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Article

Assessing the Relationship between Cerebral Metabolic Rate of Oxygen and Redox Cytochrome C Oxidase During Cardiac Arrest and Cardiopulmonary Resuscitation

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Abstract: Understanding brain oxygen metabolism is crucial for evaluating overall health, particularly in high-stress medical situations such as cardiac arrest and cardiopulmonary resuscitation (CPR). This study focuses on two key indicators of brain oxygen metabolism: the cerebral metabolic rate of oxygen ($CMRO_2$) and the oxidation state of redox cytochrome c oxidase ($rCCO$). Using advanced techniques like hyperspectral near-infrared spectroscopy (hNIRS) and laser doppler flowmetry (LDF), we have conducted a comprehensive analysis of their relationship in pigs during and after cardiac arrest and CPR. We investigated both the entire duration of these experiments and specific time intervals, providing a detailed view of how these metrics interact. The data reveals non-linear relationship between $rCCO$ and $CMRO_2$. Our findings contribute to a deeper understanding of how the brain manages oxygen during critical episodes, potentially guiding future interventions in neurological care and improving outcomes in emergency medical settings.

Keywords: near-infrared spectroscopy; NIRS; brain; neuronal activity; cardiac arrest; resuscitation; CPR; hyperspectral near-infrared spectroscopy; microvasculature; laser doppler flowmetry; diffuse correlation spectroscopy; cerebral blood flow; cerebral metabolic rate of oxygen; redox cytochrome c oxidase; hemodynamic model

1. Introduction

Out-of-hospital cardiac arrest is a significant medical emergency, affecting more than 350,000 individuals annually in the United States. Despite extensive advancements in emergency care, the survival rates for these events remain low: less than 23% of patients who experience out-of-hospital cardiac arrest survive long enough to be admitted to the hospital, and only about 10% ultimately survive to hospital discharge [1]. Among those who do survive, brain injury remains a leading cause of morbidity and mortality, with over two-thirds of survivors enduring irreversible neurological damage during their hospital stay. These dire outcomes highlight the urgent need for a deeper understanding of the physiological changes in the brain during and after cardiac arrest.

The brain's vulnerability to oxygen deprivation during cardiac arrest makes it particularly susceptible to ischemic injury. Although the brain comprises only 2% of body weight, it requires 15–20% of total cardiac output to sustain homeostasis [2]. Any disruption in oxygen delivery can lead to severe consequences, including secondary brain injury in conditions such as ischemia, severe traumatic brain injury, and sub-arachnoid hemorrhage [3]. Post-cardiac arrest brain injury is the leading cause of mortality in patients revived from cardiac arrest and the primary cause of long-term

disability in survivors [4]. Cardiac arrest (CA) and cardiopulmonary resuscitation (CPR) introduce significant fluctuations in brain oxygen metabolism, posing a severe challenge to preserving cerebral function during these critical events.

Brain function relies heavily on the precise balance between its metabolic needs, the adequate delivery of oxygen and nutrients, and the efficient removal of cellular waste. Achieving this balance necessitates constant regulation of cerebral blood flow (CBF) [5]. Rapid and precise restoration of CBF and oxygenation is crucial for pre-serving brain function, yet the complex interactions between cerebral perfusion, oxygen metabolism, and neuronal survival during these critical moments are not fully understood. Monitoring cerebral perfusion parameters—such as cerebral tissue oxygen saturation, cerebral blood volume, CBF, the cerebral metabolic rate of oxygen ($CMRO_2$), and redox cytochrome c oxidase ($rCCO$) activity—is essential for improving patient outcomes. These metrics offer vital insights into the brain's oxygen metabolism, a process critical for maintaining cerebral health, especially under the stress conditions of cardiac arrest.

Understanding the relationship between $CMRO_2$, mitochondrial activity, and oxygen delivery is crucial for assessing cerebral health and function. Mitochondrial activity, particularly involving $rCCO$, plays a key role in oxygen consumption within the electron transport chain, with its oxidation state influenced by factors such as oxygen availability and electron flow [3, 6-8]. This understanding, provides insight into neurovascular coupling and how these processes are altered in pathological conditions. It is a key step in neurocritical care, as it can guide interventions aimed at preserving brain function and reducing neurological damage.

Advancements in measurement techniques have enhanced our ability to quantify $rCCO$, with hyperspectral near-infrared spectroscopy (hNIRS) providing detailed in-sights into its activity. This advancement is critical for understanding how $rCCO$ activity correlates with oxygen delivery and consumption. However, not everyone has access to these sorts of technologies. To overcome this issue, mathematical models play a huge role. Mathematical models have been employed to study the interactions among CBF, $CMRO_2$, and $rCCO$, providing a framework to understand these relationships more comprehensively. These models help in analyzing how changes in one parameter can affect the others, offering deeper insights into neurovascular coupling and alterations in pathological conditions.

Previous studies have explored the coupling between oxygen consumption, mitochondrial function, and oxygen delivery in the brain. For instance, research utilizing a multimodal NIRS-MRI approach has quantified the interplay between CBF, $CMRO_2$, and the redox state of $rCCO$ in response to physiological challenges such as hypercapnia and varying oxygenation levels [9]. This research has revealed that the relationship between CBF and $rCCO$ oxidation is dynamic and can vary depending on the type of physiological challenge, thereby providing valuable insights into the complex dynamics of neurovascular coupling under different conditions [9].

Some researchers employed advanced imaging techniques to probe these relationships with greater precision. For example, functional MRI (fMRI) studies in macaques have measured laminar $\Delta CMRO_2$ in the primary visual cortex during visual stimulation, showing that $\Delta CMRO_2$ peaks in the middle cortical layers, which corresponds with autoradiographic measures of metabolism. This study also highlighted a strong relationship between the peak $CMRO_2$ and the highest cytochrome oxidase in-tensity, further refining our understanding of oxygen metabolism in the brain [10].

In addition to these studies, research using an hNIRS-DCS (Diffuse Correlation Spectroscopy) setup has investigated the interdependence of CBF, $CMRO_2$, and $rCCO$ during minor perturbations such as hypoxia [11]. These studies have focused on the brain's responses to less extreme conditions, offering a nuanced understanding of how these critical variables interact in relatively stable environments.

In the present study, we analyze the data obtained invasively from a pig model of cardiac arrest to examine the intricate relationships between $rCCO$, $CMRO_2$, and CBF during CA and CPR. We used laser doppler flowmetry (LDF) to measure CBF, and intracerebral hNIRS to assess changes in oxyhemoglobin (HbO_2), deoxyhemoglobin (HHb), and $rCCO$. These measurements pro-vided insights

into variations in cerebral blood volume (CBV), $rCCO$, and $CMRO_2$ throughout the critical phases of cardiac arrest and resuscitation. By investigating these relationships in a model that closely mimics the human condition, we aimed to deepen understanding of the brain's metabolic responses to such critical events. Ultimately, the findings of this research could contribute to the development of more effective strategies for managing brain injury in cardiac arrest patients, potentially improving survival rates and long-term neurological outcomes.

In our previous work [12], we employed the inverse of a dynamic hemometabolic model (CHS) [13] to analyze hNIRS data collected during cardiac arrest and CPR in a pig model. This study allowed us to measure critical physiological parameters, including the rate of oxygen diffusion from blood to tissue, arteriole and venule volume fractions, and the Fåhræus factor. By identifying significant differences between baseline and post-cardiac arrest values, we gained insights into the microvascular and metabolic perturbations that occur during these critical events. The findings demonstrated the utility of the hemometabolic model in capturing dynamic changes in cerebral oxygenation and blood flow, reinforcing its relevance for studying cerebral responses to cardiac arrest. Building upon this foundation, the present study delves deeper into the relationships between $CMRO_2$, CBF , and $rCCO$ activity. In this study we continue using the CHS model to calculate the absolute values of $CMRO_2$ and CBF .

2. Materials and Methods

2.1. Cardiac Arrest and CPR Setup

Ten female pigs (6–9 week old, 33–39kg) were selected for this study to eliminate the influence of sex differences in susceptibility to ventricular fibrillation (VF) and because female pigs typically exhibit a higher rate of return of spontaneous circulation (ROSC). The animals were fasted overnight before being sedated with an intramuscular injection of ketamine (20 mg/kg; "Ketalean," Bimeda-MTC Animal Health, Cambridge, ON, Canada). After sedation, the pigs were intubated and maintained under anesthesia with continuous isoflurane (1–3% mixed with oxygen). Ventilation was supported using an Ohmeda ventilator (Ohio Medical Products, Madison, WI, USA) with settings adjusted to stabilize physiological parameters such as pH (7.35–7.45), PCO_2 (35–45 mm Hg), and PO_2 (>100 mm Hg). To prevent hypovolemia, normal saline (NS) was administered intravenously through a cannulated ear vein at a rate of 2–4 mL/kg/h. Electrocardiogram (ECG) leads were attached, and defibrillation patches (Zoll Medical, Inc., Chelmsford, MA, USA) were applied to monitor the cardiac activity. Aortic and right atrial pressures were continuously monitored via femoral artery catheters (Mikro-Tip Transducer; Millar Instruments, Houston, TX, USA), providing real-time data to detect the onset of cardiac arrest (VF) [14]. Figure 1 shows the experimental setup, including the positioning of the automatic compression device on the left and the hNIRS system on the right.

All experimental protocols adhered to the guidelines for reporting in vivo experiments [15]. Ethical approval was obtained from the Animal Care Committee of St. Michael's Hospital (Toronto, ON, Canada), and all procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as outlined by the U.S. National Institutes of Health (NIH Publication number 85–23, revised 1996).

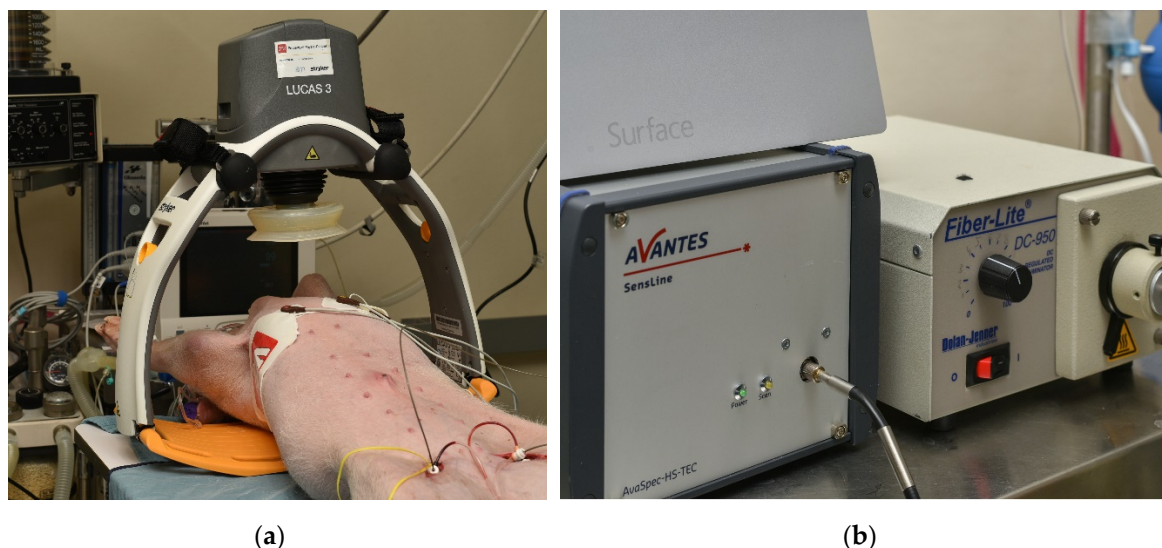


Figure 1. (a) Experiment setup and (b) hNIRS system.

2.2. Resuscitation Protocol

Before inducing VF, the administration of anesthetic gases was halted for 15 minutes, and the animals were sedated with propofol and fentanyl to minimize any anti-arrhythmic effects from the anesthetic gases. VF was then induced by burst pacing at a frequency of at least 300 Hz with a 10 mV pulse, using a pacing catheter (AM-2200, AD Instruments, Castle Hill, Australia) inserted into the right ventricle. CPR was initiated after 2 minutes of VF, consisting of chest compressions at a rate of 100 per minute using an automatic piston device (LUCAS; Physio-Control Inc./Jolife AB, Lund, Sweden). Mechanical ventilation was maintained at a rate of 10 breaths per minute with pure oxygen using the Ohmeda ventilator. An intravenous bolus of 0.015 mg/kg of epinephrine in NS (0.1 mg/mL concentration) was administered after 2 minutes of CPR, followed by a 10 mL NS flush, with additional doses given every 4 minutes for a total of three doses. Additionally, a continuous infusion of NS equivalent to the volume of epinephrine was started after 2 minutes of CPR and continued for a total duration of 12 minutes.

2.3. Cerebral hNIRS and LDF Setup and Measurements

We used a custom-built hyperspectral near-infrared spectroscopy (hNIRS) system [16,17] to invasively measure the absolute concentrations of HbO_2 , HHb , and the differential signal between oxidized and reduced cytochrome c oxidase ($\Delta rCCO$) in the brain tissue. With the hNIRS optodes were placed on the dura mater at a 20 mm interoptode distance/through the holes drilled in the skull. With a differential path length factor (DPF) of 5 and a 20 mm optode spacing, the average optical path length was calculated to be approximately 100 mm, with a penetration depth of about 16 mm [17–19], chosen to minimize interference from the dura mater, which is approximately 1 mm thick. The hNIRS system included a highly sensitive spectrometer (AvaSpec, Avantes, Lafayette, CO, USA) with a custom 0.5-mm slit that captured spectral data in the 700–1000 nm wavelength range (Figure 1). The hNIRS diffusion model was applied to analyze the data, allowing the determination of changes in cerebral HbO_2 , HHb , and $\Delta rCCO$ [14]. The dynamic changes in chromophore concentrations were extracted using a multi-step data-fitting algorithm, based on an analytical solution to the diffusion equation for a semi-infinite medium, incorporating an extrapolated boundary condition. This algorithm provided tissue concentrations of HbO_2 , HHb , and $\Delta rCCO$. The total tissue hemoglobin concentration ($[tHB]$) in μM per liter and tissue oxygen saturation (tSO_2) in % were computed using the following equation:

$$[tHB] = [HbO_2] + [HHb], \quad (1)$$

$$tSO_2 = [HbO_2] / ([tHb]) \times 100\%. \quad (2)$$

Cerebral blood flow velocity index (CBFi) was measured using a laser Doppler flowmeter (LDF) (Periflux; Perimed Inc., Ardmore, PA, USA). The LDF probe was placed on the dura mater through a hole drilled in the pig's skull. Throughout the experiments, mean aortic pressure and right atrial pressure were continuously monitored using catheters equipped with micro-manometer tips.

2.4. Analysis Methods

The data analysis was conducted using MATLAB R2023a (MathWorks, Natick, MA, USA). We analyzed data during periods of cardiac arrest or ventricular fibrillation (VF), CPR, and, in cases where it occurred, ROSC. Each of these phases was examined individually to assess physiological responses under different conditions. Outliers were systematically identified and excluded to ensure the accuracy and reliability of the results.

The analysis included the use of hNIRS diffusion modeling [16-21] to assess cerebral oxygenation, deoxygenation, and $rCCO$ levels.

The absolute values of CBF and the $CMRO_2$ were calculated using the nonlinear version of the Coherent Hemodynamic Spectroscopy (CHS) model [22]. This approach integrates experimental data obtained from hNIRS and LDF, allowing for accurate assessment during significant physiological perturbations, such as cardiac arrest and CPR [12].

CBF was derived from the model's fitted capillary blood flow factor (BFf) and the cerebral blood flow index (CBFi) measured by LDF. The velocity of capillary blood flow ($c^{(c)}$) was calculated as:

$$c^{(c)}(t) \left(\frac{mm}{s} \right) = BFf \left(\frac{mm}{s} \right) \cdot CBFi(t). \quad (7)$$

Using the capillary cross-sectional area ($3.85 \times 10^{-7} \text{ cm}^2$), the brain tissue density (1.081 g/ml) and the total volume of capillaries in the brain tissue (2.7%), the absolute CBF per 100 g of brain tissue was computed as:

$$CBF(t) \left(\frac{ml \text{ blood}}{100 \text{ g tissue} \cdot min} \right) = 18.75 \left(\frac{s \cdot cm^2}{100 \text{ g tissue} \cdot min} \right) \times c^{(c)}(t) \left(\frac{mm}{s} \right). \quad (8)$$

This value falls within accepted ranges reported in similar studies, supporting its validity. However, further validation is part of our ongoing work [23-26].

$CMRO_2$ was calculated using the equation called "the Fick's principle" [27]:

$$CMRO_2(t) \left(\frac{ml \text{ } O_2}{100 \text{ g tissue} \cdot min} \right) = k \left(\frac{ml \text{ } O_2}{g \text{ Hb}} \right) \times [tHb] \left(\frac{g}{l} \right) \times CBF(t) \left(\frac{ml \text{ blood}}{100 \text{ g tissue} \cdot min} \right) \times (S_0^{(a)} - S^{(v)}(t)), \quad (9)$$

where S^a and S^v represent the arterial and venous oxygen saturations, respectively and k is a factor describing the amount of O_2 bound to hemoglobin when completely saturated (1.39 ml of O_2 per g Hb). $CMRO_2$ is expressed in unit of ml O_2 /100 g/min.

Venous's oxygen saturation was calculated using the CHS model [13]. To do so, the non-linear version of this model was used [12,22]. The results closely matched the equations suggested by the authors [27], as follows:

$$S^v = \frac{4}{3} tSO_2 - \frac{1}{3} S^a. \quad (4)$$

We investigated possible nonlinearities among the variables of interest, $rCCO$ and $CMRO_2$, $rCCO$ and CBF, and $CMRO_2$ and CBF using the power law:

$$X = a + kY^\alpha, \quad (5)$$

where k was a scaling factor, $\alpha > 0$ was the power exponent, X and Y were either the change in $CMRO_2$, CBF or $rCCO$. In the cases of the nonlinear relationships α was significantly different from 1.

2.5. Statistical Analysis

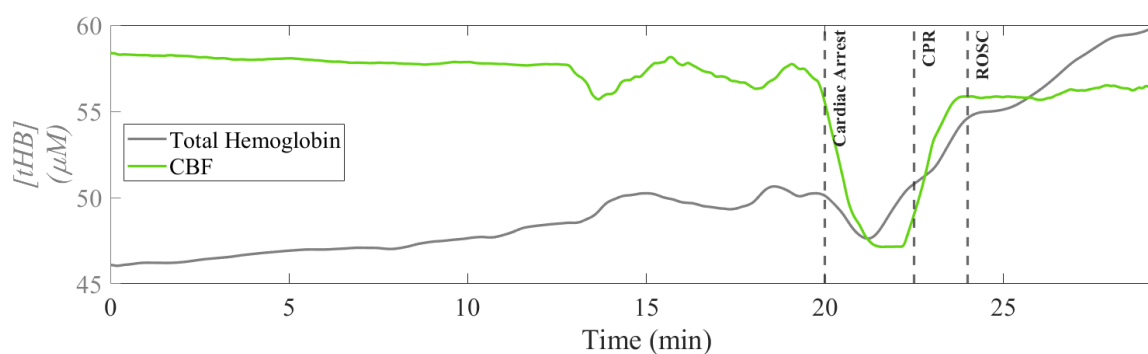
We applied statistical techniques to ensure the robustness and reliability of identifying relationships among CBF vs. $CMRO_2$, CBF vs. $rCCO$, and $CMRO_2$ vs. $rCCO$. Correlation coefficients (R) and p -values were calculated using MATLAB's `fitnlm` function, with statistical significance defined as $p < 0.05$. To evaluate the power-law exponent (α) for each relationship, we conducted a one-sample t -test to determine if the mean value of α was statistically different from 1 or 0. This analysis tested whether the observed relationships deviated from linearity ($\alpha = 1$) or if the exponent indicated no significant relationship ($\alpha = 0$). This approach provided valuable insight into the nature of coupling between the variables. A similar method was applied to assess whether the intercept term (a) and the scaling factor (k) were significantly different from 0, further verifying the strength and validity of the fitted models.

3. Results

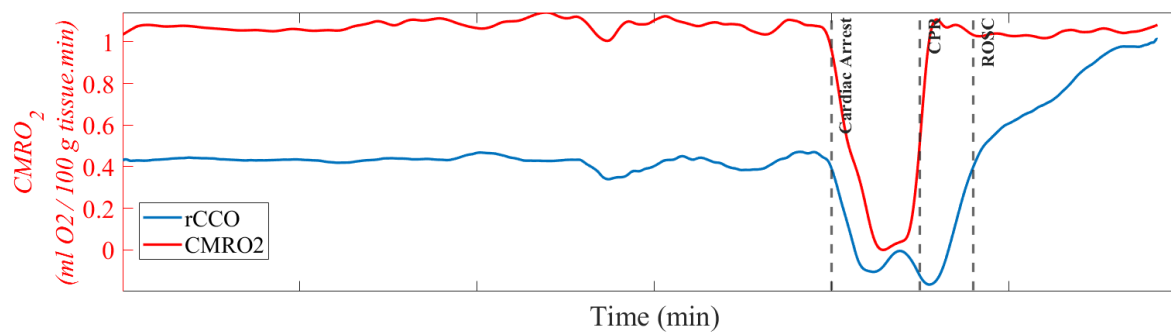
Figure 2 presents representative time traces of $[tHB]$, $rCCO$, $CMRO_2$, and CBF for one of the pigs, serving as an example of absolute value calculations as well as normalized CBF , $rCCO$ and $CMRO_2$ in pairs. The top two plots show $[tHB]$ versus CBF , and $rCCO$ versus $CMRO_2$ in absolute values. $[tHB]$ and $rCCO$ values are given in μM , while CBF and $CMRO_2$ are expressed in mL blood/100 g tissue/min and mL O_2 /100 g tissue/min, respectively.

Figure 3 illustrates these relationships during cardiac arrest, with each dataset normalized to its maximum value for comparability. Each relationship is represented by a unique color, with median lines shown as solid lines and data ranges depicted by shaded areas. These plots highlight the linear and non-linear dynamics between $rCCO$, $CMRO_2$, and CBF under the extreme physiological conditions of cardiac arrest.

To further analyze the data shown in Figure 4 and to compare it to similar studies reporting approximately 70% reductions from baseline, we investigated the data by dividing it into two zones. During cardiac arrest, zone I represents the period from baseline to a 70% drop relative to baseline, while zone II corresponds to a 60% to 100% drop (complete cessation of blood flow). The results indicate that as blood flow approaches zero, the parameters exhibit increasingly random behavior. This is reflected in the standard deviation of the power-law α values, with $CMRO_2$ vs. $rCCO$ showing a standard deviation of 0.388, CBF vs. $rCCO$ at 0.350, and CBF vs. $CMRO_2$ at 0.314. These findings highlight the variability and instability of physiological responses during severe reductions in perfusion. This suggests that during severe ischemia, the relationships between these parameters become less predictable, reflecting the breakdown of normal regulatory mechanisms. While variability at this level is expected under extreme conditions, it highlights the need for careful interpretation and potentially more complex modeling to accurately capture metabolic dynamics during CA. Table 1 provides the median and standard deviation values for α across both zones and for all three relationships.

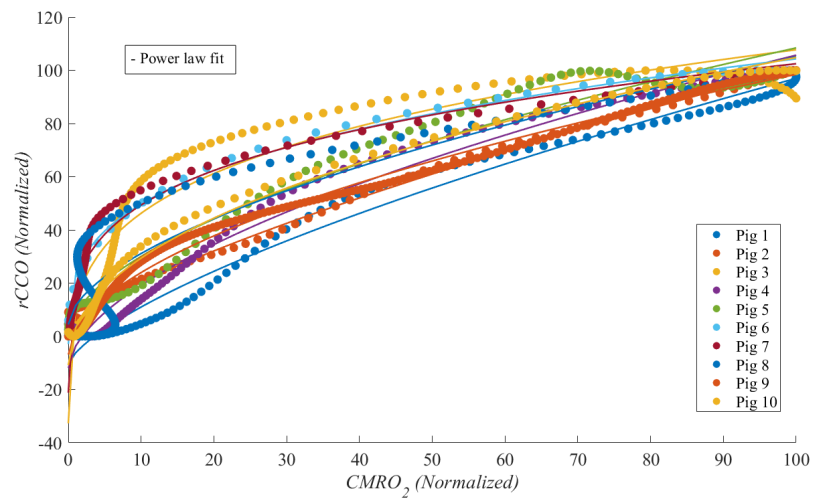


(a)

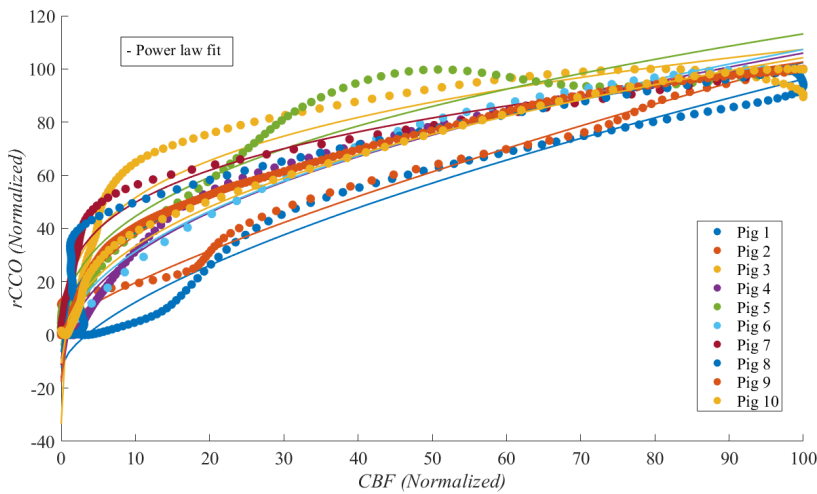


(b)

Figure 2. Time traces of $[tHB]$, $rCCO$, $CMRO_2$, and CBF for one pig are shown in pairs: (a), $[tHB]$ vs. CBF ; and (b), $rCCO$ vs. $CMRO_2$; $[tHB]$ and $rCCO$ values are in μM , CBF ($\frac{ml\ blood}{100\ g\ tissue\ .min}$) and $CMRO_2$ in ($\frac{ml\ O_2}{100\ g\ tissue\ .min}$).



(a)



(b)

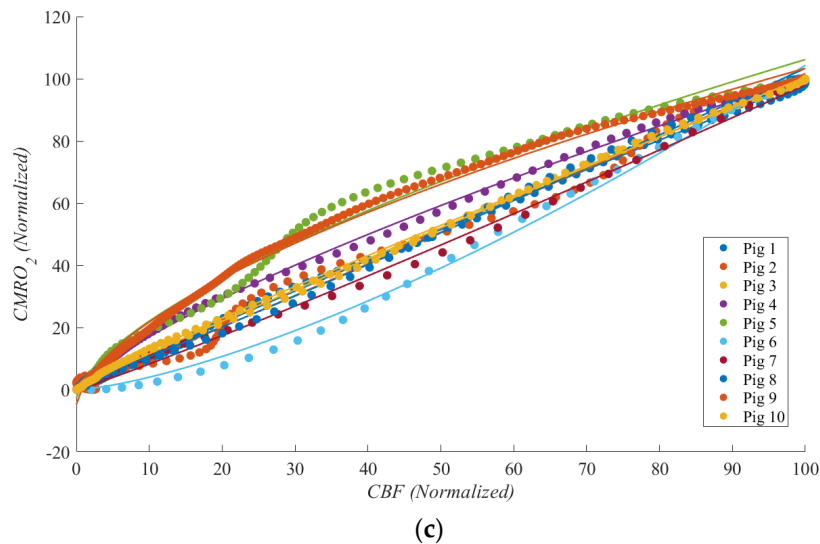


Figure 3. Scatter plots for all the pigs (each color represents one pig) during cardiac arrest from the correlations between: (a) $rCCO$ vs $CMRO_2$; (b) CBF vs $rCCO$; c) CBF vs $CMRO_2$.

Table 1. This is a table. Tables should be placed in the main text near to the first time they are cited.

	Relationship / Parameter	R^2	α	p-value ($\alpha \neq 1$)
Zone I	$CMRO_2$ vs $rCCO$	0.986 ± 0.158	0.590 ± 0.277	0.003
	CBF vs $rCCO$	0.989 ± 0.253	0.475 ± 0.356	0.005
	CBF vs $CMRO_2$	0.996 ± 0.005	0.909 ± 0.339	0.827
Zone II	$CMRO_2$ vs $rCCO$	0.974 ± 0.147	0.707 ± 0.388	0.135
	CBF vs $rCCO$	0.961 ± 0.112	0.608 ± 0.350	0.023
	CBF vs $CMRO_2$	0.998 ± 0.011	0.888 ± 0.314	0.999

Due to the volatile conditions during CPR, characterized by intense movements and mechanical compressions, analyzing physiological markers was challenging compared to stable periods for most experiments. As a result, no clear relationships between $rCCO$ and other variables were observed, and the data appeared random. This is reflected in the low R^2 values for the power-law fits. Figure 5 shows a scatter plot of $CMRO_2$ vs. $rCCO$, illustrating this lack of correlation. However, as expected, CBF and $CMRO_2$ maintained a strong linear relationship. In most experiments, MATLAB was unable to fit the power-law model for $rCCO$ -related data, resulting in fewer than 10 pigs being represented in Figure 5. Nonetheless, R^2 values and the median power-law α , along with their standard deviations, are presented in Table 3.

Table 2 and Table 3 summarize the R^2 values and power-law α medians with standard deviations for each relationship during cardiac arrest and CPR respectively. The consistently strong relationships underscore the importance of these variables in understanding cerebral metabolism during cardiac arrest and CPR. These findings reveal that while the relationship between CBF and $CMRO_2$ is predominantly linear, the interactions involving $rCCO$ exhibit significant non-linear dynamics, underscoring the complexity of cerebral metabolism during cardiac arrest.

Figure 6 shows CBF vs. $CMRO_2$, CBF vs. $rCCO$, and $CMRO_2$ vs. $rCCO$ for two of the experiments, ROSC was achieved, enabling the study of physiological relationships during this phase. Although statistical analysis was not feasible due to the limited sample size, strong non-linear relationships were observed between $rCCO$ and $CMRO_2$, as well as between $rCCO$ and CBF . Notable differences compared to the cardiac arrest phase included the emergence of a weak non-linear relationship between CBF and $CMRO_2$ and a marked change in curvature for the other two relationships (α shifting from approximately 0.5 during cardiac arrest to around 3 during ROSC).

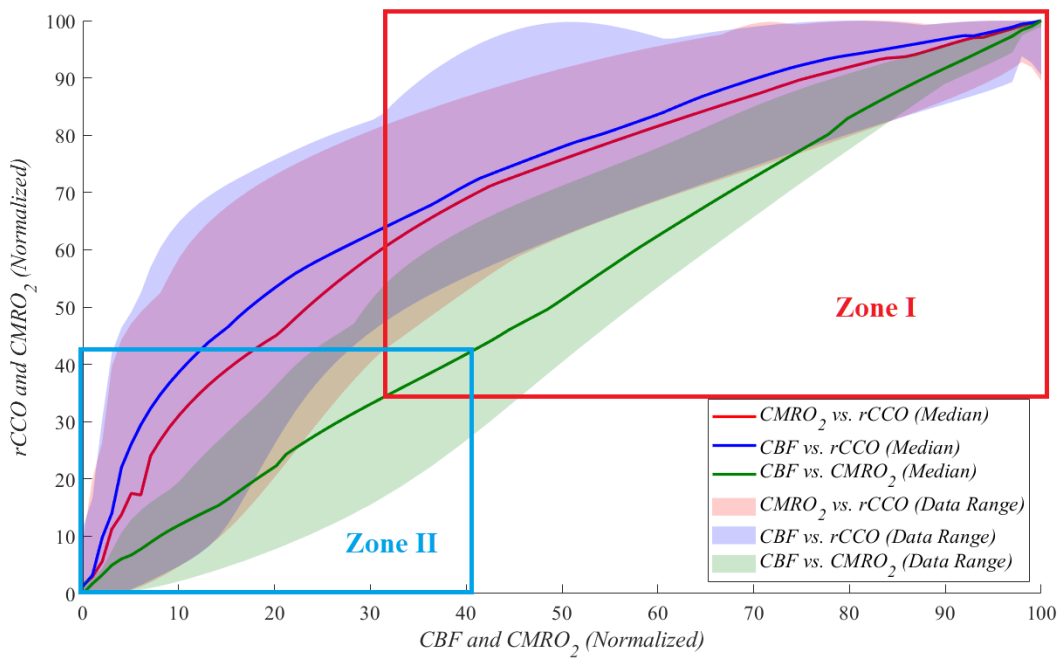


Figure 4. Correlation plots for all the pigs (each color represents one relationship) during cardiac arrest divided to two zones, zone I represents the period from baseline to a 70% drop relative to baseline, while zone II corresponds to a 60% to 100% drop (complete cessation of blood flow): (red) $CMRO_2$ vs. $rCCO$ during; (blue) CBF vs. $rCCO$; (green) CBF vs. $CMRO_2$.

Table 2. Power-law fit R^2 and power law α means $\pm$ standard deviations for relationships between $rCCO$ and $CMRO_2$, CBF and $CMRO_2$ as well as $rCCO$ and CBF during Cardiac Arrest.

Relationship / Parameter	R^2	α	p-value ($\alpha \neq 1$)	p-value ($\alpha \neq 0$)
$CMRO_2$ vs $rCCO$	0.981 ± 0.057	0.550 ± 0.169	0.000	0.000
CBF vs $rCCO$	0.980 ± 0.038	0.401 ± 0.157	0.000	0.000
CBF vs $CMRO_2$	0.998 ± 0.003	0.944 ± 0.222	0.428	0.000

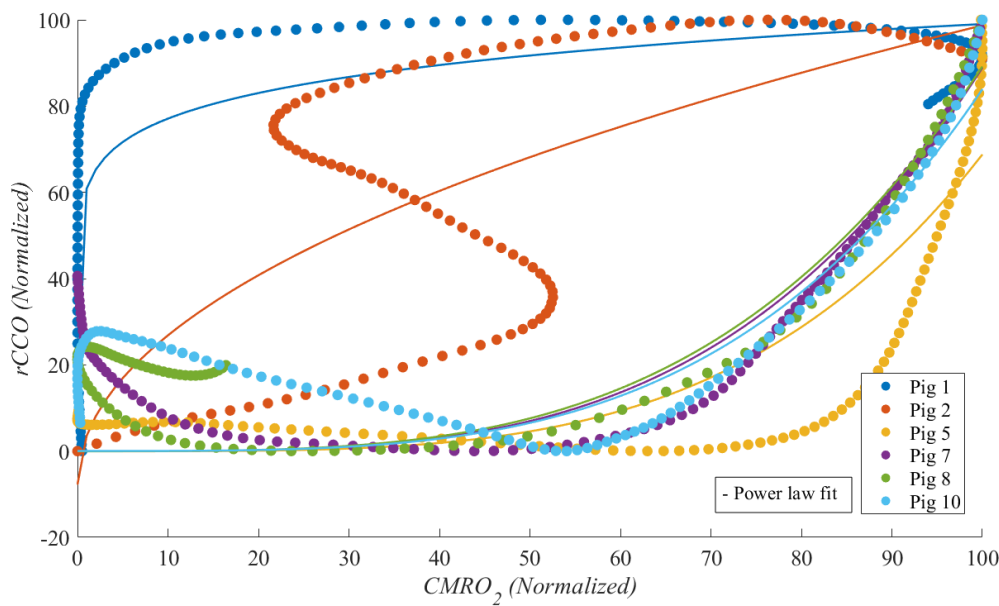
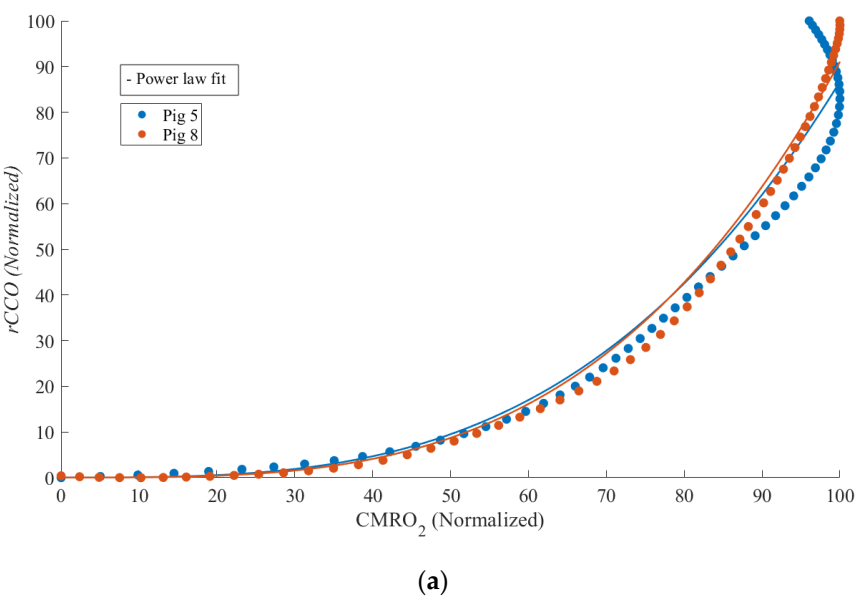


Figure 5. Scatter plot for 6 pigs (each color represents one pig) from the correlations between $CMRO_2$ and $rCCO$ during CPR.

Table 3. Power law fit R^2 and power law α means $\pm$ standard deviations for relationships between $rCCO$ and $CMRO_2$, CBF and $CMRO_2$ as well as $rCCO$ and CBF during CPR.

Relationship / Parameter	R^2	α	p-value ($\alpha \neq 1$)	p-value ($\alpha \neq 0$)
$CMRO_2$ vs $rCCO$	0.681 ± 0.137	3.614 ± 1.608	0.080	0.016
CBF vs $rCCO$	0.694 ± 0.204	3.531 ± 1.628	0.084	0.017
CBF vs $CMRO_2$	0.996 ± 0.028	0.942 ± 0.154	0.831	0.000

In addition to the power-law exponent α , the intercept a and the scaling factor k were calculated for each relationship during cardiac arrest and CPR. A one-sample t-test confirmed that k and α were positive and significantly different from zero ($p < 0.05$) during cardiac arrest, reflecting strong coupling between $CMRO_2$, CBF , and $rCCO$ under ischemic conditions. The p-values for $\alpha \neq 1$ (non-linearity) and $\alpha \neq 0$ (presence of coupling) are presented in Tables 2 and 3, indicating significant deviations from linearity during cardiac arrest ($p < 0.05$), except for the CBF vs. $CMRO_2$ relationship, where $\alpha \approx 1$ ($p = 0.428$), suggesting a predominantly linear flow-metabolism coupling. Conversely, during CPR, a and k were not significantly different from zero ($p > 0.05$) in the CBF vs. $rCCO$ and $CMRO_2$ vs. $rCCO$ relationships, underscoring the absence of meaningful coupling. However, k in the CBF vs. $CMRO_2$ relationship remained significant ($p < 0.05$), reinforcing that partial linearity persists between flow and metabolism, even under the dynamic and mechanically unstable conditions introduced by chest compressions. These findings highlight the non-linear and disrupted nature of cerebral metabolism during CPR, with select preservation of linear flow-metabolism interactions between CBF and $CMRO_2$.



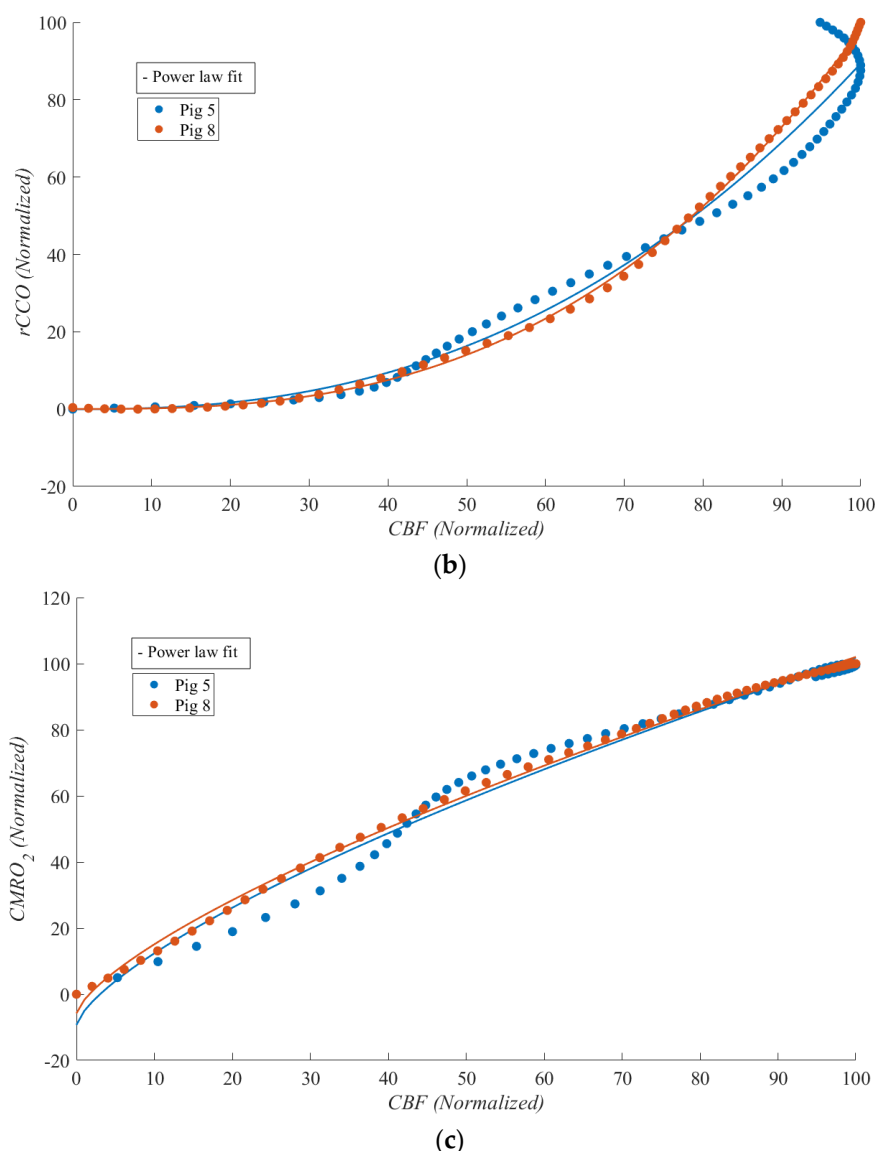


Figure 6. Scatter plots for two pigs (each color represents one pig) during ROSC from the correlations between: (a) $CMRO_2$ and $rCCO$; (b) CBF and $rCCO$; (c) CBF and $CMRO_2$. The median R^2 values for CBF vs. $CMRO_2$, CBF vs. $rCCO$, and $CMRO_2$ vs. $rCCO$ were 0.989, 0.982, and 0.968, respectively. The median α values for these relationships were 0.711, 3.293 and 2.629 respectively.

4. Discussion

Near-infrared spectroscopy (NIRS) has demonstrated the potential to detect cardiac arrest (CA) and monitor tissue oxygenation even in the absence of a pulse waveform. This suggests its application in identifying CA outside hospital settings and continuously monitoring patients until circulation is restored [28]. In this study, we investigated CA and cardiopulmonary resuscitation (CPR) in pigs using hyperspectral NIRS combined with laser Doppler flowmetry (LDF). Broadband NIRS captures the full NIR spectrum at each time point, providing: (1) the ability to detect chromophores beyond HbO_2 and HHb , such as cytochrome-c-oxidase (CCO) and water; (2) improved accuracy in chromophore concentration by resolving full spectral features; and (3) the capability to determine pathlength and absolute concentrations using second differential spectral methods [29].

Our study aimed to analyze the relationship between redox cytochrome c oxidase ($rCCO$), the cerebral metabolic rate of oxygen ($CMRO_2$), and cerebral blood flow (CBF) during CA, CPR, and return of spontaneous circulation (ROSC). Our results obtained invasively directly from the brain reveal distinct patterns of interaction between these variables, shedding light on the non-linear dynamics governing cerebral metabolism under extreme physiological conditions.

The strong linear relationship observed between CBF and $CMRO_2$, with R^2 values approaching 1.0, aligns with expectations based on Fick's principle, indicating a robust coupling between oxygen delivery and consumption. This consistency underscores the fundamental role of CBF in sustaining cerebral oxygen metabolism. In contrast, the relationships involving $rCCO$ deviated from linearity, particularly during CA. The power-law fits for $CMRO_2$ vs. $rCCO$ and CBF vs. $rCCO$ exhibited lower R^2 values and α exponents around 0.5, suggesting that mitochondrial function and oxygen utilization become less predictable as blood flow diminishes. During cardiac arrest, the scaling factor k and power-law exponent α were consistently positive and significant ($p < 0.05$), reflecting strong coupling between $CMRO_2$, CBF , and $rCCO$, while the intercept a was not significant for CBF vs. $CMRO_2$, suggesting proportional flow-metabolism coupling without a baseline offset. In contrast, during CPR, a and k were not significant for $rCCO$ -related relationships, indicating disrupted mitochondrial coupling, likely due to mechanical chest compressions. However, k remained significant for CBF vs. $CMRO_2$, highlighting partial preservation of linear flow-metabolism interaction even under unstable conditions. This suggests that while cerebral blood flow and oxygen metabolism coupling persists during CPR, mitochondrial dynamics are more sensitive to resuscitative efforts, emphasizing the need to protect mitochondrial function for better neurological outcomes.

Interestingly, during CPR, the relationships between $rCCO$ and $CMRO_2$ or CBF weakened, likely due to the physical disturbances and hemodynamic fluctuations induced by mechanical chest compressions. The low R^2 values during CPR reflect the challenges of accurately capturing cerebral metabolism under such turbulent conditions. This finding highlights the need for refined measurement techniques to mitigate motion artifacts and enhance data reliability.

The p-values for $\alpha \neq 1$ across the entire cardiac event reveal distinct patterns in the relationships between $CMRO_2$, CBF , and $rCCO$ during CA, CPR, and ROSC. The relationship between CBF and $CMRO_2$ consistently showed $p > 0.05$ ($p = 0.404$ during CA and $p = 0.391$ during CPR), indicating that the coupling between oxygen delivery and consumption persists even during mechanical compressions. Conversely, the relationships involving $rCCO$ demonstrated $p < 0.05$ for $\alpha \neq 1$ during CA and ROSC ($p = 0.001$ and $p = 0.015$ respectively), reflecting significant deviations from linearity. This suggests that mitochondrial function follows non-linear behavior under ischemic and reperfusion conditions. During CPR, the relationships between $rCCO$ and $CMRO_2$ or CBF weakened, as indicated by $p > 0.05$, which may reflect the hemodynamic instability introduced by chest compressions. Despite this, p-values for $\alpha \neq 0$ remained highly significant ($p < 0.001$), highlighting the ongoing metabolic contribution of $rCCO$ even during turbulent periods.

The scaling factor k , which represents the proportional relationship between the variables of interest, was consistently positive and statistically significant across all phases of the study (CA, CPR, and ROSC). This finding aligns with physiological expectations, suggesting that even during severe reductions in cerebral blood flow, a positive relationship between $CMRO_2$, CBF , and $rCCO$ persists, reflecting the brain's ongoing metabolic activity and mitochondrial function. The significance of k during CPR highlights the resilience of cerebral metabolism, even under the dynamic and mechanically unstable conditions introduced by chest compressions. This reinforces the importance of preserving mitochondrial function during resuscitation efforts, as it may play a critical role in improving neurological outcomes post-ROSC.

To further analyze these relationships, we separated the data during CA in two zones: Zone I (baseline to 70% drop) and Zone II (60% to 100% drop in blood flow). The results revealed increasing randomness in physiological responses as blood flow approached zero, with greater variability in the power-law α values observed in Zone 2. The standard deviations of α for $CMRO_2$ vs. $rCCO$ (0.388), CBF vs. $rCCO$ (0.350), and CBF vs. $CMRO_2$ (0.314) suggest heightened instability in mitochondrial and metabolic responses near complete cessation of perfusion.

The segmentation of the data into Zone I and Zone II enabled a more detailed analysis of metabolic responses. This division allows for direct comparison with prior non-invasive studies in small piglets by Milej et al. [11], which focused on smaller perturbations in CBF from the baseline values. In Zone I, For the $CMRO_2$ vs. $rCCO$ relationship, $p = 0.07$, suggesting near-linearity. This result

corresponds closely to [11], where the relationship between $rCCO$ and $CMRO_2$ was found to be not significantly different from one. The relationship between $CMRO_2$ and CBF remained linear with $\alpha = 0.941 \pm 0.299$ and $p = 0.813$ for $\alpha \neq 1$, indicating that oxygen delivery and consumption are tightly coupled under moderate reductions in blood flow. Interestingly, this result contrasts with the findings of [11], where the same relationship exhibited mild non-linearity ($\alpha = 1.39 \pm 0.31$) during carotid occlusion. One possible explanation for this difference is the nature of the ischemic event. In Milej et al. [11], ischemia was induced gradually under anesthesia, which may have allowed for compensatory mechanisms that introduced non-linearity. In contrast, our study involved CA, characterized by a rapid and complete cessation of blood flow, likely overwhelming autoregulatory mechanisms and preserving a direct linear relationship between CBF and $CMRO_2$ during the initial phase of ischemia.

However, in Zone II, as blood flow approached zero, non-linear responses emerged. The relationships between $CMRO_2$ and $rCCO$ ($p = 0.004$) and between CBF and $rCCO$ ($p = 0.016$) showed significant deviations from linearity, reflecting increased variability in mitochondrial responses under severe ischemia. These findings contrast with the linearity observed during moderate occlusion in [11] partially and emphasize the need for non-linear modeling to fully capture metabolic dynamics during extreme reductions in blood flow. The comparison between Zone I and Zone II highlights the shift from linear to non-linear behavior as ischemia deepens. While prior studies concentrated on milder ischemic conditions, our data illustrate the breakdown of linear relationships during extreme drops in blood flow, emphasizing the need for non-linear modeling to fully capture the metabolic dynamics of CA and CPR.

Unfortunately, in our study we had a very limited number of ROSC cases (two ROSC out of ten total). During ROSC, the non-linear relationship between $CMRO_2$ and $rCCO$ persisted but exhibited a significant shift, with α values increasing to approximately 3. This sharp contrast to the α values observed during CA suggests a dynamic change in mitochondrial activity and oxygen utilization as circulation is restored. The observed non-linearity may reflect the brain's attempt to rapidly restore homeostasis following prolonged ischemia. However, possible differences between CA, CPR, and ROSC phases require further investigations.

Additionally, in this study we focused on the relative changes in $rCCO$ rather than on the absolute values, as we did not have baseline measurements of oxidized and reduced CCO concentrations. This limitation should be considered when interpreting the results, as absolute values would provide a more comprehensive understanding of the physiological changes occurring during CA and CPR. To advance our understanding, leveraging mathematical models presents several advantages. By identifying and quantifying the mathematical relationships between physiological variables, we can predict one variable based on measurements of another. This approach becomes particularly valuable in situations where advanced measurement technologies are unavailable or impractical. Mathematical models can provide meaningful insights and predictions in such contexts.

In our future research we will focus on evaluating the validity and applicability of existing hemometabolic models to describe the mathematical relationships between $rCCO$, $CMRO_2$, and CBF . A key part of this effort will involve applying the BrainSignal model [30], which provides a comprehensive framework for analyzing these interactions. Unlike CHS, which lacks the capacity to calculate $rCCO$, BrainSignal incorporates mitochondrial metabolism, allowing for the quantification of $rCCO$. In this study, the availability of hNIRS enabled us to directly measure $rCCO$, offering insights into mitochondrial function during CA and CPR.

Moving forward, we aim to leverage the BrainSignal model to mathematically estimate $rCCO$ in contexts where direct measurements are not feasible. By integrating this model with our experimental data, we seek to uncover more precise correlations between $rCCO$, $CMRO_2$, and CBF under various physiological conditions. This dual approach—combining experimental data from hNIRS with predictive modeling—will not only deepen our understanding of cerebral metabolism but also enhance the development of tools capable of predicting cerebral hemodynamic responses in both clinical and research settings. Ultimately, these efforts could bridge the gap between experimental

measurements and real-time predictive monitoring, furthering the potential for non-invasive assessment of brain metabolism in critical care environments.

5. Conclusions

This study provides novel insights into the interactions between $CMRO_2$, $rCCO$, and CBF during cardiac arrest, CPR, and ROSC. The results confirm a linear relationship between CBF and $CMRO_2$ during CA, reflecting the dependence of oxygen metabolism on cerebral perfusion. In contrast, the relationships involving $rCCO$ exhibited significant non-linearity, highlighting the complex role of mitochondrial function in oxygen utilization under extreme physiological stress.

The division of data into two zones during CA revealed increasing variability in metabolic responses as blood flow declined, with greater randomness observed near complete cessation of perfusion. These findings underscore the importance of studying cerebral metabolism across different phases of cardiac arrest and resuscitation to better understand the mechanisms underlying brain injury. The emergence of non-linear relationships during ROSC suggests dynamic changes in mitochondrial activity as circulation is restored. This highlights the potential for leveraging advanced NIRS technologies and mathematical models to predict cerebral metabolic responses and guide clinical interventions during and after CA.

Future research involving CHS and BrainSignal hemometabolic models should focus on refining measurement techniques to minimize motion artifacts during CPR and validating the observed relationships in larger animal models or clinical settings. By enhancing our understanding of cerebral metabolism under extreme conditions, these efforts may contribute to improved strategies for mitigating brain injury and enhancing patient outcomes during cardiac arrest and resuscitation.

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Institutional Review Board Statement: All animals in the project will receive humane and proper treatment in accordance with the policies formulated by St. Michael’s Hospital Animal Care Committee, the Animals for Research Act 1968-69 and with the recommendations of the Canadian Council on Animal Care. PI accepts responsibility for keeping the foregoing information current especially with respect to methodology. The protocol code is 245ACC #245.

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