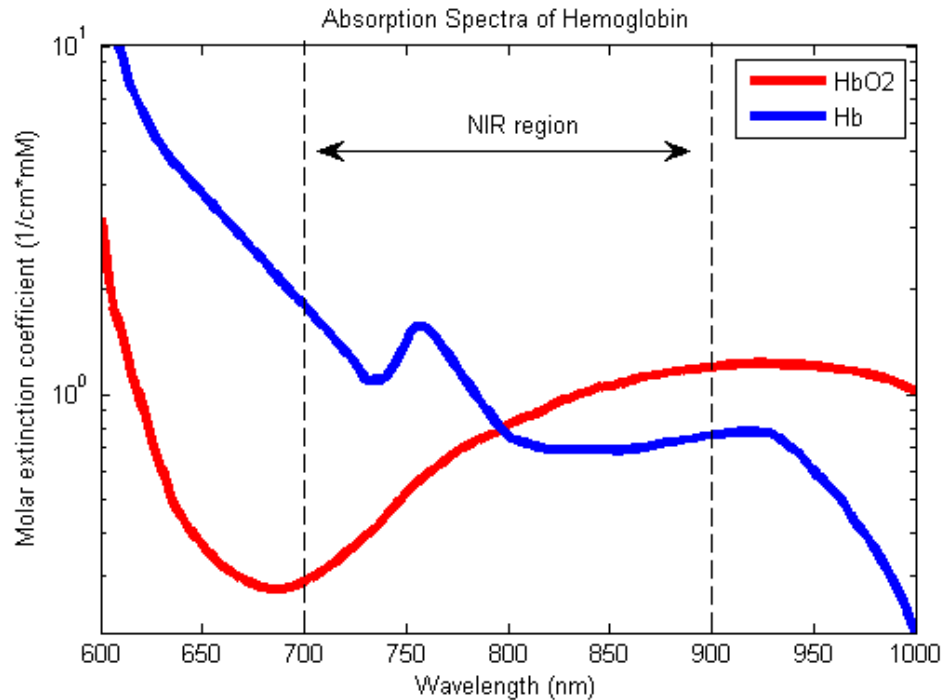
## 4.2 METHODS

### Study Design

This is a feasibility prospective cohort study that met the approval of the local Human Ethics Board at Western University. Informed written consent was obtained on the day of surgery from nineteen cardiac surgical patients undergoing non-pulsatile CPB at University Hospital, London Health Sciences Centre. All adult patients admitted for elective cardiac surgery involving any valve repair/replacement, and aortic replacement procedures were invited in this study. Exclusion criteria included patients requiring coronary artery bypass grafting and cases where pre-operative consent was unattainable.

### Near Infrared Spectroscopy

A continuous wave broadband NIRS device was previously designed and built by our lab. This new technology was successfully used to measure variability in MHC or total Hb concentration in skeletal muscle of critically ill patients in the ICU (Mendelson et. al, 2021). In our previous study we successfully transferred this technology to the cardiac surgical operating room theatre to track how MHC varies with time in patients undergoing CPB. We do this by processing the light intensity signal from the spectrometer at high temporal resolution (8-10 Hz) at the Isosbestic point in the NIR range (798 nm). This means that the amount of light absorbed by Hb is independent of whether its oxygenated or not. We do not measure Hb directly; we measure  $\Delta OD$  at the isosbestic wavelength which gives a measure of changes in Hb over time (Fig 4.1).



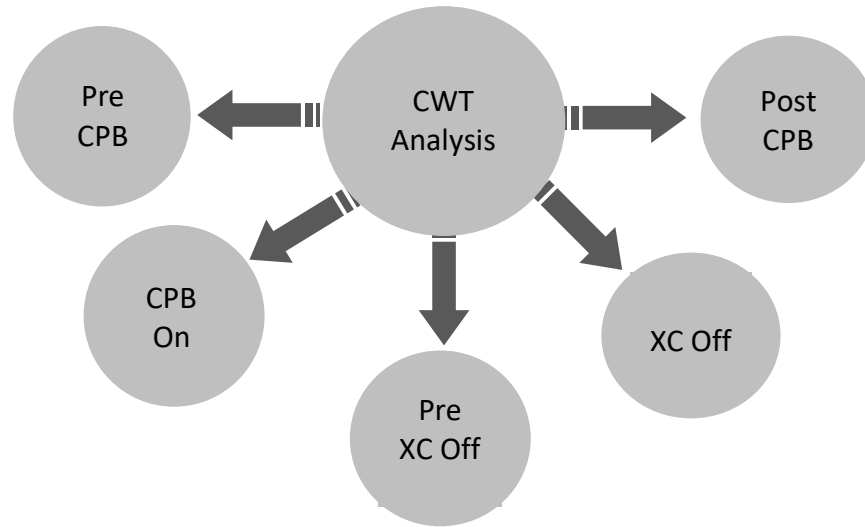
**Figure 4.1:** Our analysis consists of changes in OD at the Isosbestic wavelength of Hb in the NIR range (798nm). [https://commons.wikimedia.org/wiki/File:Oxy\\_and\\_Deoxy\\_Hemoglobin\\_Near-Infrared\\_absorption\\_spectra.png](https://commons.wikimedia.org/wiki/File:Oxy_and_Deoxy_Hemoglobin_Near-Infrared_absorption_spectra.png)

The NIRS device consists of a modified QE65000 spectrometer (Ocean Optics, Dunedin, Florida), a broadband light source (Ocean Optics HL-2000-HP), custom detection fiber bundles (Fiberoptics Technology, Inc., Pomfret, Connecticut) and prisms (Thorlabs, Newton, NJ). A 3D printed probe holder was used to secure the fiber bundles and allow for proper positioning on the right thigh of the patient. As the light source travels down the fiber bundles and illuminates the tissue, a small fraction of light returns to the spectrometer via the detection fibers after being scattered within the tissue and absorbed by Hb and other chromophores. The returning light intensities are recorded using Spectra Suite™ software (Ocean Optics, Inc.). The spectral analysis of light collected yields quantitative data on Hb and oxygen saturation levels over a wide range of

wavelengths from 600 to 900 nm. A custom MATLAB program was used to convert isosbestic wavelength intensities captured by Spectra Suite to MATLAB files, and subsequently converted to a  $\Delta OD$  time series. A steady state two minute time interval of light intensity values was used as the baseline for  $\Delta OD$  calculations.

### **Data Collection**

Patient demographics including age, sex, body surface area, and pre-operative risk factors were obtained for all participants. Hemodynamic monitoring for heart rate, mean arterial pressure (MAP), temperature, and cardiac index were recorded continuously throughout the study. Arterial blood samples were drawn at various time intervals for analysis of acid/base and oxygenation status, hemoglobin, and lactate levels. Continuous NIRS sampling of the right thigh was initiated following general anesthesia, and concluded beyond termination of CPB just prior to chest closure. A steady state two minute time interval of baseline  $\Delta OD$  values were recorded for each patient following NIRS probe application. A CWT analysis was applied to all patients at specific 10 minute time intervals during bypass: Pre CPB; CPB On (beginning of pump flow); Pre Aortic Cross-Clamp Off (Pre XC Off); Cross-Clamp Off (XC Off); Post CPB Off (Fig 4.2).



**Figure 4.2:** CWT analysis was performed for a duration of 10 minutes at various time points throughout the study. Pre CPB time interval occurred following induction of anesthesia; CPB On time interval occurred at initiation of CPB (including XC application), Pre XC Off time interval ended two minutes prior to XC removal, XC Off time interval began as XC was removed, and Post CPB time interval occurred following termination of bypass.

### Cardiopulmonary Bypass

CPB was performed using the Jostra HL 20 heart-lung machine (Maquet-Dynamed Inc.). The extra-corporeal circuit was composed of: x-coating custom tubing pack (Terumo Cardiovascular Systems), Capiiox FX25 integrated membrane oxygenator (Terumo Cardiovascular Systems), and the Myocardial Protection System for microplegic arrest (Quest Medical Inc.). The ascending aorta was the preferred site for arterial inflow to the patient during conventional bypass cases, while the femoral artery was used for all minimally invasive cardiac procedures. Approximately 1.5 L of Lactated Ringers was used as a standard priming solution for all CPB circuits.

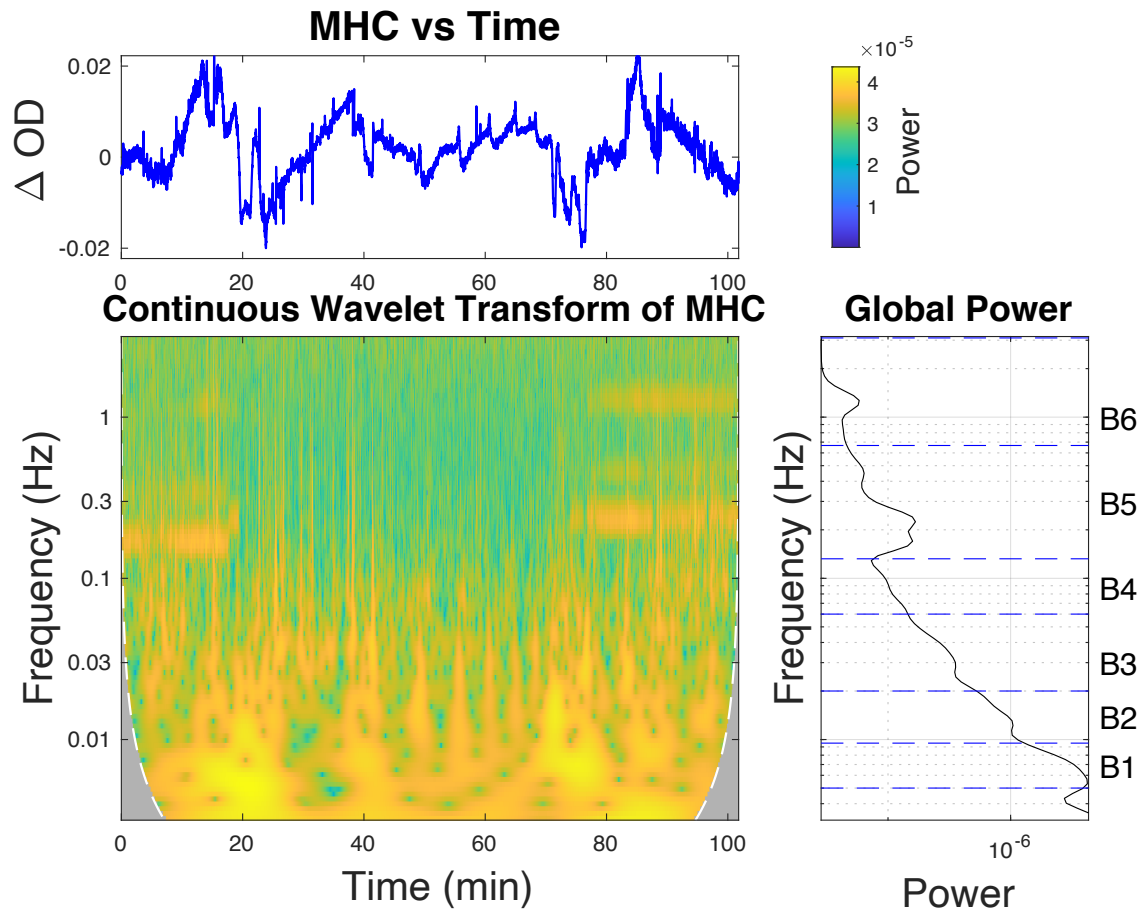
Prior to initiation of bypass, patients were heparinized to a safe level of anticoagulation with an activated clotting time greater than 480 seconds. Following initiation of CPB and the mechanical ventilator turned off, the aortic cross clamp was applied to dissociate the cardiopulmonary circulation from the system circulation to enable the heart to be arrested using a microplegia potassium based cardioplegia solution. During the course of CPB, patients were cooled to a systemic nasopharyngeal temperature of 34-35°C unless directed otherwise by the cardiac surgeon. A minimum cardiac index of 2.2-2.4 L/min/m<sup>2</sup> was maintained for all patients by adjusting the pump flow rate (FR) of the heart-lung machine. A minimum MAP of 60 mmHg was targeted with administration of phenylephrine boluses as needed. Anesthetic maintenance during CPB was managed with an inline sevoflurane gas mixture which was blended in with gas flow delivery to the oxygenator. Following the surgical repair, the aortic cross clamp was removed, allowing the ischemic heart to be perfused with blood and convert back to a normal sinus rhythm. Upon weaning from bypass, the ventilator was turned back on and the pump FR was slowly turned down and off. Following CPB, the administration of vasopressors and inotropic support medications deemed necessary by the anesthesiologist was given to maintain adequate MAP and heart function.

### **Continuous Wavelet Transform**

A CWT was applied to  $\Delta OD$  (MHC) time series of the skeletal muscle for continuous perfusion monitoring. Periodic oscillations that occur as a result of rhythmic tone changes within the microvasculature can be detected and categorized into specific bands, each associated with a proposed physiological regulatory mechanisms of the cardiovascular system (Table 1). Figure 4.3 shows a single patient data set, with  $\Delta OD$  time series represented by the blue curve at the top. The coloured image is a plot of power vs frequency vs time. The colour tells us what power is

associated with the wavelet at every frequency over the time interval. The figure to the right shows the global power averaged for each frequency band over time starting with highest frequencies at the top. Bands 2-4 represent the microvascular bands which will be the primary focus in this study.

All patients were managed routinely except for the intra-operative application of a NIRS probe placed on the right thigh muscle following general anesthesia, with continuous sampling concluding post bypass. CWT analysis was also applied to all patients at specific 10-minute time intervals during the course CPB. Since the heart is arrested and the ventilator is turned off during CPB, very little NIRS signal is associated with B5 and B6. Therefore microvascular bands (B2-B4) are interrogated and reported during CPB as median power and the percent contribution of each frequency band (% power). The total power for each frequency band was determined as the sum over each interval and represented by median power. Median power is less impacted by extreme values when compared to the mean, therefore this was the measurement of choice in this study. The % power for each frequency band was calculated as the median power for that band, divided by the sum of all median powers in B2-B4, therefore the contribution of each band to the total signal power is reported as a percentage.



**Figure 4.3:** Single patient analysis of skeletal muscle of the right thigh using isosbestic NIRS. MHC is calculated from the change in  $\Delta OD$ . CWT is a function of power vs frequency vs time, GWPS is a function of frequency vs power, indicating the average power for each frequency over the course of the entire procedure. Data collection outside the dotted lines within the CWT is considered invalid. The dashed lines within the GWPS represent the range of each frequency band used for analysis (B1 metabolic; B2 endothelial; B3 neurogenic; B4 myogenic; B5 respiratory; B6 cardiac).

## Statistical Analysis

In all tables, the data are presented as mean  $\pm$  standard error of the mean, median {25<sup>th</sup>-75<sup>th</sup> percentile}, or absolute number percentages as specified. Results are considered significant when corresponding *P*-value was  $< 0.05$ . A Repeated Measures One-way ANOVA using Dunnett's multiple comparison test was used to calculate significance in both median and % power ( $P < 0.05$ ) at various time points. Simple linear regression analysis was used to calculate the coefficient of determination ( $R^2$ ) between delta % power vs several independent variables. Statistical tests were performed using GraphPad prism9 (GraphPad Software, San Diego, CA, USA).

## 4.3 RESULTS

### Data Collection

The final study cohort of 19 patients had a mean recording time of  $4.7 \pm .61$  hours (range: 1.7 – 13.02) with an average sample rate of  $9.272 \pm 0.128$  Hz of the right thigh muscle. A subset of patients ( $n=4$ ), underwent minimally invasive surgery requiring peripheral cannulation strategies, while the remaining patients ( $n=15$ ) received conventional CPB with central cannulation. There were no statistical differences found between groups with respect to any of the data collected. A full list of operative procedures are listed in Table 4.2. Demographics and notable risk factors for the final study cohort are provided in Table 4.3. Intraoperative data and important blood values are also recorded in Table 4.4.

**Table 4.2: Type of Procedure**

<b>Conventional CPB (n=15)</b>	<b>Minimally Invasive CPB (n=4)</b>
AVR, n=11	MV Repair, n=2
AVR/Aortic Root Enlargement	MV Repair/TR Repair
AVR/ECMO	Left Atrial Myxoma
AVR/MVR/TR Repair	
MVR/Ablation	

AVR = Aortic Valve Replacement; ECMO; extracorporeal membrane oxygenation;

MVR = Mitral Valve Replacement; MV = Mitral Valve; TR = Tricuspid Valve.

**Table 4.3: Demographic Data**

Characteristic	Cardiopulmonary Bypass (n = 19)
<b>Patient Demographics</b>	
Age (y)	67.2 ± 1.90
Males (%)	12 (63%)
BSA (m <sup>2</sup> )	2.04 ± 0.06
<b>Risk Factors</b>	
NYHA II-III	13 (68%)
Hypertension	11 (58%)
Diabetes Mellitus	7 (37%)
Hyperlipidemia	11 (58%)

Data are presented as mean ± SEM, or absolute numbers (percentage); p value < 0.05 using students t-test and Fishers Exact test. BSA = body surface area; NYHA = New York Heart Association classification.

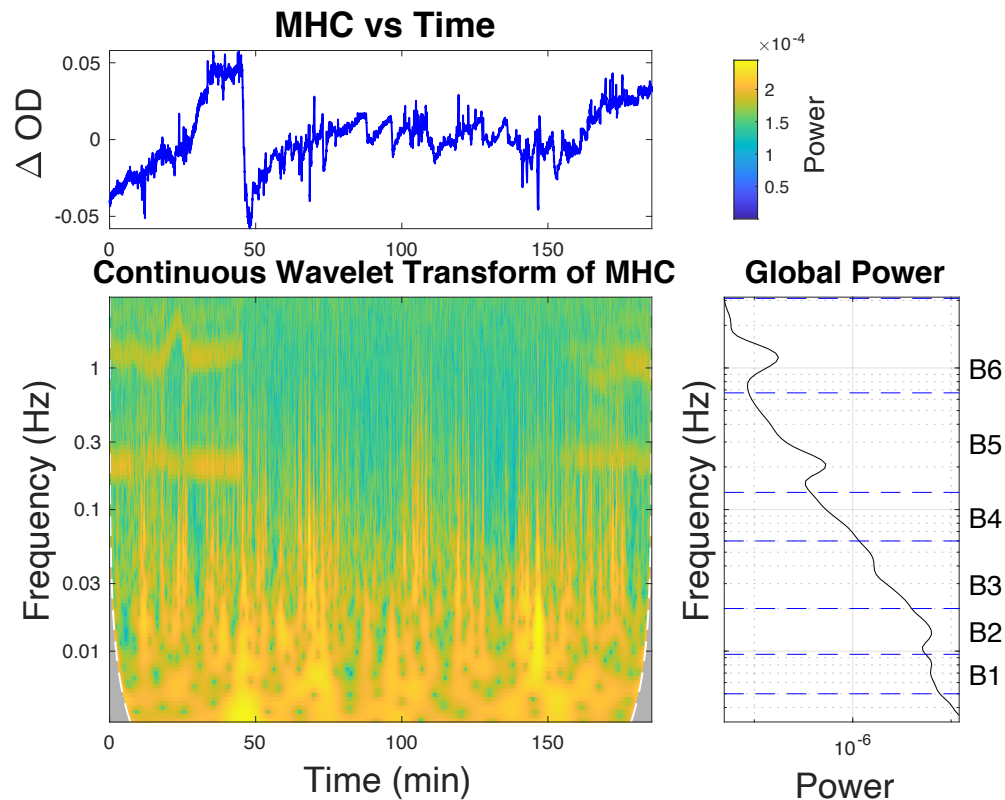
**Table 4.4: Intra-operative Data**

Characteristic	Cardiopulmonary Bypass (n = 19)
CPB time (min.)	189.0 $\pm$ 39.6
XC time (min.)	102.6 $\pm$ 15.3
Lowest Temp Celsius (NP)	33.3 $\pm$ 0.6
Phenylephrine usage (ug/min)	26.9 $\pm$ 4.9
Pre CPB Hb	126.8 $\pm$ 3.89
On CPB Hb (lowest)	95.8 $\pm$ 4.21
Off CPB Hb	101.2 $\pm$ 4.32
Pre CPB lactate	1.4 $\pm$ 0.14
On CPB lactate (peak)	2.9 $\pm$ 0.55
Off CPB lactate	3.7 $\pm$ 0.69

Data are presented as mean  $\pm$  SEM, or absolute numbers (percentage); p value < 0.05 using students t-test and Fishers Exact test. CPB = cardiopulmonary bypass; Hb = hemoglobin; NP = nasopharyngeal; XC = cross clamp.

### Single Patient Continuous Dataset

A continuous NIRS signal for a single patient is shown in Figure 4.4. The CWT is represented by power vs frequency vs time, and the global wavelet power spectrum (GWPS) during the entire course of signal acquisition. This single patient data is to be analyzed in 10-minute intervals at five various time points throughout the procedure.



**Figure 4.4:** Single patient application of CWT over time. MHC time series (blue line), CWT time series (power vs frequency vs time), and GWPS time series over entire time of data acquisition. Subsequent time intervals taken from the above full time series.

### Single Patient Time-Interval Dataset

The continuous NIRS signal acquired from a single patient (Fig 4.4) was interrogated further at various 10 minute time intervals associated with specific events during CPB.

**Pre CPB** (Fig 4.5A-B): This time period is inclusive of steady state baseline  $\Delta OD$  values from our previous study. **B6**: CWT analysis show clear steady oscillations associated with the cardiac signal, that translate into a total global power value of  $5.4 \times 10^{-7}$  seen in the GWPS; **B5**: A similar pattern is seen within the respiratory band with a higher global power of  $1.1 \times 10^{-6}$  in the GWPS; **B2-B4**: The microvascular bands reveal a balanced distribution of power of  $2.3 \times 10^{-6}$  in B2,  $3.2 \times 10^{-6}$  in B3, and  $1.5 \times 10^{-6}$  in B4 respectively.

**CPB On** (Fig 4.5C-D): This time period includes the initiation of bypass (hemodilution effect) and application of the aortic XC. **B5-B6**: Very minimal global power is seen in these bands as the ventilator is soon to be turned off and the heart arrested. **B2-B4**: A shift in global power towards lower frequency bands occur within the microvasculature, with significant increase in peak value of  $1.1 \times 10^{-5}$  dominating in B2. An increase in global power to  $4.9 \times 10^{-6}$  was seen in B3, and a slight decrease in B4 to  $1.3 \times 10^{-6}$ .

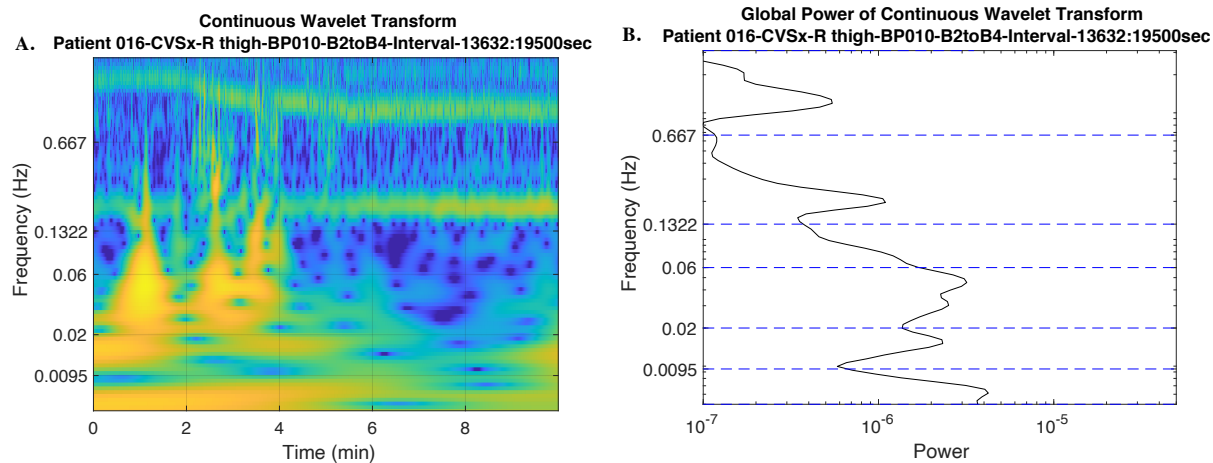
**Pre XC Off** (Fig 4.5E-F): This time point represents maintenance of CPB. **B5-B6**: At this time point global power associated with B6 continues to be absent as the heart remains arrested. Despite the ventilator being off, there is very minimal global power value of  $4.1 \times 10^{-7}$  in B5. **B2-B4**: Total global power within the microvascular bands seemed to equilibrate to pre CPB values with  $2.5 \times 10^{-6}$  in B2,  $4.4 \times 10^{-6}$  in B3, and  $1.4 \times 10^{-6}$  in B4.

**XC Off** (Fig 4.5G-H): Following the removal of the aortic XC we see a shift in global power that looks very similar to CPB On values. **B5-B6**: Global power in B6 remains absent without a beating heart, and there is slight respiratory activity in B5 with a global power of  $1.7 \times$

$10^{-7}$ . **B2-B4:** A shift in power towards lower frequency bands occur within the microvasculature upon reperfusion of the ischemic heart. Global power significantly increased to  $5.0 \times 10^{-5}$  in B2 and  $3.2 \times 10^{-5}$  in B3, whereas B4 essentially unchanged with a value of  $1.6 \times 10^{-6}$ .

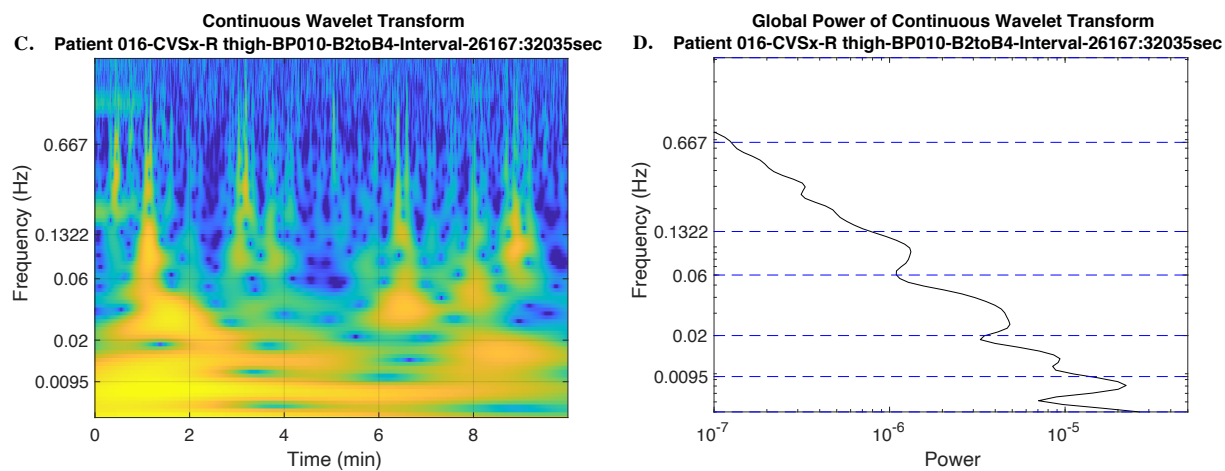
**CPB Off** (Fig 4.5I-J): This time interval is without CPB hemodynamic support. **B6:** The CWT time-frequency projection show a return of steady oscillations in B6 as the native heart supports circulation. We see a very slight increase in cardiac global power values at B6 ( $6.2 \times 10^{-7}$ ) compared to pre CPB. **B5:** Return of the ventilator signal is also seen within the CWT, however, compared to pre CPB there is a decrease in global power to  $4 \times 10^{-7}$ . **B2-B4:** The microvascular bands again reveal a balanced distribution of power with a return to pre CPB values of  $2.2 \times 10^{-6}$  in B2, a slight decrease in B3 to  $1.1 \times 10^{-6}$ , and decrease in power values in B4 to  $5 \times 10^{-7}$ .

Both cardiac (B6) and ventilator (B5) signals are steadily periodic, meaning they repeat with a very distinct and regular frequency. The constant frequency and power seen over time gives us confidence in the accuracy of global power measurements in these two bands with CWT analysis. The microvascular bands show a great deal of variability over time, not only in power but also within frequency at peak power. Microvascular bands are pseudo periodic, meaning they remain steady for brief periods of time only. They do seem to have a wave pattern but the amplitude may vary and gradually shift in frequency, therefore global power is useful for showing where power is concentrated.



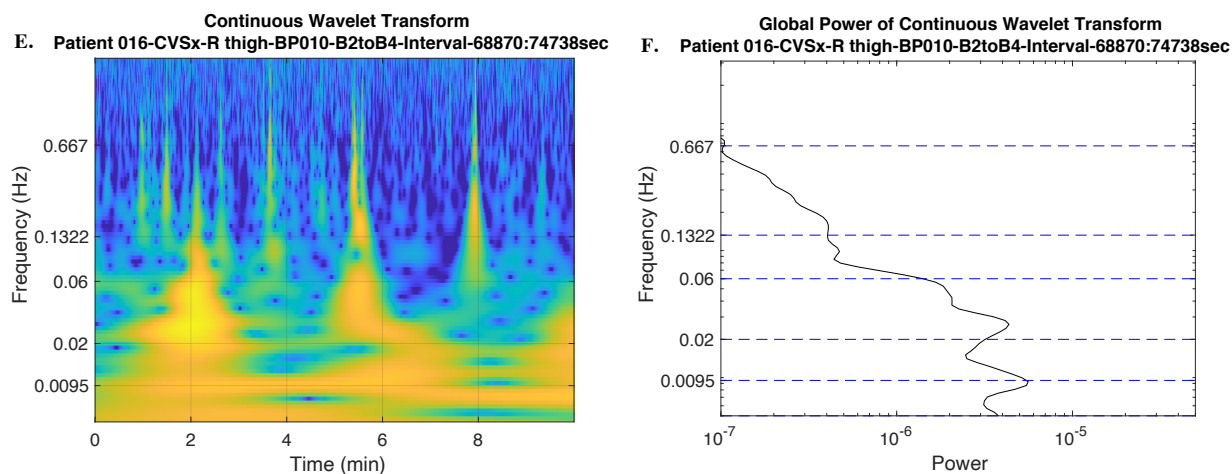
**Figure 4.5A:** Pre CPB (CWT time frequency projection)

**Figure 4.5B:** Pre CPB (GWPS)



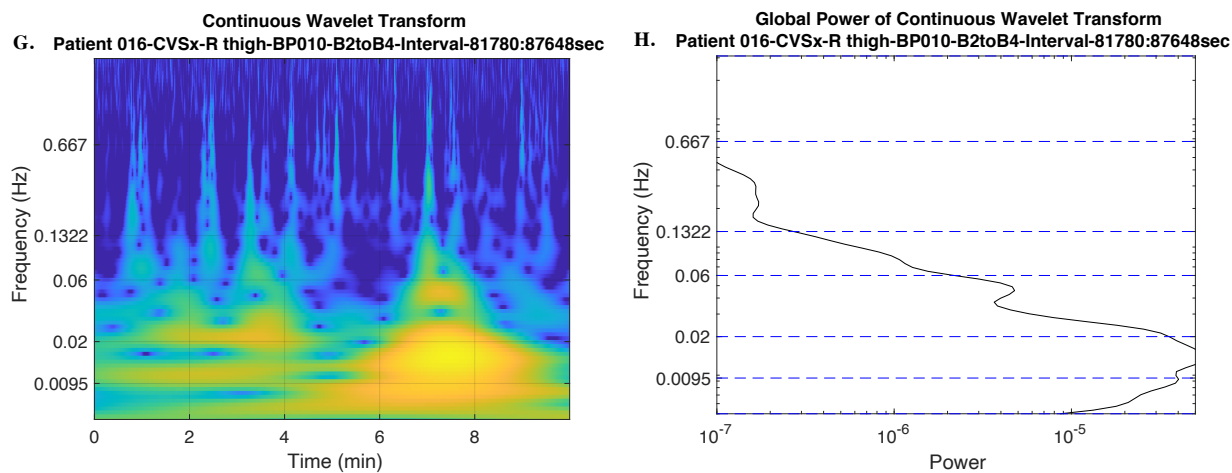
**Figure 4.5C:** CPB On (CWT time frequency projection)

**Figure 4.5D:** CPB On (GWPS)



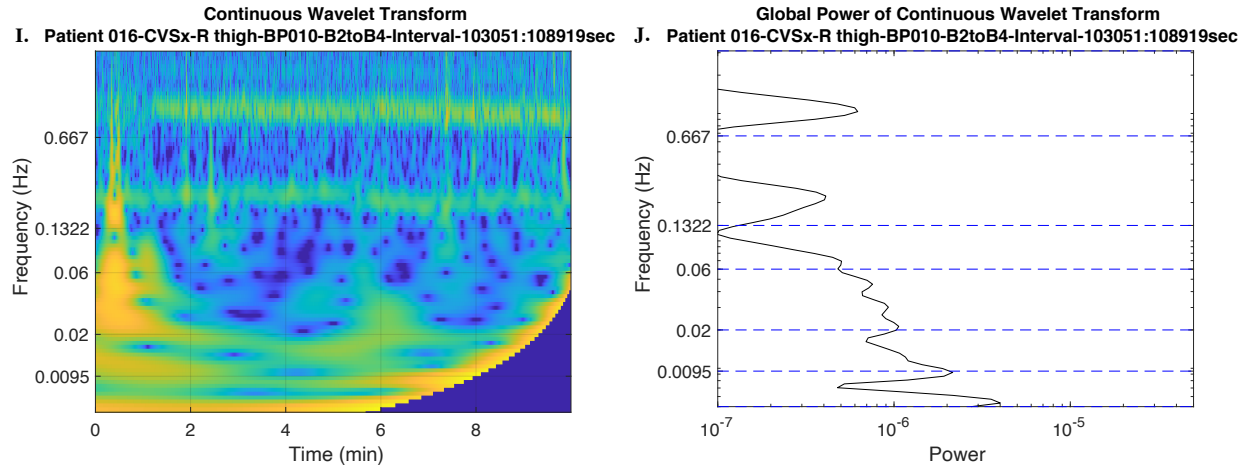
**Figure 4.5E:** Pre XC Off (CWT time frequency projection)

**Figure 4.5F:** Pre XC Off (GWPS)



**Figure 4.5G:** XC Off (CWT time frequency projection)

**Figure 4.5H:** XC Off (GWPS)

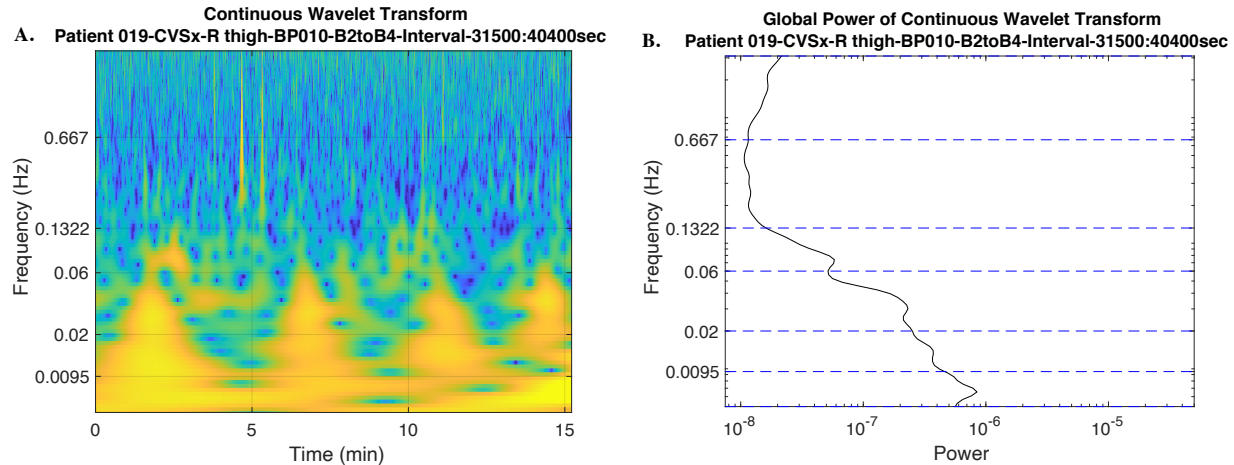


**Figure 4.5I:** Post CPB (CWT time frequency projection)

**Figure 4.5J:** Post CPB (GWPS)

### Effect of Phenylephrine Bolus on Microvascular Bands B2-B4

During the course of CPB, boluses of phenylephrine were administered as needed by the perfusionist to maintain MAP. Figures 4.6A-B show the effects of four boluses of phenylephrine spread over a 15-minute time interval during CPB. The CWT time-frequency projection reveal similar global power triangular shapes for each bolus. (Fig 4.6A). The GWPS shows very little power captured in B4 with each peak of the bolus signal. Most global power is concentrated at the base in B2 and B3 with each bolus of phenylephrine (Fig 4.6B). Phenylephrine boluses cause vasoconstriction, and therefore abrupt changes in MHC. This may not reflect a regulatory change in each frequency band, but rather a reflection of a simple time domain event. However, as the time between boluses becomes less, the amount of global power seems to be decreasing despite an increase in the dosage administered. In other words, as phenylephrine becomes less effective or refractory, the global power decreases.

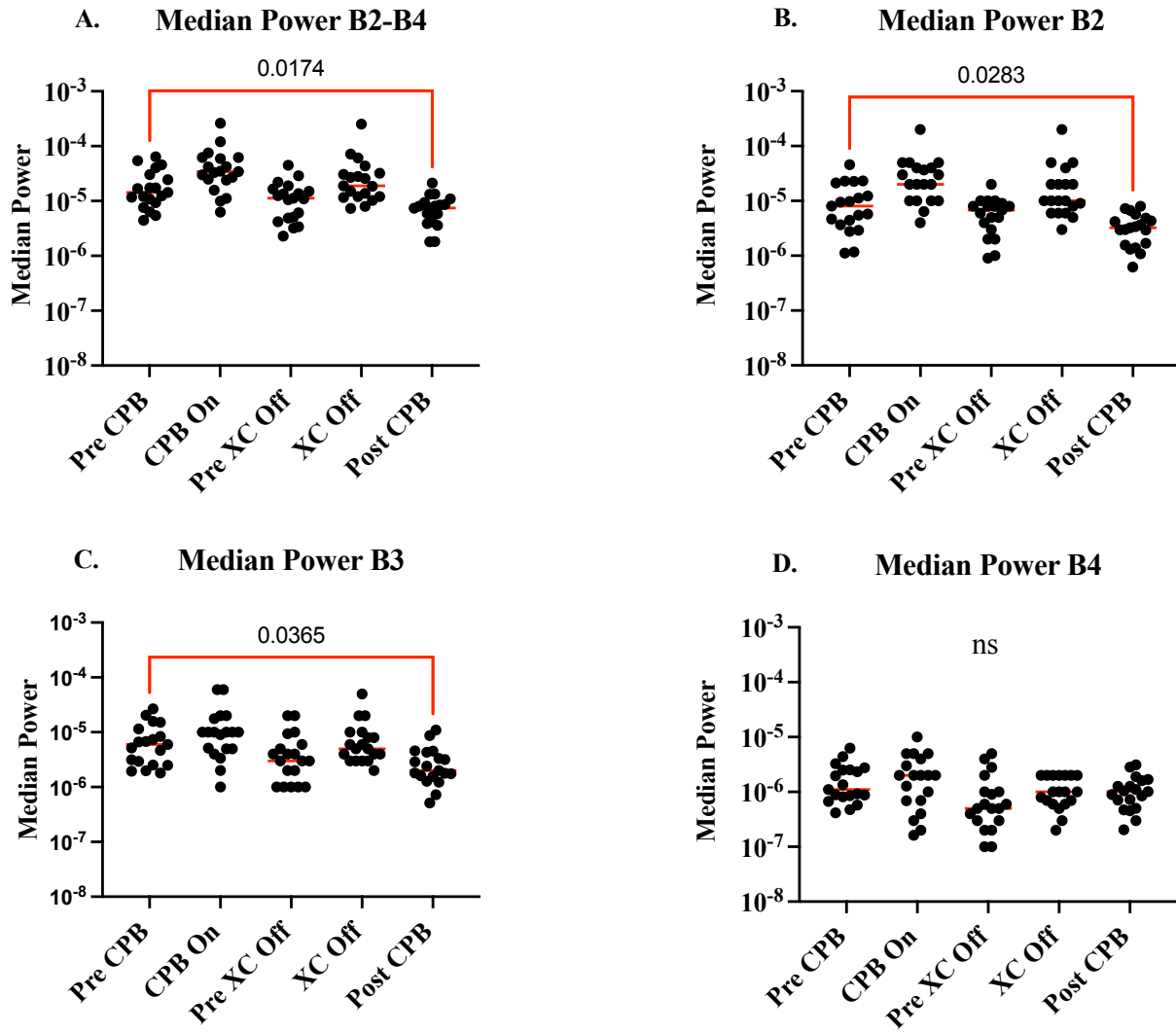


**Figure 4.6A:** Phenylephrine bolus (CWT time frequency projection)

**Figure 4.6B:** Phenylephrine bolus (GWPS)

### Continuous Wavelet Transform Median Power

MHC signal characteristics are expressed as median power, and the % power for 19 patients in the final dataset. Frequency bands B2 endothelial; B3 neurogenic; B4 myogenic comprise the microvascular bands. The median power for each band was calculated as the sum of power over a 10-minute frequency range at various time points. The total median power of the microvascular bands was determined as the sum of all three frequency bands (B2-B4) over the same time interval. Figure 4.7A reveals a significant decrease in the total median power (B2-B4) post CPB compared to pre CPB values. The total decrease in power was a result of a significant decrease in each of B2 and B3 respectively (Fig 4.7B-C) post bypass compared to pre bypass. There were no significant differences in median power for B4 at any time points (Fig 4.7D).



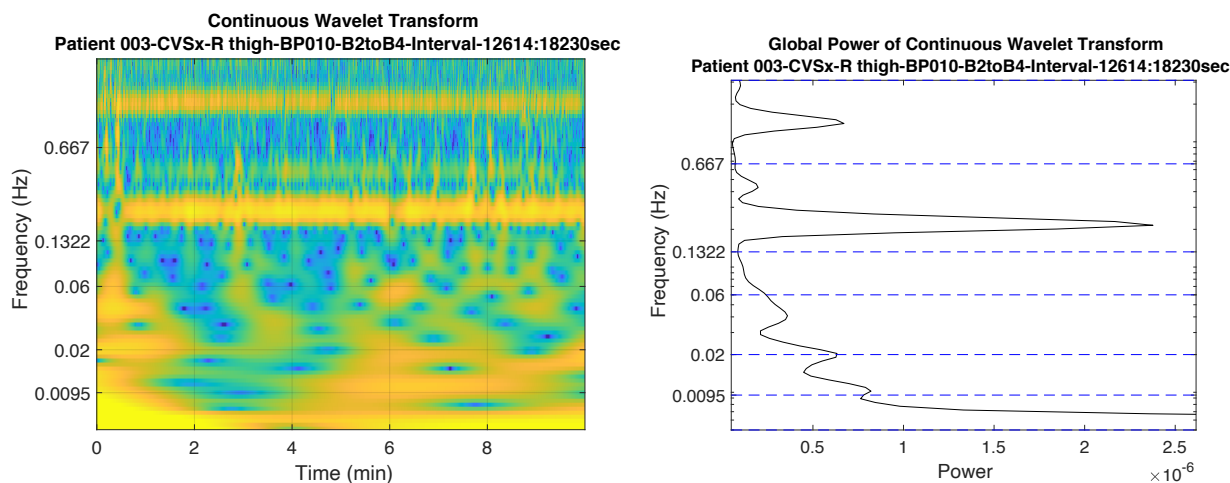
**Figure 4.7:** MHC median power values for microvascular bands at various time points (n=19).

**Fig 4.7A;** total median power for B2–B4; **Fig 4.7B;** median power for B2 (endothelial);

**Fig 4.7C;** median power for B3 (neurogenic); **Fig 4.7D;** median power for B4 (myogenic).

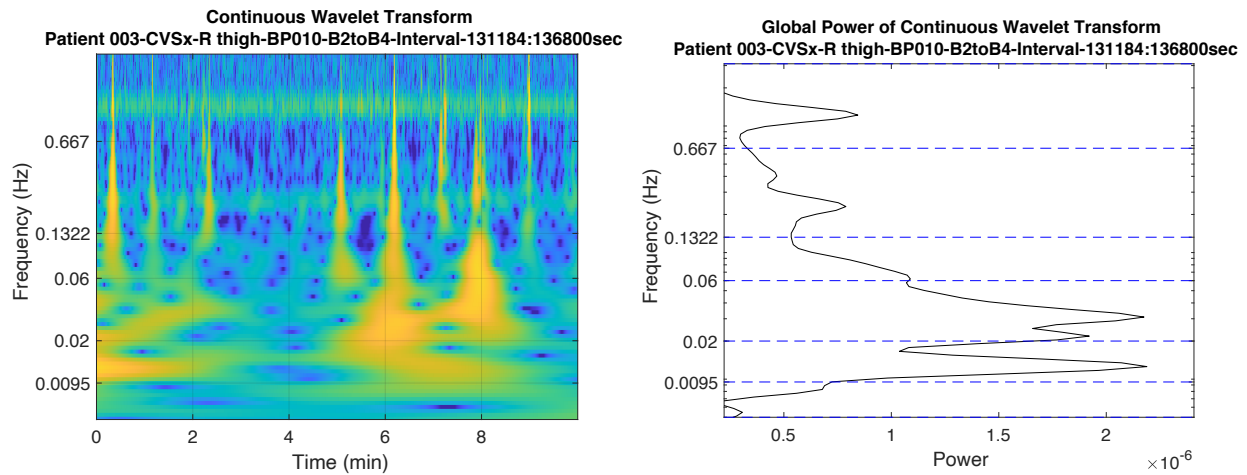
### Median Power High Frequency Bands B5-B6

The coloured images are single patient plots of power vs frequency vs time. The colour tells us what power is associated with the wavelet at every frequency over the time interval. In figure 4.8A we see clear cardiac oscillations in B6, along with a distinct ventilatory signal in B5 pre bypass. Figure 4.8B shows the resultant global power for the 10-minute time interval pre CPB. Similarly, figure 4.8C shows both cardiac and ventilatory signals post CPB, along with resultant global power for the 10-minute time interval post bypass (Fig 4.8D). In this patient we can see that the global power in B6 pre CPB is similar to the global power in B6 post CPB. Conversely, the global power in B5 is significantly reduced post CPB compared to the pre bypass oscillatory signal. Finally, in figure 4.9A we see a significant decrease in median power at B5 post CPB compared to pre bypass. In addition there is also a significant decrease in median power at B6 post CPB compared to pre bypass (Fig 4.9B).



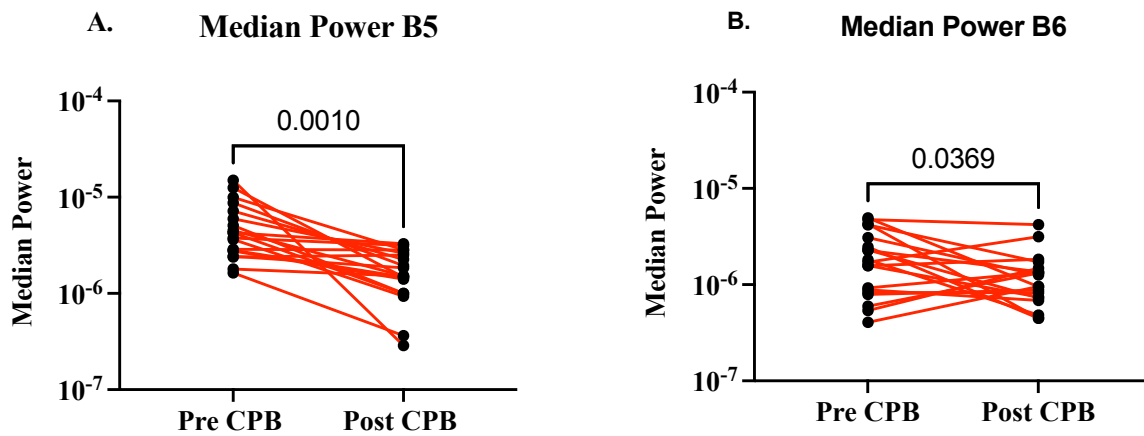
**Fig 4.8A:** Pre CPB (CWT time frequency projection) show clear cardiac oscillations in B5-B6

**Fig 4.8B:** Pre CPB (GWPS) for B5-B6



**Fig 4.8C:** Post CPB (CWT time frequency projection) show clear cardiac oscillations in B5-B6

**Fig 4.8D:** Pre CPB (GWPS) for B5-B6



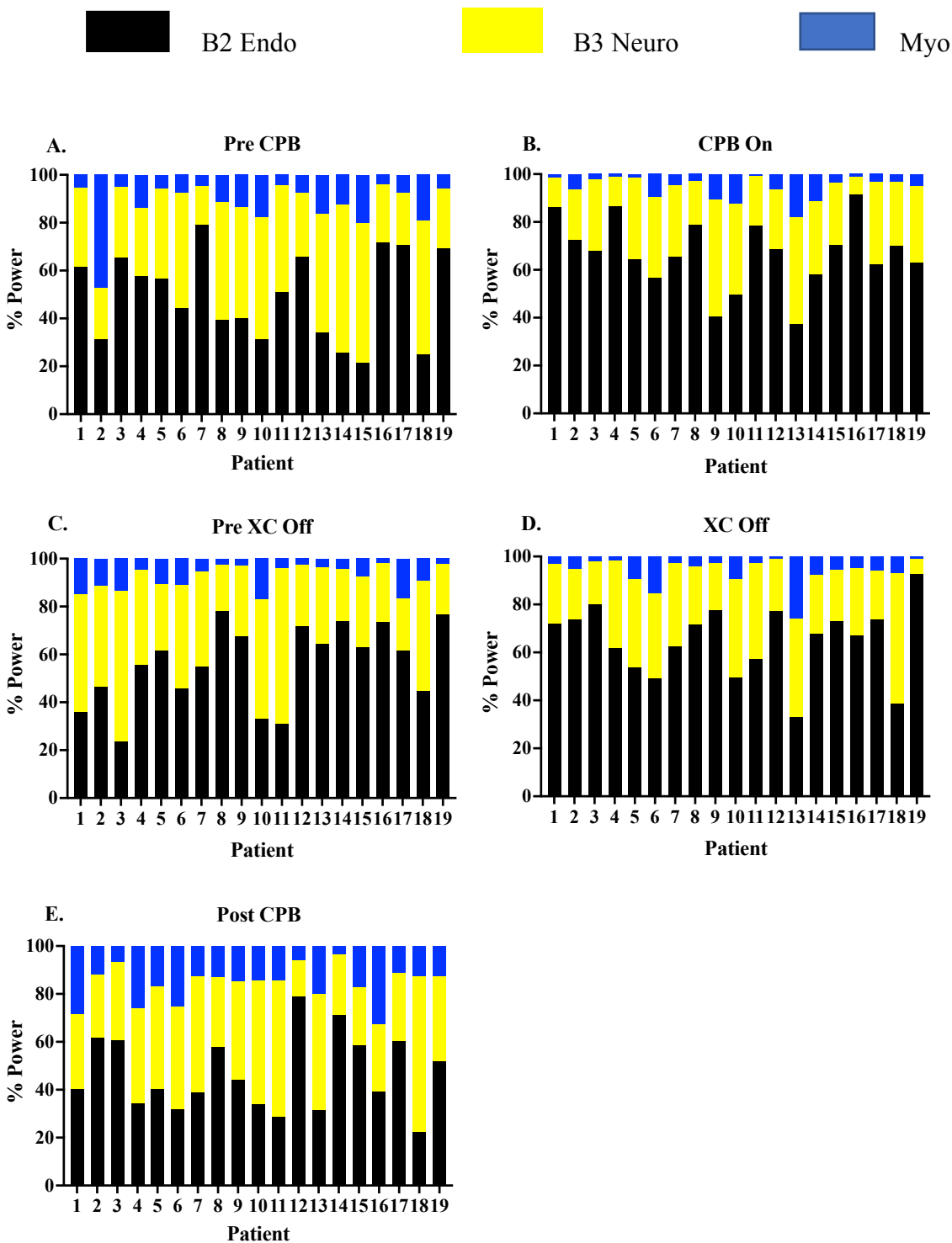
**Fig 4.9A:** Pre CPB vs Post CPB median power at B5

**Fig 4.9B:** Pre CPB vs Post CPB median power at B6.

Paired t-test  $P < 0.05$ .

### **Continuous Wavelet Transform % Power**

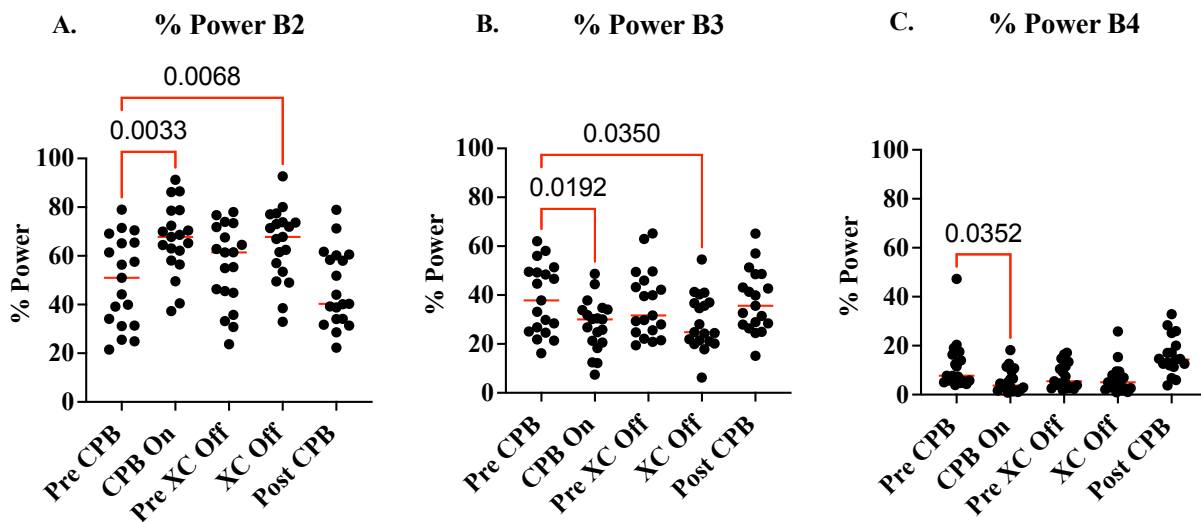
The % power for each frequency band was calculated as the median power for that band, divided by the sum of all median powers in B2-B4, therefore the contribution of each band to the total signal power is reported as a percentage. The % power composition of the MHC signal for each patient is shown in figure 4.10A-E. Results show a very diverse distribution in the % power between patients at individual time points, as well as within patients across all time points during the surgical procedure. All patients are dominated by % power within B2 or B3, with B4 being less involved. At specific time points, such as CPB On and XC Off, the % power in low frequency B2 is most prevalent (Fig 4.10B, Fig 4.10D). The % power contribution of B4 is also increased post CPB when compared to pre CPB values (Fig 4.10A, Fig 4.10E).



**Figure 4.10A-E:** The individual patient % power from each frequency band relative to overall signal power B2-B4. Power varies between patients at individual time points, and also within patients across all time points.

### % Power Microvascular Bands B2-B4

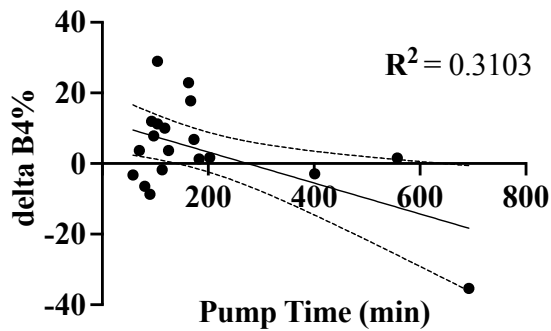
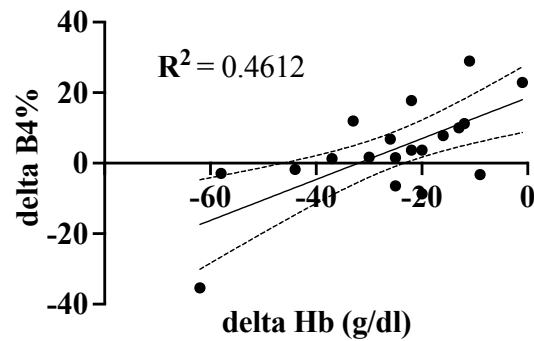
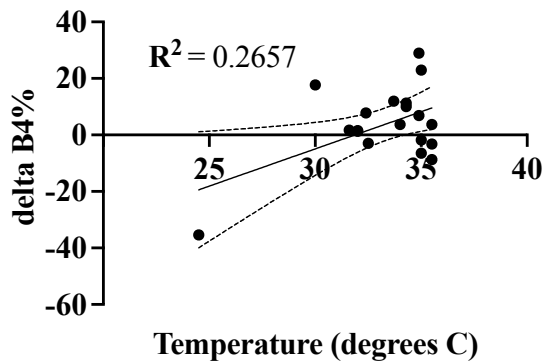
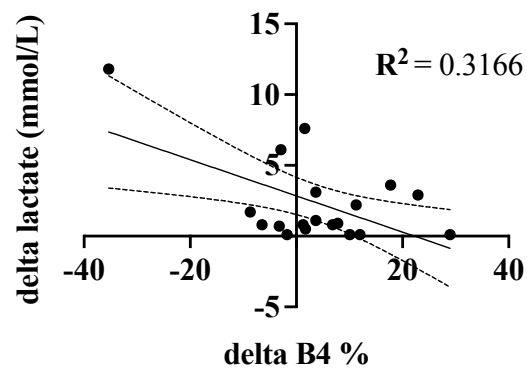
The % power for each band at various time intervals is shown in figure 4.11A-C. For all time intervals the % power from B2 dominates the MHC signal, followed by band B3, and the least amount of power from B4. Relative to pre CPB, B2 shows a significant increase at pump on, and XC off, with a return to pre CPB values following bypass (Fig 4.11A). Alternatively, a significant decrease in B3 at CPB on and XC off relative to pre bypass values is seen, with a return to pre CPB values following bypass (Fig 4.11B). In high frequency band B4 there was a significant decrease at pump on only, with a slightly higher % power post bypass compared to pre CPB values (Fig 4.11C).



**Figure 4.11:** The % power contribution from each frequency band relative to overall signal power within microvascular bands. **Fig 4.11A;** B2 endothelial; **Fig 4.11B;** B3 neurogenic; **Fig 4.11C;** B4 myogenic. Power composition is not consistent within or between patients across all time points.

### **% Power versus Intraoperative Data**

When comparing the difference between pre CPB and post CPB % power (delta % power) to intraoperative data, there were no significant correlations with low frequency B2 or B3. However, variability seen at a higher frequency in B4 was associated with certain independent variables. Figure 4.12A shows a decrease in % power at B4 as pump time is prolonged (31% of the variability in delta B4% was associated with the length of CPB time). Figure 4.12B shows a decrease in % power at B4 as the patient systemic Hb levels decrease (46% of the variability in delta B4% was associated with a decrease in systemic Hb). Figure 4.12C shows a decrease in % power at B4 as patients are cooled during CPB (27% of the variability in delta B4% was associated with a decrease in temperature). Figure 4.12D shows an increase in lactate post bypass as the % power at B4 decreases over the course of CPB (32% of the variability in lactate was associated with a decrease in delta B4 %).

**A. delta % Power B4 vs Pump Time****B. delta % Power B4 vs delta Hb****C. delta % Power B4 vs Temperature****D. Lactate vs delta % Power B4**

**Figure 4.12:** Univariate linear regression shows significant correlation with solid line. Dotted line represents 95% confidence interval. **Fig 4.12A:** delta % B4 vs Pump Time shows significant correlation,  $R^2 = .3103$ ; **Fig 4.12B:** delta %B4 vs delta Hb shows significant correlation,  $R^2 = .4612$ ; **Fig 4.12C:** delta %B4 vs temperature shows significant correlation,  $R^2 = .2657$ ; **Fig 4.12D:** delta lactate vs delta %B4 shows significant correlation,  $R^2 = .3166$ .

#### 4.4 DISCUSSION

In this study continuous wavelet transform (CWT) was applied to the microvascular hemoglobin content (MHC) signal to pinpoint frequency ranges that correspond to physiological oscillations most likely to originate within the cardiovascular system. NIRS algorithms were used to apply a variability analysis to provide insight on blood flow reactivity using high resolution data sampling. This second to second variability adds information of how well the microvasculature maybe regulating blood flow, whereas commercial NIRS devices act mostly as a trend monitor over minutes to hours with StO<sub>2</sub> values being recorded every 2-5 seconds (Mendelson et al., 2021). Rather than tracking absolute StO<sub>2</sub> values, we are interested in the dynamic change of RBC distribution within the microvasculature. We hypothesize that variability in MHC reflects the redistribution of RBC flow within the microvasculature as the microvascular regulatory system maintains tissue oxygenation. Hence changes in the dynamic characteristics of MHC would reflect changes in the regulatory system, by responding to external challenges induced by CPB, or to impaired regulatory function.

In our previous study we focused solely on the feasibility of tracking changes in MHC over time, whereas here we apply a continuous wavelet analysis to study the dynamic variability between low and high frequency oscillations in the microvasculature over specific time intervals. This is the first successful study utilizing CWT to a NIRS signal to assess peripheral perfusion and microvascular hemodynamics in cardiac surgical patients undergoing CPB. A CWT analysis was applied to the  $\Delta OD$  time series data, and revealed that signal power composition varied within each patient, as well as between patients across all time points. This CWT time-frequency projection of the microcirculation represents the intrinsic and extrinsic mechanisms involved in the dynamic variability of RBC distribution (Kvandal et al., 2006). By looking at the dynamics of

MHC we gain insights in terms of the regulatory system that may not be reflected in typical macrohemodynamic data.

Distribution of power occurs from high to low frequency, and vice versa, across several time points during the course of the surgical procedure. Upon initiation of CPB, one could argue that variability seen within microvascular bands (B2-B4) is mostly due to the tremendous change in systemic Hb content and MHC caused by hemodilution. The shift in % power to low frequency bands (increase in B2, decrease in B3-B4) may be a reflection of hemodilution, as opposed to the impaired ability of the microvasculature to redistribute RBC. However, we do speculate the microcirculation is under stress as power shifts toward lower frequency oscillations at subsequent time points.

As the surgical procedure progresses to Pre XC Off time point, the microvasculature appears to recover from the sudden effects of hemodilution, and returns closely to Pre CPB values (decrease in B2, increase in B3-B4). As the aortic XC is removed, a similar response to CPB On occurs. However, we suspect the change in power may be affected internally by an ischemic reperfusion (IR) event causing vasodilation, decrease in SVR, and reduction in MAP (Bronicki & Hall, 2016; Tempe & Cooper, 1994), rather than an external stimulus such as hemodilution. The heart and lungs are excluded from the circulation once the aortic cross clamp is applied. During ischemia, a decrease in arterial blood flow can result in a reduction of oxygen and the supply of energy to its cells, therefore stored levels of ATP within the mitochondria are utilized for energy, and are ultimately reduced to hypoxanthine (Granger, 1988). Following removal of the aortic XC clamp, the re-introduction of oxygen drives the xanthine oxidase-mediated conversion of hypoxanthine to xanthine. A byproduct of this reaction is the formation of an oxygen free radical called superoxide (Cañas, 1999; Granger, 1988).

Superoxide is a highly reactive oxidant that causes damage to cells and can also activate local inflammatory mediators and cytokines (Badhwar et al., 2004). Systemic vascular tone is regulated by neurohormonal systems and endothelial function, both of which may be adversely affected by the inflammatory response (Landry & Oliver, 2001). The releases of cytokines can upregulate the production of endothelial anti-oxidants such as heme oxygenase, superoxide dismutase, and endothelial-cell-derived nitric oxide. Through a negative feedback mechanism, production of anti-oxidants such as NO can efficiently scavenge increased levels of superoxide, and protecting the cell from free radical damage (Badhwar et al., 2004). Therefore, the significant increase we see in endothelial B2 global power following removal of the aortic XC may be due to this upregulation in endothelial activity brought on by I/R injury. This sudden increase in low frequency power (increase in B2, decrease in B3) demonstrates a substantial redistribution of RBCs in the microvasculature as well as a shift in dynamics towards lower frequencies as the XC is removed. This shift in global power is unlikely due to the transient decrease in pump flow that occurs during de-clamping of the aorta, as we found in our previous study there were no changes in MHC at this particular time point.

As CPB is terminated and the pump is turned off, B2 returns to pre CPB values and a shift toward high frequency oscillations occur (increase in B3-B4). Unlike the I/R effect seen at XC off with an elevation in B2 during NP flow, the elevation in B4 seen post CPB could reflect I/R under P flow conditions. The fact we saw an increase in microvascular responsiveness under P flow conditions in our second study may help corroborate this theory (O'Neil et al., 2018). When we look at post CPB, there is a similar balance of power within B2-B4 compared to pre CPB. It is during bypass when the signal shifts away from baseline values, potentially reflecting a suppressed or impaired microvascular regulatory control mechanism. This could be due to the

transition to NP flow during CPB, and the resumption of P flow post CPB. This is in line with results from a previous study by O'Neil using OPS imaging who reported that P perfusion is superior to NP perfusion at preserving the microcirculation, which may reflect attenuation of the systemic inflammatory response during CPB (O'Neil et al., 2012). A study by DeBacker also revealed that cardiac surgery without CPB exhibited less severe microcirculatory alterations in the immediate post-operative period compared to those patients who experienced NP CPB (De Backer et al., 2009). The use of P flow during CPB may be associated with less severe microvascular alterations due to a decrease in the inflammatory response (Orime et al., 1999). Our results suggest there is a change in the way the microcirculation is distributing RBC's over the course of surgery with power shifting from one band to another. What we speculate from this trend is that the microvasculature is considered to be unhealthy or under stress when a shift from high frequency to low frequency oscillations occur. This is indicative by an increased shift towards low frequency B2 upon initiation of CPB and aortic XC removal, versus an increase in high frequency band power at pre CPB, steady state pre XC Off, and post CPB time points.

As stated earlier, the concept of frequency bands was derived from high resolution LDF studies of the skin, each associated with a proposed physiological event (Holowatz et al., 2008). What exactly these bands mean in skeletal muscle remains unclear, however we attempt to interrogate their relationship with physiological data collected on pump. When patients undergo CPB, the body adapts to many physiological insults, such as hemodilution, change in temperature, MAP, and perfusion in the form of non-pulsatile flow. With the focus being on oxygen delivery and the regulation of blood flow by means of RBC oxygen saturation dependent release of ATP mechanism, we looked for relationships between hemodilution and lactate levels with microvascular analysis using CWT.

Hemodilution as a result of a crystalloid priming solution is inevitable during CPB, and low hematocrit (Hct) levels on pump have been associated with poor outcomes (Fang et al., 1997; Habib et al., 2003; Karkouti, Beattie, et al., 2005; Karkouti, Djaiani, et al., 2005). Severe hemodilution during bypass is a recognized risk factor for induced kidney injury (Ranucci, Aloisio, et al., 2015; Swaminathan et al., 2003), mostly due to poor oxygen delivery to the ischemic damaged renal medulla (De Somer, 2009; Ranucci et al., 2005). The colloid osmotic pressure has been shown to drop as much as 44% during hemodilution, with albumin being the primary protein depressed by 32% post CPB (Hall, 1995). A single Hct value of 19% or less during CPB was also reported to have double the mortality rate compared to patients with a nadir Hct value of 25% (DeFoe et al., 2001). Another study found that stroke, myocardial infarction, low cardiac output, cardiac arrest, renal failure, prolonged ventilation, pulmonary edema, reoperation caused by bleeding, sepsis, and multi organ failure were all significantly increased as the lowest Hct value on CPB decreased to less than 22% (Habib et al., 2003).

In this study we looked at correlations comparing delta % power for each band (the difference between pre and post CPB % power), to delta Hb level (difference between pre and post CPB values). There were no statistical differences when comparing delta % power in either B2 or B3 to Hb levels, however B4 showed significant correlation. Figure 4.12B shows a decrease in % power at B4 as the patient systemic Hb levels decrease (46% of the variability in delta B4% was associated with a decrease in systemic Hb). When we look at delta Hb versus % power in B4, there was an increase in B4 power relative to pre-CPB with only a small reduction in Hb (20-30%). This mild reduction in Hb would be considered ideal in reducing blood viscosity beneficial to cooling patients, along with a reduction in RBC hemolysis and the systemic inflammatory response that can occur during CPB. With an additional decrease in Hb of more than 30% we see further

decline of power in B4. As indicated by these changes in B4 due to hemodilution, an increase of power in B4 seems to be beneficial whereas a decrease in power may not be. There may be some variability between patients, but we speculate that relative changes in B4 in this scenario may be used as a marker of how well the microvascular is regulating, with hemodilution less than 30% suggesting an active regulatory system and hemodilution greater than 30% proposing abnormal regulation with an  $R^2$  value close to 50% (Fig. 12B). Given the poor outcomes associated with a Hct level of 22% or less (Habib et al., 2003), the ability to monitor changes in the microcirculation in real time during events of hemodilution could be advantageous.

If our interpretation is correct our results suggest that on pump hemodilution should be maintained above a certain point, therefore prime reduction techniques should be individualized and planned out methodically for each patient. Currently, the advancement of perfusion technology has led to the development and use of mini circuits to reduce hemodilution and SIRS, while preserving platelet function and minimizing the need for allogeneic blood products. Other techniques to reduce hemodilution include retrograde autologous priming (RAP), ultrafiltration, and albumin administration when indicated.

The literature suggests that with significant hemodilution comes the loss of shear stress-mediated vasodilation, and reduces capillary perfusion pressure and functional capillary density (Martini et al., 2006). Hematocrit has the greatest effect on blood viscosity, as a 10 % increase in hematocrit increases high shear blood viscosity by approximately 5 % (Cho et al., 2014). An increase in blood viscosity due to an elevated hematocrit increases peripheral vascular resistance (Cho et al., 2014), therefore a decrease in viscosity associated with hemodilution can result in a decrease in MAP, and a subsequent increase in vasopressors. Maintenance of MAP with vasoconstrictors may worsen complications already associated with hypoperfusion (Sato et al.,

2006; Tsai et al., 1998). Phenylephrine is a vasoconstricting agent that is going to have a large effect in the arteriolar tree, increase MAP and therefore abrupt changes in MHC. When applying a CWT analysis to phenylephrine boluses we found the time-frequency projection to reveal similar triangular shaped increases in global power (Fig. 6A). This external event imposed onto the microcirculation may not reflect a regulatory change in each frequency band, but rather a reflection of a simple time domain event. However, as the time between boluses becomes less, the amount of global power seems to be decreasing despite an increase in the dosage administered. In other words, as phenylephrine becomes less effective or refractory, the global power decreases.

This may be a sign of vasoplegia, characterized by a low systemic vascular resistance (SVR) and severe vasodilation refractory to vasopressors during and after CPB. Vasoplegia is a relatively common complication of CPB with an incidence of 5-25%. Outcomes associated with vasoplegia after CPB include renal failure, prolonged ICU and hospital stay, and increased mortality (Barnes et al., 2020). The GWPS also shows very little power captured in B4 with each peak spike of the bolus signal. Most global power is concentrated in the lower frequency bands (B2-B3) with phenylephrine administration (Fig 4.6B). Increases in global power and shifting between bands with vasopressor administration may indicate a microvasculature attempting to maintain oxygen regulation. In contrast, MHC signal power was significantly reduced in patients receiving vasopressors in critically ill patients in the ICU (Mendelson et al., 2021). This would suggest an already dysfunctional microvasculature in these patients if MAP could not be maintained initially with fluids, and perhaps refractory to vasopressors due to a severe systemic inflammatory response.

The lowest Hb level on bypass has been identified as a risk factor for postoperative acute kidney injury (Karkouti, Beattie, et al., 2005; Ranucci et al., 1994), stroke (Habib et al., 2003;

Karkouti, Djaiani, et al., 2005), and mortality (Habib et al., 2003). This is due to poor oxygen delivery leading to dysoxia and end organ failure (Ranucci, Aloisio, et al., 2015). Inadequate oxygen delivery during CPB can also trigger anaerobic metabolism, leading to increased levels of blood lactate. Following cardiac surgery, hyperlactatemia is also a recognized marker of impaired hemodynamics associated with increased morbidity and mortality (Hajjar et al., 2013; Maillet et al., 2003). Another study explored the relationship between lowest Hct on CPB and early postoperative hyperlactatemia. It was demonstrated the lowest Hct on CPB is independently correlated with moderate and severe hyperlactatemia, strengthening the hypothesis that poor oxygen delivery with consequent organ ischemia is the mechanism leading to hemodilution-associated adverse outcomes (Ranucci, Carboni, et al., 2015).

We also investigated relationships between lactate and our CWT analysis. There were no statistical differences when comparing delta B2 and B3 with delta lactate levels. Figure 4.12D shows an increase in delta lactate as the delta % power at B4 decreases over the course of CPB (32% of the variability in lactate was affected by a decrease in delta B4 %). This would indicate that a higher microvascular frequency is preferred as those patients that have higher power in B4 tend to have lower lactate levels. B4 is a higher frequency band that we believe is characteristic of a very active regulatory system maintaining proper oxygen delivery in tissue that is potentially tied to RBC signalling the vascular endothelium. By losing power in B4, our data would suggest losing the functional ability to regulate efficiently as patients with poor regulation in B4 have increased lactate levels. Post pump lactate generally reflect inadequate oxygen delivery during CPB, in particular during the rewarming phase (Demers et al., 2000; Ranucci et al., 2010). It is impossible to draw conclusion about the exact onset of hyperlactatemia from one blood measures. Lactate production occurs within an organ very rapidly under low oxygen concentration, however

lactate clearance takes more time by the liver, especially if hampered by low hepatic blood flow on CPB (Takala et al., 1996). Therefore lactate levels measured on CPB are an indicator of what was happening much earlier on pump. Low perfused vessel density and high microcirculatory heterogeneity are associated with an increased intensity and duration of lactic acidosis after cardiac surgery with CPB (Greenwood et al., 2021). Therefore, a marker such as B4 using CWT could perhaps be used on pump to help guide for earlier interventions simultaneously with lowest Hct on CPB.

For the procedures investigated in this study cohort, expected CPB times are typically 120-150 minutes with patient temperatures about 35 degrees Celsius. When complications arise and pump times are extended, patients are often cooled below target temperatures and lactate levels tend to increase postoperatively (Luz & Auler Junior, 2002). In this study, as we interrogate the % power associated with B4, the microvasculature does not seem to regulate as well with increased pump times and cooler temperatures. Figure 4.12A shows a decrease in % power at B4 as pump time is prolonged (31% of the variability in delta B4% was affected by the length of CPB time). Figure 4.12C shows a decrease in % power at B4 as patients are cooled during CPB (27% of the variability in delta B4% was affected by a decrease in temperature). There were no statistical differences when comparing % power at B2-B3 to either CPB time or temperature.

When we look at B5-B6, oscillations are very precise and steady at one frequency compared to B2-B4 where the microvasculature is dynamically regulated. Figure 4.9A reveals a significant decrease in median power at B5 post CPB compared to pre CPB and Fig 4.9B demonstrates a significant decrease in median power at B6 post CPB compared to pre CPB. The decreased effects of ventilation and cardiac contractility on the skeletal muscle signal following CPB indicates something has changed compared to pre CPB. This change in power could be due

to a lack of blood volume, as patients are slightly hypovolemic upon separation of bypass, or a result of vasodilation from protamine administration for the reversal of heparin. When this occurs the ventilatory and cardiac signal transmitted to the skeletal muscle may be depressed, therefore less power is generated in B5-B6. Conversely, a lower median power in B5-B6 could also be due to postoperative pulmonary and cardiac complication that have been documented following CPB (Ji et al., 2013; Messent et al., 1992). Increased interstitial edema and dysfunction of end organs such as the brain, heart, and lungs can occur as a result of hemodilution and the SIRS (DeFoe et al., 2001). It is possible the decrease in power seen at B5-B6 may be due to lung and heart injury following CPB. If so, this would affect oxygen delivery to the tissues, which may explain the elevation in delta B4 post op, which we speculate is an important index for the regulation of oxygen to meet the increase demand. This may be the reason for an increase in B4 post op to help the regulation of oxygen to meet the increase in demand.

As B4 is affected by changes in levels of hemodilution, temperature, pump time, and contributes to changes in lactate, we feel this may reflect a regulatory system besides myogenic, one that is related to maintaining appropriate levels of oxygen delivery to tissues. This study is designed to find relationships using CWT, therefore we can only speculate that the oxygen saturation dependent release of ATP within the RBC may be the mechanism behind our results. There are still unanswered questions regarding CWT analysis, but the fact we see a trend in B4 related to changes in lactate is very compelling. Further studies are needed to improve our ability to monitor the microcirculation with this technology, and to see if B4 may be an important marker for us in tracking the microvascular status of our patients in the operating room. This technology should continue to be worked on with the goal of verifying that changes in MHC and CWT could

be a surrogate for blood flow regulation and oxygen delivery to tissues, in addition to investigating potential correlations with clinical outcomes and potential therapeutic targets.

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## FINAL SUMMARY

### 5.1 OVERVIEW

To help guide the perfusionist during cardiac surgery they rely heavily on monitors that reflect global perfusion status or macro-hemodynamics. The primary objective of this thesis was to investigate and monitor the microcirculation in cardiac surgery patients undergoing cardiopulmonary bypass (CPB).

Direct visualization of the sublingual mucosal microcirculation was performed in our first study using a bedside version of intra-vital video microscopy (IVVM) called Orthogonal Polarization Spectral (OPS) imaging. The sublingual mucosa is the preferred site for obtaining OPS images due to its accessibility (Hamilton et al., 2008), and has been documented to correlate with that of vital internal organs (Creteur et al., 2006; Verdant et al., 2009). Along with OPS measurements, we simultaneously used a near infra-red spectroscopy (NIRS) device (Hutchinson In Spectra) that uniquely measures tissue hemoglobin oxygen saturation ( $StO_2$ ) of the thenar muscle, and the microvascular responsiveness following an ischemia reperfusion (I/R) challenge induced by a vascular occlusion test (VOT).

In our second experiment we elected to use a custom broadband continuous wave NIRS monitor that was previously designed and built by our lab. This device was successfully tested on patients in the intensive care unit (ICU) to measure the temporal changes in microvascular hemoglobin content (MHC) of skeletal muscle, which can be used as a surrogate for red blood cell (RBC) flow within the microcirculation (Mendelson et al., 2021). We were very fortunate to transfer this technology into the cardiac surgery operating room to determine the feasibility for continuous real time monitoring of MHC in patients undergoing CPB. NIRS algorithms were used to detect changes in optical density ( $\Delta OD$ ) at the isosbestic wavelength of hemoglobin (Hb) in the

NIRS range (798nm) continuously over the course of surgery with analysis focused on specific time points.

In our third study we applied a continuous wavelet transform (CWT) analysis to the  $\Delta OD$  time series with the goal of reflecting the time-dependent variability in MHC. The microcirculation of the skin has been studied and documented to correlate with the systemic microcirculation using Laser Doppler Flowmetry (LDF) (Holowatz et al., 2008). This widely accepted technology provides non-invasive continuous microcirculatory perfusion measurements that allow researchers to study the periodic oscillations which occur as a result of rhythmic tone changes within the microvasculature (Reynès et al., 2020). There are six frequency bands that can be derived from high resolution laser doppler studies, each associated with a proposed physiological event (Kvandal et al., 2006; Stefanovska et al., 1999). We were successful in applying CWT analysis to detect power within various frequency bands over the course of the surgical procedure. Variability seen within and between these bands may ultimately reflect dynamic information on microvascular regulation in response to balancing oxygen supply and demand.

## **5.2 NEED FOR MONITORING THE MICROCIRCULATION**

Microcirculatory alterations may exist in the presence of preserved global hemodynamics (De Backer et al., 2010), and can result in the loss of hemodynamic coherence or decoupling of the macrocirculation and microcirculation (De Backer et al., 2010; Ince, 2015). As we know, microvascular perfusion can be severely impaired during cardiac surgery as a result of reduced cardiac output, the switch to non-pulsatile (NP) flow, systemic inflammatory response syndrome (SIRS), hemodilution, hypothermia, and length of bypass time (Haase-Fielitz et al., 2017).

Microvascular impairment as a result of the SIRS may lead to the development of post-operative complications such as multiple organ failure (Murphy & Angelini, 2004). Following cardiac surgery, the prolonged treatment of multiple organ failure in ICU leads to excess mortality and cardiovascular morbidity after discharge (Mazzoni et al., 2006), therefore having the ability to monitor the microcirculation has the potential to be extremely valuable for improving patient outcomes.

In clinical practice, detection of these microcirculatory alterations can be challenging given the current technology available to researchers. Many observational studies using bedside versions of IVVM have reported microvascular dysfunction in cardiac surgery patients due to the effects of CPB (Bauer et al., 2007; De Backer et al., 2009; Koning et al., 2012; O'Neil et al., 2012). However, these studies are underpowered to consider their consequence on patient outcomes due to small sample size, the lack of time points under investigation, and the requirement of complex and time consuming analysis. Therefore more research needs to be done, using clinically friendly and sophisticated non-invasive devices that can be easily interpreted by the clinician and perfusionist in real time to adequately assess the microcirculation during the surgery and document patient outcomes.

### **The Ideal Microvascular Monitor**

With many potential factors affecting the microcirculation and patient outcomes during cardiac surgery, a need exists for improved microvascular monitors in the operating room that can continuously track real-time changes in the microvasculature without intervention from a clinician to obtain data. Ideally a monitor that can provide information on the variability of microvascular blood flow, and its dynamic regulation due to oxygen supply and demand. The clinical implications of such a monitor would permit earlier, non-invasive detection of significant

physiological derangements, and allow for more accurate and timely therapeutic interventions in the attenuation of I/R injury and the SIR response linked to CPB. Therefore, a resuscitation strategy of the microcirculation could be performed based on a bedside monitoring device, potentially reducing the risk of multiple organ failure following cardiac surgery, the length of hospital stay and the associated health care costs.

### **5.3 KEY FINDINGS**

#### **Study #1**

The significance of the pulse during CPB continues to be debated. Adding to the controversy is whether perfusion mode during CPB has any beneficial effect on the microcirculation (Ji & Undar, 2007). Earlier studies have shown that CPB with NP flow is associated with a decrease in capillary density and microvascular flow, most likely as a result of hemodilution, hypothermia, inflammation, and endothelial dysfunction (Atasever et al., 2011; Bauer et al., 2007; De Backer et al., 2009; Koning et al., 2014; Koning et al., 2012; O'Neil et al., 2012; Verdant et al., 2009).

In our first study we used OPS imaging and NIRS/VOT to determine whether pulsatile (P) flow generated by the roller pump during CPB improves microcirculatory blood flow compared to NP flow in high-risk cardiac surgical patients. Key findings from this study showed that sublingual microvascular perfusion at 90 minutes on CPB in the NP group had 17% fewer normally perfused vessels than those in the P group, with a secondary increase in hyper-dynamically flowing vessels. At both one hour and 24 hour periods following CPB, the NP group continued to have significantly fewer normally perfused vessels, as well as an increase in slow continuous/intermittent blood flow characteristics compared to the P group. NIRS/VOT measurements also revealed a significant

decrease in reperfusion slope, indicating a reduction in microvascular responsiveness in the NP group at 24 hours post CPB compared to P flow. These microvascular measurements also coincided with an increase in lactate in the NP group at 24 hours post CPB compared to P group.

These findings support the hypothesis that P flow may attenuate microcirculatory heterogeneity during extracorporeal circulation compared with NP flow, although the underlying mechanism remains unknown. Using side-stream dark field (SDF) imaging of the sublingual microcirculation, a study by Koning showed that P flow during the aortic cross-clamp period was also associated with preservation of microvascular perfusion in the early postoperative period but not during pump. The fact they had not witnessed any changes during CPB was likely due to a low risk study population that underwent short lasting, uncomplicated procedures, whereas our focus was on high risk patients with expected prolonged pump times. Near-infrared spectroscopic measurements of StO<sub>2</sub> and the VOT were also utilized in a study by Smith on elective cardiac surgical patients undergoing NP CPB (Smith & Murkin, 2014). This pilot study demonstrated a significant decrease in reperfusion slopes during CPB when compared to pre-bypass and post-bypass, suggesting impaired peripheral microvascular reactivity. Reperfusion slopes also exhibited a successive decline with duration of CPB, implying worsening microcirculatory dysfunction that returned to baseline values in all patients within one hour of separation from CPB. Similarly, we also show a significant decrease in the reperfusion slope during CPB, that when compared to P flow continues for 24 hours postoperatively.

One of the proposed mechanisms of the SIRS during CPB is alteration in arterial blood flow patterns in the form of NP flow. This deviation from the normal physiological pulse generates less shear stress on the vessel wall resulting in less endogenous nitric oxide (NO) production (Baskurt et al., 2004). Preservation of NO levels during P flow has been reported to

attenuate the inflammatory response (Lanzarone et al., 2009), and vasodilation produced by shear-stress induced NO is important in the regulation of SVR and tissue perfusion (Balligand et al., 2009). The mechanisms related to shear stress and endothelial NO production may have been a contributing factor to our results, leading to impaired microvascular perfusion and increased lactate levels in the NP group. The maintenance of more normal levels of NO, or at least higher levels relative to NP flow, may have attenuated the inflammatory response and improved the microcirculatory perfusion profile and vasoreactivity we see in the P group. However, this is purely speculative as we did not measure NO levels, or know whether the mechanism was due to shear stress (endothelial nitric oxide synthase, eNOS), or from ongoing inflammation (inducible nitric oxide synthase, iNOS). One thing we do know is OPS imaging ultimately reflects the capacity of oxygen delivery to the tissues by determination of RBC velocity and functional capillary density (FCD) in the microcirculation (Groner et al., 1999), and that NIRS technology has been successfully used to measure StO<sub>2</sub> values of tissue.

### **Limitations**

Although development of OPS imaging and NIRS monitoring technologies have helped us to better understand the pathophysiology of acute circulatory failure in surgical patients, they provide only a snapshot of the microcirculation and carry many limitations. Application of too much pressure of the OPS probe may underestimate blood flow in the area under investigation (den Uil et al., 2008) and repeated measurements from the same vascular bed are nearly impossible due to movement artifacts between the tongue and the OPS probe (Lindert et al., 2002). In cardiac surgical patients it can be very challenging for the investigator to gain access to the tongue while having both an endotracheal tube and transesophageal echo probe in place, as well as the small spatial footprint shared with anesthesia at the head of the bed. Additionally,

NIRS values are also limited as StO<sub>2</sub> measurements combine arterial, venous, and capillary blood. Since venules contain the majority of blood in the microcirculation, StO<sub>2</sub> can be viewed primarily as a measure of regional venous oxygen saturation which is still not a true indicator of microvascular blood flow (Mesquida et al., 2013). Venous blood oxygen saturation values may appear normal despite maldistribution of microvascular blood flow resulting in heterogeneous distribution of over and under perfused regions of the organ. Thus NIRS acts mostly as a trend monitor over minutes to hours with values being recorded every 2-5 seconds (Mendelson et al., 2021).

Therefore, the challenge to accurately monitor the state of tissue oxygenation still remains. With NIRS devices only providing relative or absolute StO<sub>2</sub> values, what they don't provide is the insight into how well the microcirculation is regulating oxygen supply. It is that control mechanism which is just as important as the oxygen saturation values themselves. Instead of focusing solely on relative changes, adding information with a functional marker of how well the microvascular is regulating can help personalize our therapies based on these markers. This leads to our subsequent studies focusing on the dynamic change of RBC distribution within the microcirculation, reflecting changes in microvascular hemoglobin content (MHC) and providing more detailed information on oxygen supply and demand. We attempted this by using a custom broadband continuous-wave NIRS monitor previously designed and built in our lab. This technology is non-invasive, operator independent, and applies continuous measures of microcirculatory function as opposed to functional snapshots.

## Study #2

Our second experiment was a feasibility study aimed to determine what changes in MHC can be detected within skeletal muscle of the thigh in cardiac surgical patients undergoing CPB. We don't measure Hb directly but we measure changes in  $\Delta OD$  at the isosbestic wavelength to give us changes in Hb over time. Following intubation and NIRS probe application, a baseline intensity was calculated for each patient over a baseline time period of two minutes. The change in OD relative to the baseline intensity was deemed as the patient's own baseline  $\Delta OD$  value of zero.

The interaction between mechanical ventilation and systemic blood flow is well described for the macrocirculation (Leung et al., 2008; Wolf et al., 1997), but rarely has it been reported to impact the microcirculation. We were able to detect the effects of mechanical ventilation demonstrated by changes in MHC in sync with each breath of the mechanical ventilator. This was previously reported in ICU patients using the same technology (Mendelson et al., 2021), therefore it was very encouraging and added strength to our study moving forward. The magnitude of MHC induced by the ventilator may be multi-factorial, and any direct consequence on organ function is unknown at this time.

Another key metric in our study is a measurement called the area below baseline, calculated as the continuous change in every  $\Delta OD$  value compared to the patient's baseline  $\Delta OD$  value over time. We then standardized the area below baseline for each patient based on the amount of time spent on CPB. Our results revealed that the area below baseline was significantly dependent on the pump duration along with the magnitude, or the average area below baseline. In fact, for every 10 minute increase in pump time, the standardized area below baseline was increased by 0.026 units. This coincides with the fact that 86 % of the variable increase in lactate

was also associated with increased CPB time. Using OPS imaging, a study by O'Neil showed that prolonged bypass time that extended beyond two hours was documented to impair microvascular perfusion (O'Neil et al., 2012). Extended CPB times have also been linked to impaired microvascular perfusion with increased levels of lactate (Ranucci, De Toffol, et al., 2006). Although we cannot control for bypass time as complications can often arise, microvascular monitoring would be very helpful to the perfusionist in these situations to help manage the patient accordingly.

The need for microvascular monitors is also evident given the difficulty in maintaining adequate tissue perfusion during CPB, as demonstrated by the fact that up to 20% of all cardiac procedures show evidence of hyperlactatemia (Demers et al., 2000; Ranucci, Isgrò, et al., 2006). Hyperlactatemia is a recognized indicator of non-optimal tissue perfusion and is a risk factor for adverse outcomes (Boldt et al., 1999; Maillet et al., 2003; Takala et al., 1996). However, it is impossible to draw conclusions as to the onset of hyperlactatemia from one blood measurement as lactate production occurs very rapidly under low oxygen concentration within organs, but its clearance by the liver takes much longer especially if hampered by low hepatic blood flow during bypass (Takala et al., 1996). Lactate levels drawn on CPB are likely a reflection of what things looked like much earlier in the pump run, therefore continuous monitoring of the microcirculation may add value in recognizing real time episodes of decreased oxygen delivery, as opposed to waiting for adverse outcomes to occur post-operatively. Potentially this could be accomplished by tracking MHC and a metric such as the area below baseline using the device in our study. A multivariable linear regression model in this study revealed that post-pump lactate was significantly dependent on the area below baseline, with every one-unit increase in the area below baseline the post-pump lactate was increased by 87.6%. A study by DeBacker using OPS

imaging also revealed that peak lactate levels significantly increased as the proportion of perfused small vessels decreased in cardiac surgery patients (De Backer et al., 2009).

The lowest Hb level on bypass has also been identified as a risk factor for postoperative acute kidney injury (Karkouti, Beattie, et al., 2005; Ranucci et al., 1994), stroke (Habib et al., 2003; Karkouti, Djaiani, et al., 2005), and mortality (Habib et al., 2003). Hemodilution causes a loss of RBC filled capillaries, thereby increasing the diffusion distance between oxygen carrying RBC's and tissue cells contributing to tissue hypoxia seen during CPB (Kara et al., 2016). Inadequate oxygen delivery due to hemodilution can also trigger anaerobic metabolism and result in elevated lactate levels (Ranucci, Carboni, et al., 2015). In this study we document a significant decrease in  $\Delta OD$  upon initiation of CPB, indicating the effect of hemodilution on the microvasculature followed by a slow return back to baseline values. We also found that 28 % of the variable increase in lactate measured postoperatively was associated with the amount of hemodilution that occurred during CPB, and that phenylephrine usage on pump was significantly dependent on pre-pump hemoglobin level, as for every 10-unit increase in pre-pump hemoglobin, the phenylephrine was decreased by 15.7%.

The microcirculation is also affected by hypothermia, due to an increase in blood viscosity and sludging of RBC's that can lead to reduced microcirculatory flow and organ ischemia. Changes in viscosity due to hypothermia can be counteracted by the effects of hemodilution. Although oxygen carrying capacity would be decreased with hemodilution, microcirculatory flow and oxygen delivery is improved with less viscosity. Hypothermia has been documented to affect microvascular perfusion and lead to heterogeneous microcirculatory blood flow (Kara et al., 2016). Results in this study show that 34% of the variable increase in the area below baseline was associated with a decrease in temperature, combined with the fact that

59% of the variable increase in lactate was also affected by temperature. This suggests that a decrease in tissue perfusion may occur due to vasoconstriction during the cooling phase, less phenylephrine maybe needed, and lactate washout occurs upon rewarming. Post pump lactate levels generally reflect inadequate oxygen delivery during CPB, in particular during the rewarming phase (Demers et al., 2000; Ranucci et al., 2010). As patients are rewarmed the capacitance vessels within the systemic circulation begin to vasodilate and phenylephrine requirements increase. In this study we found that for every one-unit increase in the temperature on pump, the phenylephrine usage was increased by 30.5%.

### **Study #3**

In our previous study we focused solely on the feasibility of tracking changes in MHC over time, whereas here we applied a continuous wavelet (CWT) analysis to the  $\Delta OD$  time series data to reflect the dynamic variability in RBC distribution. By looking at the dynamics of MHC we gain insights in terms of the regulatory system that may not be reflected in typical macrohemodynamic data.

CWT analysis revealed that signal power composition varied within each patient, as well as between patients across all time points. For all time intervals the % power from B2 (lowest frequency band associated with microvascular dynamics) dominates the MHC signal, followed by band B3, with the least amount of power from B4. Relative to pre CPB, the shift in % power to low frequency bands (increase in B2, decrease in B3-B4) at pump on may reflect the initial effects of hemodilution on MHC as well as the microvascular response as the microvasculature recovered to pre CPB values (decrease in B2, increase in B3-B4) following this event. However, a similar response to pump on occurred as the aortic XC was removed, only this time

hemodilution was not the factor (increase in B2, decrease in B3). A shift in dynamics towards lower frequencies demonstrates a substantial redistribution of RBCs in the microvasculature and a shift from mechanisms responsible for the higher frequencies. As the pump is turned off, power within B2-B4 shift again towards pre CPB values with the exception of an elevation seen in B4. There was also a decrease in power post CPB within B5-B6 compared to pre CPB values. This may be due to reperfusion of the heart and lungs following an extended period of ischemia during CPB, as well as the washout of inflammatory mediators upon resumption of P flow. Unlike the I/R effect seen at XC off with an elevation in B2 during NP flow, the elevation in B4 seen post CPB could be the result of I/R with P flow. If so, this would affect oxygen delivery to the tissues, which may explain the elevation in delta B4 post op, which we speculate is an important index for the regulation of oxygen to meet the increase demand. These results likely reflect changes in microvascular regulatory control mechanisms, meaning there is a change in the way the microcirculation is distributing RBC's over the course of surgery with power shifting from one band to another. What we speculate from this trend is that the microvasculature is considered to be unhealthy or under stress when a shift from high frequency to low frequency oscillations occur.

We also looked at correlations between delta % power for each band (the difference between pre and post CPB % power), to various independent variables such as delta Hb, temperature, and pump time, as well as the effects of delta % power on delta lactate levels. There were no statistical differences when comparing delta % power in B2 or B3 to any of the above, however delta B4 % power (high frequency band) did show significance. There was a significant decrease in % power at B4 with hemodilution. Although variability exists between patients, based on our results we speculate that relative changes in B4 in this scenario may be

used as a marker of how well the microvascular is regulating following CPB, with hemodilution less than 30% suggesting an active regulatory system and hemodilution greater than 30% proposing abnormal regulation.

When complications arise and pump times are extended, patients are often cooled below target temperatures. In this study we showed a decrease in % power at B4 as patients are cooled during CPB, as well as decrease in % power at B4 as pump time is prolonged. With the reduction in high frequency B4 activity, we speculate that the microvasculature may not regulate as well with increased pump times and cooler temperatures. As patients are cooled and pump times are extended, lactate levels tend to increase postoperatively (Luz & Auler Junior, 2002). Here we show an increase in delta lactate as the delta % power at B4 decreases over the course of CPB. This would indicate that a higher microvascular frequency is preferred as those patients that have higher power in B4 tend to have lower lactate levels. By losing power in B4, our data would suggest the microvasculature may be losing the functional ability to regulate as efficiently as patients with reduced power in B4 have increased lactate levels.

As B4 is affected by changes in levels of hemodilution, temperature, pump time, and contributes to changes in lactate, we feel this may reflect a regulatory system besides myogenic which is associated with B4 in the literature; one that is related to maintaining appropriate levels of oxygen delivery to tissues. This study is designed only to find relationships, therefore we can only speculate that the oxygen saturation dependent release of ATP within the RBC is the mechanism behind our results since there are multiple regulatory mechanisms which might be affected. What we see in B4 may be an important marker for us to track the microvascular status of our patients in the operating room, given how lactate levels drawn on CPB are a reflection of what was happening much earlier in the pump run. This may provide real time values that could

help to guide for earlier interventions and improve patient outcomes. Further studies are needed to improve our ability to monitor the microcirculation with this technology.

## 5.4 CONCLUSION

Current use of NIRS at the bedside is a non-optimal way of assessing oxygen delivery to a patient. A more practical monitor tracking MHC along with CWT analysis could be very useful in providing information about oxygen supply and local tissue demand. A clinically friendly and sophisticated device that can be easily interpreted by the clinician and perfusionist in real time, incorporating the patient's own baseline values into an algorithm, and alert the perfusionist to potential interventions that may improve microvascular perfusion. Our current research and related future work is an important first step and compelling pre-requisite for such a monitor. There are some key metrics established from this thesis which could be helpful in guiding the perfusionist to improve best practices. For example, maintaining MHC at or above relative to baseline levels, as the amount of time and magnitude spent below a certain threshold may be prognostic for adverse outcomes. This technology should continue to be worked on with the goal of verifying that MHC below baseline values could be a surrogate for low oxygen delivery, and studied to see if there is a correlation with clinical outcomes and potential therapeutic targets. The hypothesis would be that superior outcomes may be observed when these values are kept as close as possible to patients own baseline. The last step would be to apply the monitor to see what if any therapeutic interventions can be done to bring these values closer to baseline. Questions raised towards the underlying mechanisms behind microvascular dysfunction can also be studied in animal models in an attempt to avoid further injury.

Our results show some very interesting relationships in the data. We already know that lactates increase with an increase in pump time, and the fact that we were able to reproduce this phenomenon gives us confidence in our results, and that we are ultimately measuring something that could be clinically important. This is also the case with our NIRS signal and CWT analysis that was affected by mechanical ventilation. It helps point to the idea that changes in  $\Delta OD$  at the isosbestic wavelength reflect changes in MHC, and provides insight within the microvasculature. Using NIRS we see dynamic changes in MHC and how blood flow is redistributed to maintain tissue oxygenation. Our speculation is that higher frequencies are particularly involved, and any deviations would suggest some type of adjustment in regulation. There are still unanswered questions regarding CWT analysis, but the fact we see a trend in B4 related to changes in lactate is very compelling. During I/R with NP flow we see an increase in B2 at XC Off, whereas an increase in B4 occurs following reperfusion with P flow post CPB. The fact we saw an increase in microvascular responsiveness under P flow conditions in our second study may help corroborate this theory. Perhaps a CWT analysis following a VOT under P and NP flow modes would shed more light on this topic.

## **5.5 FUTURE STUDIES**

The NIRS device used in our second and third experiments was specifically designed for skeletal muscle use, providing a depth of measurement of roughly 1cm and a 15mm separation of light. The device itself was not the feasibility aspect of this thesis, but rather the successful analysis using an algorithm program created in our lab was the main focus. One interesting thing about our device is the fact it is hyperspectral, not just dealing with several wavelengths but rather a continuous capture over a wide range of 600-850nm, which can ultimately be used to

perform other analysis besides just MHC. We can also perform coherence analysis between MAP and the NIRS data to evaluate if MAP is within the patient's own autoregulatory range, and whether hypoperfusion occurred from a low MAP or not.

There are many interventions that can be applied during the course of CPB in order to keep new metric values close to baseline and improve microvascular perfusion. Things such as increasing pump flow rate, the use of vasoconstrictors and vasodilators to maintain vascular tone, the application of P flow, increasing systemic Hb content by administration of packed RBC's, hemoconcentration of plasma water in the blood which can also eliminate increased levels of cytokines, leukocyte filtration, minimizing ischemia reperfusion events with improved cardioplegia delivery, and reducing the amount of suction blood returning to the venous reservoir. Technological advancements have evolved over the years to reduce the impact of the SIRS such as surface coating modification and circuit miniaturization. Important to the mini circuit is the attempt to attenuate the SIRS by removal of the venous reservoir, decreasing prime volumes, and reducing the blood to circuit component interface. By incorporating NIRS monitoring during any of these interventions, we can see if changes to important metrics occur in real time, therefore personalizing the conduct of perfusion for individual patients.

Using similar NIRS protocols within this thesis, we can perform full clinical studies comparing phenylephrine boluses versus a continuous infusion, P versus NP flow, and conventional CPB circuits versus mini-circuits. We could also look to monitor the microvasculature in eligible cardiac surgical patients that undergo acute normovolemic hemodilution therapy prior to CPB or follow their progress during recovery over subsequent months.

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## Appendix A: Ethics Approval



### Use of Human Participants - Ethics Approval Notice

**Principal Investigator:** Dr. Christopher Ellis  
**Review Number:** 18498  
**Review Level:** Full Board  
**Approved Local Adult Participants:** 20  
**Approved Local Minor Participants:** 0  
**Protocol Title:** Microvascular Responsiveness to Pulsatile and Non-Pulsatile Cardiopulmonary Bypass: A Validation Study using Orthogonal Polarization Spectral Imaging and Near-Infrared Spectroscopy during Vascular Occlusion Testing.  
**Department & Institution:** Medical Biophysics, University of Western Ontario  
**Sponsor:**  
**Ethics Approval Date:** December 08, 2011  
**Ethics Expiry Date:** January 31, 2013

#### Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
UWO Protocol		
Letter of Information & Consent		2011/12/08

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/CH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Signature

#### Ethics Officer to Contact for Further Information

Janice Sutherland	Grace Kelly	Shantel Walcott
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*This is an official document. Please retain the original in your files.*

#### The University of Western Ontario

Office of Research Ethics

Support Services Building Room 5150 • London, Ontario • CANADA - N6G 1G9  
 PH: 519-661-3036 • F: 519-850-2466 • [ethics@uwo.ca](mailto:ethics@uwo.ca) • [www.uwo.ca/research/ethics](http://www.uwo.ca/research/ethics)



**Western  
Research**

Research Ethics

**Western University Health Science Research Ethics Board  
HSREB Delegated Initial Approval Notice**

**Principal Investigator:** Dr. Linrui Guo

**Department & Institution:** Schulich School of Medicine and Dentistry\Surgery, London Health Sciences Centre

**Review Type:** Delegated

**HSREB File Number:** 108858

**Study Title:** Microvascular Responsiveness to Cardiopulmonary Bypass

**HSREB Initial Approval Date:** August 08, 2017

**HSREB Expiry Date:** August 08, 2018

**Documents Approved and/or Received for Information:**

Document Name	Comments	Version Date
Change in Study Personnel	Change of PI	2017/05/26
Revised Western University Protocol	Received June 7, 2017	
Letter of Information & Consent		2017/08/03
Data Collection Form/Case Report Form	Received June 7, 2017	

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

~~Ethics Officer~~, on behalf of Dr. Marcelo Kremenchutzky, HSREB Vice Chair

EO: Erika Basile ☐ Grace Kelly ☐ Katelyn Harris ☐ Nicola Morphet ☐ Karen Gopaul ☒ Patricia Sargeant ☐

## **Appendix B: Copyright Permission**

### **RE: copyright request**

Yaklin Melinda  
Tue 2022-08-09 4:03 PM

To: Michael O'Neil

Hi Mike and thanks. I understand that the original circuit design works better for your audience.

Per the copyright request, please make sure the circuit diagram and photo reference the copyright:  
The notice of copyright font may be no smaller size than eight point and shall take the form "© Terumo Cardiovascular Systems Corporation".

Thanks and let me know if you need any further support. Best of luck with the publication!  
Melinda

## Appendix C: Curriculum Vitae

Michael P. O'Neil MSc., CCP, CPC

### EDUCATION AND TRAINING

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2011-2013 2018-2022	Western University PhD Candidate – Dr. Chris Ellis Department of Medical Biophysics Schulich School of Medicine London, ON
2008-2010	Western University Masters of Science – Dr. Amit Badhwar Department of Medical Biophysics Schulich School of Medicine London, ON
1999-2000	The Michener Institute Advanced Diploma Cardiovascular Perfusion Technology Toronto, ON
1990-1992	Henry Ford Community College Associates Degree in Science Respiratory Therapy Dearborn, MI, USA
1986-1990	University of Windsor Bachelor of Human Kinetics Major: Applied Kinesiology Windsor, ON

### CERTIFICATION

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2001	American Board of Cardiovascular Perfusion Certified Clinical Perfusionist (CCP)
2000	Canadian Society of Clinical Perfusion Clinical Perfusionist Certified (CPC)
1998	Certified Open Water Scuba Diver (PADI)

1994	Basic Life Saver (BLS) Instructor American Heart Association
1994	Advance Cardiac Life Saver (ACLS) Provider American Heart Association
1992	Basic Life Saver (BLS) Provider American Heart Association
1992	National Board for Respiratory Care (NBRC) Registered Respiratory Therapist (RRT)

#### EMPLOYMENT

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2001-present	Senior Clinical Perfusionist Department of Clinical Perfusion London Health Sciences Center London, ON
2000-2001	Clinical Perfusionist Department of Clinical Perfusion Sudbury Regional Hospital Sudbury, ON
1998-2000	Registered Respiratory Therapist Department of Respiratory Therapy Windsor Regional Hospital Windsor, ON
1992-1998	Registered Respiratory Therapist Department of Respiratory Therapy Detroit Medical Center, Sinai Hospital Detroit, MI

#### CONTINUING EDUCATION

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2021	Virtual 4 <sup>th</sup> Minimally Invasive Extracorporeal Technologies Symposium
2019	Canadian Society of Clinical Perfusion, Eastern Region Meeting Saint Andrews, NB, Canada

2018	Advanced Minimally Invasive Extracorporeal Technologies Training Kerkrade, Netherlands
2017	Canadian Society of Clinical Perfusion Annual General Meeting and Scientific Sessions Vancouver, BC, Canada
2015	Canadian Society of Clinical Perfusion Annual General Meeting and Scientific Sessions Toronto, ON, Canada
2014	The Society of Thoracic Surgeons Extracorporeal Membrane Oxygenation Symposium Chicago, Illinois, USA
2013	International Society for Minimally Invasive Cardiothoracic Surgery Prague, Czech Republic
2012	Mechanisms of Perfusion XXVII Lake Buena Vista Florida, USA
2011	Canadian Society of Clinical Perfusion Annual General Meeting and Scientific Sessions Vancouver, BC, Canada
2011	11 <sup>th</sup> European Conference on Perfusion Education and Training Lisbon, Portugal
2010	Case Reports in the Sun, The Florida Society of Clinical Perfusion Annual General Meeting Tampa Bay, Florida, U.S.A.
2009	Canadian Society of Clinical Perfusion Annual General Meeting and Scientific Sessions Edmonton, AB, Canada
2009	Outcomes, The Barbados Meeting Barbados, B.W.I

- 2008 Canadian Society of Clinical Perfusion Annual  
General Meeting and Scientific Sessions  
Toronto, ON, Canada
- 2008 Ontario Society of Clinical Perfusion Annual  
General  
Meeting and Scientific Sessions  
Markham, ON, Canada
- 2008 American Society of Extra-Corporeal  
Technology Annual General Meeting and  
Scientific Sessions, Left Ventricular Assist  
Device Seminar and Workshop  
Orlando, FL, U.S.A.
- 2007 Canadian Society of Clinical Perfusion Annual  
General Meeting and Scientific Sessions  
Quebec City, QC, Canada
- 2007 Ontario Society of Clinical Perfusion Annual  
General Meeting and Scientific Sessions  
London, ON, Canada
- 2006 Outcomes Key West Meeting,  
Key West, FL, U.S.A
- 2005 Canadian Society of Clinical Perfusion Annual  
General Meeting and Scientific Sessions  
Montreal, QC, Canada
- 2004 Ontario Society of Clinical Perfusion Annual  
General Meeting and Scientific Sessions  
Markham, ON, Canada
- 2004 American Society of Extra-Corporeal  
Technology Annual General Meeting and  
Scientific Sessions  
Hollywood, FL, U.S.A.
- 2003 Canadian Society of Clinical Perfusion Annual  
General Meeting and Scientific Sessions  
Edmonton, AB, Canada
- 2003 Ontario Society of Clinical Perfusion Annual  
General Meeting and Scientific Sessions  
Markham, ON, Canada

2003	Outcomes Key West Meeting Key West, FL, U.S.A
2002	Ontario Society of Clinical Perfusion Annual General Meeting and Scientific Sessions Markham, ON, Canada
2001	Ontario Society of Clinical Perfusion Annual General Meeting and Scientific Sessions Markham, ON, Canada
2001	American Society of Extra-Corporeal Technology Annual General Meeting and Scientific Sessions Miami, FL, U.S.A
2000	Canadian Society of Clinical Perfusion Annual General Meeting and Scientific Sessions, Vancouver, BC, Canada

#### OTHER EXPERIENCE

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Internships	1999-2000	Clinical Perfusion Educational Rotation, London Health Sciences Center, London, ON, Canada
	1999	Clinical Perfusion Educational Rotation, Montreal General Hospital, Montreal, QC, Canada
	1992	Respiratory Therapy Educational Rotation, Detroit Medical Center, Harper Hospital, Detroit, MI, U.S.A.
	1991	Respiratory Therapy Educational Rotation, Providence Hospital, Southfield, MI, U.S.A.
	1990– 1991	Respiratory Therapy Educational Rotation, Oakwood Hospital, Dearborn, MI, U.S.A.

Teaching	2010-present	Clinical Adjunct Professor, The Michener Institute, Toronto, ON, Canada
	2010	ECMO Lectures Respiratory Therapy Program Fanshawe College, London, ON, Canada
	2007-2011	Career Speaker Series: Perfusion as a Career School of Kinesiology, University of Western Ontario, London, ON, Canada
	2006-2007	Overview of Cardiopulmonary Bypass Respiratory Therapy Students London Health Sciences Center, London, ON, Canada
	2001-present	Clinical Training to Perfusion Students of the Michener Institute on rotation at London Health Sciences Center, London, ON, Canada
	2001-present	Teaching Residents and Nursing Staff on Cardiopulmonary Bypass, Intra-aortic Balloon Pumps, ECMO London Health Sciences Center, London, ON, Canada
	1992 - 1998	Clinical Training of Respiratory Therapy Students from surrounding programs on rotation at Sinai Hospital, Detroit, MI, U.S.A.
	1994 - 1998	Instruction of Basic Life Saving Courses and Certification to New Residents and Staff at Sinai Hospital, Detroit, MI, U.S.A.
Volunteer	1994 - 1998	Instruction of Infant Basic Life Saving Course to parents of children residing in the Neonatal Intensive Care Unit, Sinai Hospital, Detroit, MI, U.S.A.
	2010 - present	Reviewer for “ <b>Perfusion</b> ”, SAGE Publications
	2015-2022	Head Coach, North London Nationals Hockey Association, London, Ontario
	2008	Moderator of CSCP Scientific Sessions, Annual General Meeting, Toronto, ON, Canada

2007-2008	Member of CSCP Perfusion Week Committee
2007	Moderator of OSCP Scientific Sessions, Annual General Meeting, London, ON, Canada
2003-2005	President of the Ontario Society of Clinical Perfusion
1994	Health-O-Rama Volunteer for the American Lung Association
1992	Worked with Chronic Obstructive Pulmonary Disease (COPD) patients at Windsor Western Hospital for the Canadian Lung Association on breathing exercises and prudent heart living
1991	Volunteer work with Physically Handicapped patients at Windsor Western Hospital

#### RESEARCH FUNDING AND AWARDS

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2022	Graduate Bursary Society of Graduate Students Western University London, ON	\$1000
2022	Ontario Student Opportunity Trust Fund Bursary Society of Graduate Students Western University London, ON	\$1500
2018-2022 2011-2013	Western Graduate Research Scholarship School of Graduate and Postdoctoral Studies Western University London, ON	\$38,287
2008-2010	Department of Surgery Internal Research Fund <b>Co-Investigator</b> Effects of Pulsatile versus Non-Pulsatile Flow During Cardiopulmonary Bypass on Sublingual Mucosal Microcirculation	\$25,000

2008-2010	Western Graduate Research Scholarship School of Graduate and Postdoctoral Studies Western University London, ON	\$13,864
2000	Baxter Cardiovascular Group Scholastic Achievement Award	\$1000
2000	Highest Academic Achievement Gold Medal Award Perfusion Class 2000 The Michener Institute Toronto, ON	\$1000

#### ACADEMIC PRESENTATIONS

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2021 - Microcirculation During CPB  
Virtual 4<sup>th</sup> Minimally Invasive Extracorporeal Technologies Symposium

2019 - Variability in Microvascular Hemoglobin Levels during Cardiopulmonary Bypass  
CSCP Eastern Region Meeting  
Saint Andrews, NB

2019 - Variability in Microvascular Hemoglobin Levels during Cardiopulmonary Bypass  
Cardiac Surgery Residents Day  
London Health Sciences Centre

2017- Simultaneous Hybrid Arch, Frozen Elephant Trunk, Thoraco-Abdominal Aortic  
Aneurysm Repair  
CSCP Annual General Meeting and Scientific Sessions  
Vancouver, BC

2017 - Microvascular Monitoring  
Perfusion Education Day  
London Health Sciences Centre

2015 - Thoraflex Hybrid Frozen Elephant Trunk Procedure for Acute Aortic Dissection  
CSCP Annual General Meeting and Scientific Sessions  
Toronto, ON

2015 - Microvascular Responsiveness to Pulsatile vs. Non-Pulsatile Flow during  
Cardiopulmonary Bypass  
CSCP Annual General Meeting and Scientific Sessions  
Toronto, ON

2013 - Microvascular Responsiveness to Pulsatile and Non-Pulsatile Cardiopulmonary Bypass  
International Society for Minimally Invasive Cardiothoracic Surgery  
Prague, Czech Republic

2011 - Pulsatile vs. Non-Pulsatile flow during Cardiopulmonary Bypass:  
Microcirculatory and Systemic Effects  
11<sup>th</sup> European Conference on Perfusion Education and Training  
Lisbon, Portugal

2009 - Pulsatile vs. Non-Pulsatile flow during Cardiopulmonary Bypass:  
Microcirculatory and Systemic Effects  
CSCP Annual General Meeting and Scientific Sessions  
Edmonton, AB

2009 - Pulsatile vs. Non-Pulsatile flow during Cardiopulmonary Bypass:  
Microcirculatory and Systemic Effects  
Outcomes, The Barbados Meeting  
Barbados, B.W.I

2003 - Supported Hepatic-Cardiac Transplantation  
CSCP Annual General Meeting and Scientific Sessions  
Edmonton, AB

## PUBLICATIONS

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Fernandes P, **O'Neil M**, Del Valle S, Cave A, Nagpal D. A 24-hour perioperative case study on argatroban use for left ventricular assist device insertion during cardiopulmonary bypass and veno-arterial extracorporeal membrane oxygenation. *Perfusion*. 2019 May;34(4):337-344.

**O'Neil MP**, Alie R, Guo LR, Myers ML, Murkin JM, Ellis CG. Microvascular Responsiveness to Pulsatile and Nonpulsatile Flow During Cardiopulmonary Bypass. *Ann Thorac Surg*. 2018 Jun;105(6):1745-1753.

Fernandes P, Walsh G, Walsh S, **O'Neil M**, Gelinas J, Chu MW. Whole body perfusion for hybrid aortic arch repair: evolution of selective regional perfusion with a modified extracorporeal circuit. *Perfusion*. 2017 Apr;32(3):230-237.

**O'Neil MP**, Fleming JC, Badhwar A, Guo LR. Pulsatile versus nonpulsatile flow during cardiopulmonary bypass: microcirculatory and systemic effects. *Ann Thorac Surg*. 2012 Dec;94(6):2046-53.

Mayer R, **O'Neil M**. Supported Hepatic – Cardiac Transplantation, *Canadian Perfusion Canadienne*, March 2004, Volume 14, Number 1.