

# Dissociated Oxygen Consumption and Carbon Dioxide Production in the Post-Cardiac Arrest Rat: A Novel Metabolic Phenotype

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**Background**—The concept that resuscitation from cardiac arrest (CA) results in a metabolic injury is broadly accepted, yet patients never receive this diagnosis. We sought to find evidence of metabolic injuries after CA by measuring O<sub>2</sub> consumption and CO<sub>2</sub> production (VCO<sub>2</sub>) in a rodent model. In addition, we tested the effect of inspired 100% O<sub>2</sub> on the metabolism.

**Methods and Results**—Rats were anesthetized and randomized into 3 groups: resuscitation from 10-minute asphyxia with inhaled 100% O<sub>2</sub> (CA-FIO<sub>2</sub> 1.0), with 30% O<sub>2</sub> (CA-FIO<sub>2</sub> 0.3), and sham with 30% O<sub>2</sub> (sham-FIO<sub>2</sub> 0.3). Animals were resuscitated with manual cardiopulmonary resuscitation. The volume of extracted O<sub>2</sub> (VO<sub>2</sub>) and VCO<sub>2</sub> were measured for a 2-hour period after resuscitation. The respiratory quotient (RQ) was RQ=VCO<sub>2</sub>/VO<sub>2</sub>. VCO<sub>2</sub> was elevated in CA-FIO<sub>2</sub> 1.0 and CA-FIO<sub>2</sub> 0.3 when compared with sham-FIO<sub>2</sub> 0.3 in minutes 5 to 40 after resuscitation (CA-FIO<sub>2</sub> 1.0: 16.7±2.2, *P*<0.01; CA-FIO<sub>2</sub> 0.3: 17.4±1.4, *P*<0.01; versus sham-FIO<sub>2</sub> 0.3: 13.6±1.1 mL/kg per minute), and then returned to normal. VO<sub>2</sub> in CA-FIO<sub>2</sub> 1.0 and CA-FIO<sub>2</sub> 0.3 increased gradually and was significantly higher than sham-FIO<sub>2</sub> 0.3 2 hours after resuscitation (CA-FIO<sub>2</sub> 1.0: 28.7±6.7, *P*<0.01; CA-FIO<sub>2</sub> 0.3: 24.4±2.3, *P*<0.01; versus sham-FIO<sub>2</sub> 0.3: 15.8±2.4 mL/kg per minute). The RQ of CA animals persistently decreased (CA-FIO<sub>2</sub> 1.0: 0.54±0.12 versus CA-FIO<sub>2</sub> 0.3: 0.68±0.05 versus sham-FIO<sub>2</sub> 0.3: 0.93±0.11, *P*<0.01 overall).

**Conclusions**—CA altered cellular metabolism resulting in increased VO<sub>2</sub> with normal VCO<sub>2</sub>. Normal VCO<sub>2</sub> suggests that the postresuscitation Krebs cycle is operating at a presumably healthy rate. Increased VO<sub>2</sub> in the face of normal VCO<sub>2</sub> suggests a significant alteration in O<sub>2</sub> utilization in postresuscitation. Several RQ values fell well outside the normally cited range of 0.7 to 1.0. Higher FIO<sub>2</sub> may increase VO<sub>2</sub>, leading to even lower RQ values. (*J Am Heart Assoc.* 2018;7:e007721. DOI: 10.1161/JAHA.117.007721.)

**Key Words:** CO<sub>2</sub> production • cardiopulmonary resuscitation (CPR) • metabolism • O<sub>2</sub> consumption • respiratory quotient

Exciting data from our laboratory have demonstrated that our current understanding of aerobic respiration cannot explain the O<sub>2</sub> consumption (VO<sub>2</sub>) measured in rats resuscitated from prolonged cardiac arrest (CA), suggesting that we have discovered a new postresuscitation metabolic phenotype.

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CA is a time-dependent pathology,<sup>1</sup> and the majority of patients with CA are found more than 10 minutes after their arrest occurred.<sup>2,3</sup> Patients with long untreated arrest times have the worst outcomes,<sup>2–4</sup> in part because there is much to learn about the injury sustained by patients resuscitated from prolonged CA. The loss of blood flow caused by CA is widely acknowledged to cause a metabolic pathology, but we struggle to measure the effect of this pathology at the bedside. The cessation of blood flow during CA stops delivery of metabolic substrates and removal of metabolic waste products, rapidly causing a variety of metabolic disorders including loss of ATP, acidemia, hypercarbia, hyperlactemia, loss of the adenosine pool, loss of ion gradients, and loss of water control.<sup>5–9</sup>

Our inability to connect our understanding of the metabolic injury caused by CA to the diagnosis and treatment of patients is a major impediment to progress in the field of resuscitation science. There are promising clinical treatments, such as therapeutic hypothermia, ischemic postconditioning, and controlled reperfusion, which improve survival in preclinical

## Clinical Perspective

### What Is New?

- Cardiac arrest altered cellular metabolism resulting in increased O<sub>2</sub> consumption with normal exhaled CO<sub>2</sub> in a rodent model.
- Increased O<sub>2</sub> consumption in the face of normal exhaled CO<sub>2</sub> suggests a significant alteration in O<sub>2</sub> utilization in postresuscitation.
- Our calculated respiratory quotient values, well outside the normally cited range of 0.7 to 1.0 have been barely reported in previous reports.

### What Are the Clinical Implications?

- Higher concentration of inspired O<sub>2</sub> may increase O<sub>2</sub> consumption, leading to even lower respiratory quotient values.
- Measuring O<sub>2</sub> consumption and exhaled CO<sub>2</sub> through mechanical ventilation is noninvasive and our calculated respiratory quotient values warrant larger animal and human studies.

studies and are hypothesized to address postresuscitation metabolic disorders.<sup>10–13</sup> However, there are a number of randomized controlled clinical trials that show no benefit,<sup>14–18</sup> in part as a result of the lack of an ability to differentiate patients with treatable metabolic injury, patients who might live with proper treatment, from patients who do not have a metabolic injury, patients who will live, or from patients with untreatable metabolic injury, patients who will not live.<sup>4,19–21</sup>

Because of the central role of VO<sub>2</sub> and CO<sub>2</sub> production (VCO<sub>2</sub>) in mitochondrial energy metabolism, and the evidence that mitochondrial function is altered after resuscitation from CA,<sup>22–24</sup> we hypothesize that the metabolic injury sustained by resuscitated animals will manifest as alterations in systemic VO<sub>2</sub> and VCO<sub>2</sub>. While ex vivo results suggest that VO<sub>2</sub> will decrease, we have purposefully left our hypothesis open because the conditions used in ex vivo experimentation do not reflect the postresuscitation cellular milieu. In order to test our hypothesis, we developed a novel system for the measurement of VO<sub>2</sub> and VCO<sub>2</sub> in intubated rats, and measured the VO<sub>2</sub> and VCO<sub>2</sub> to enable the calculation of the respiratory quotient (RQ) in a rat model of severe CA. VO<sub>2</sub> and VCO<sub>2</sub> were measured after return of spontaneous circulation (ROSC) at an inspired O<sub>2</sub> concentration of 30% and were compared with the results from animals that received a sham surgery. In addition, we tested whether the supplementation of inspired 100% O<sub>2</sub> gas affected the VO<sub>2</sub>, VCO<sub>2</sub>, or RQ in survivors of CA and compared results from animals receiving 100% O<sub>2</sub> with animals receiving 30% O<sub>2</sub> or the sham surgery. We tested the effect of supplemental O<sub>2</sub> on aerobic respiration after CA to determine whether the toxic

effects of supplemental O<sub>2</sub> found in other injury models<sup>25</sup> are replicated in this CA model.

## Methods

The Institutional Animal Care and Use Committees of the University of Pennsylvania, the Children's Hospital of Philadelphia, and the Feinstein Institute for Medical Research approved the study protocol. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Animal Preparation

We performed all instrumentation according to the previously described protocol.<sup>26</sup> In brief, 34 adult male Sprague-Dawley rats (450–550 g, Charles River Laboratories) were anesthetized with 4% isoflurane (Isosthesia, Butler-Schein AHS) and intubated with a 14-gauge plastic catheter (Surflo, Terumo Medical Corporation). We used male rats to avoid potential differences among animal subjects that may be caused by hormonal or genetic differences rather than differences from the experimental intervention (ie, to minimize potential sources of variability). Animals were mechanically ventilated (Ventilator Model 683, Harvard Apparatus) at a minute ventilation (MV) volume of 180 mL/min and a respiratory rate of 45 breaths per minute. In this study, we used 1 ventilation setting for all animals at all times and did not change the MV or respiratory rate over the experiments. All measured end-tidal CO<sub>2</sub> values were within a range of 35 to 45 mm Hg during preparation. Anesthesia was maintained with isoflurane 2% and fraction of inspired O<sub>2</sub> (FIO<sub>2</sub>) 0.3. The left femoral artery was cannulated (sterile polyethylene-50 catheter inserted for 20 mm) for continuous arterial pressure monitoring (MLT844, ADInstruments; Bridge Amplifier ML221, ADInstruments). A temperature probe (T-type thermocouple probes, ADInstruments) was placed in the esophagus for continuous temperature monitoring. The core temperature was maintained at 36.5±0.5°C during the surgical procedure. The left femoral vein was cannulated with a polyethylene-50 catheter, which was advanced into the inferior vena cava for drug infusion. This catheter was flushed with 150 U of heparin (Heparin, SAGENT Pharmaceuticals). At the end of the preparation, a blood sample (0.5 mL) was collected from the arterial catheter line and a blood gas analysis (i-STAT, Heska) was performed.

## Experimental Protocol: CA and Sham Animals

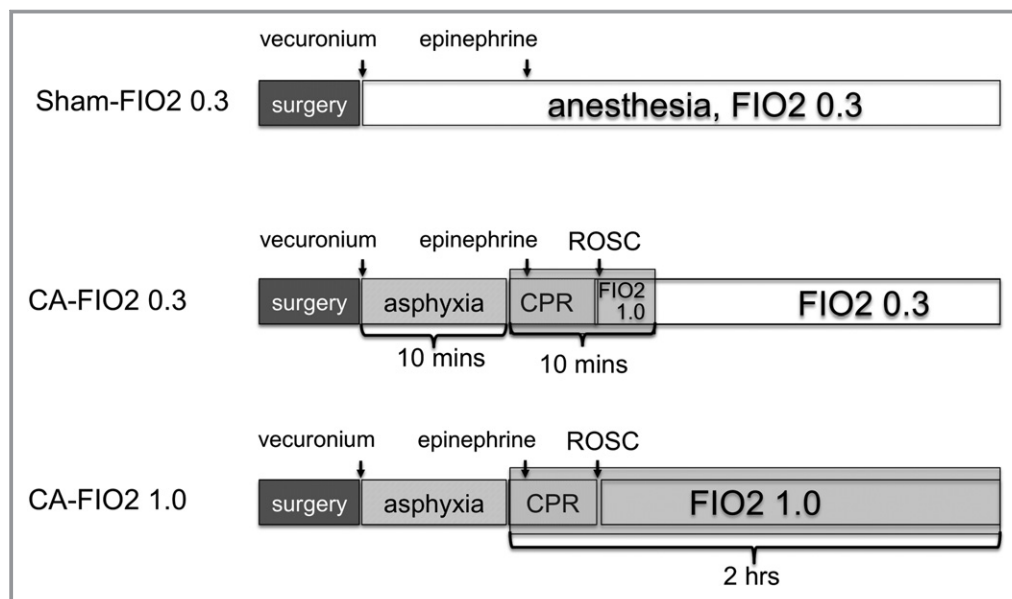
Animals were randomized into 3 groups at the end of surgery: (1) successful resuscitation from 10-minute asphyxia arrest treated with inhaled 100% O<sub>2</sub>: CA-FIO<sub>2</sub> 1.0 group (n=12);

(2) successful resuscitation from 10-minute asphyxia arrest treated with inhaled 30% O<sub>2</sub>: CA-FIO<sub>2</sub> 0.3 group (n=10); and (3) surgical sham control treated with inhaled 30% O<sub>2</sub>: sham-FIO<sub>2</sub> 0.3 group (n=12) (Figure 1). After instrumentation, neuromuscular blockade was achieved by slow intravenous administration of 2 mg/kg of vecuronium bromide (Hospira) for all groups of animals. For animals in the CA groups, asphyxia was induced by switching off the ventilator and CA occurred 3 to 4 minutes after asphyxia started. We defined CA as a mean arterial pressure of <20 mm Hg; CA was completely untreated during this initial 10-minute interval. After the initial 10 minutes, mechanical ventilation was restarted at an FIO<sub>2</sub> of 1.0 and manual cardiopulmonary resuscitation (CPR) was delivered to all CA animals. Isoflurane inhalation was discontinued after the induction of asphyxia and was not given to CA animals after successful resuscitation. Chest compressions were performed with 2 fingers over the sternum at a rate of 260 to 300 per minute. Immediately after beginning CPR, a 20 µg/kg bolus of epinephrine was given to animals through the venous catheter. Following ROSC, defined as systolic blood pressure >60 mm Hg, CPR was discontinued. If ROSC did not occur by 5 minutes of CPR, resuscitation was terminated. Ten minutes after CPR started, FIO<sub>2</sub> was switched back to 0.3 in the CA-FIO<sub>2</sub> 0.3 group, while it was kept at 1.0 in the CA-FIO<sub>2</sub> 1.0 group. We monitored animal physiology, VO<sub>2</sub>, and VCO<sub>2</sub> for 120 minutes after resuscitation. For animals in the sham-FIO<sub>2</sub> 0.3 group, the same surgical procedures were performed, including vecuronium and epinephrine injections, without asphyxia or CPR. The animals

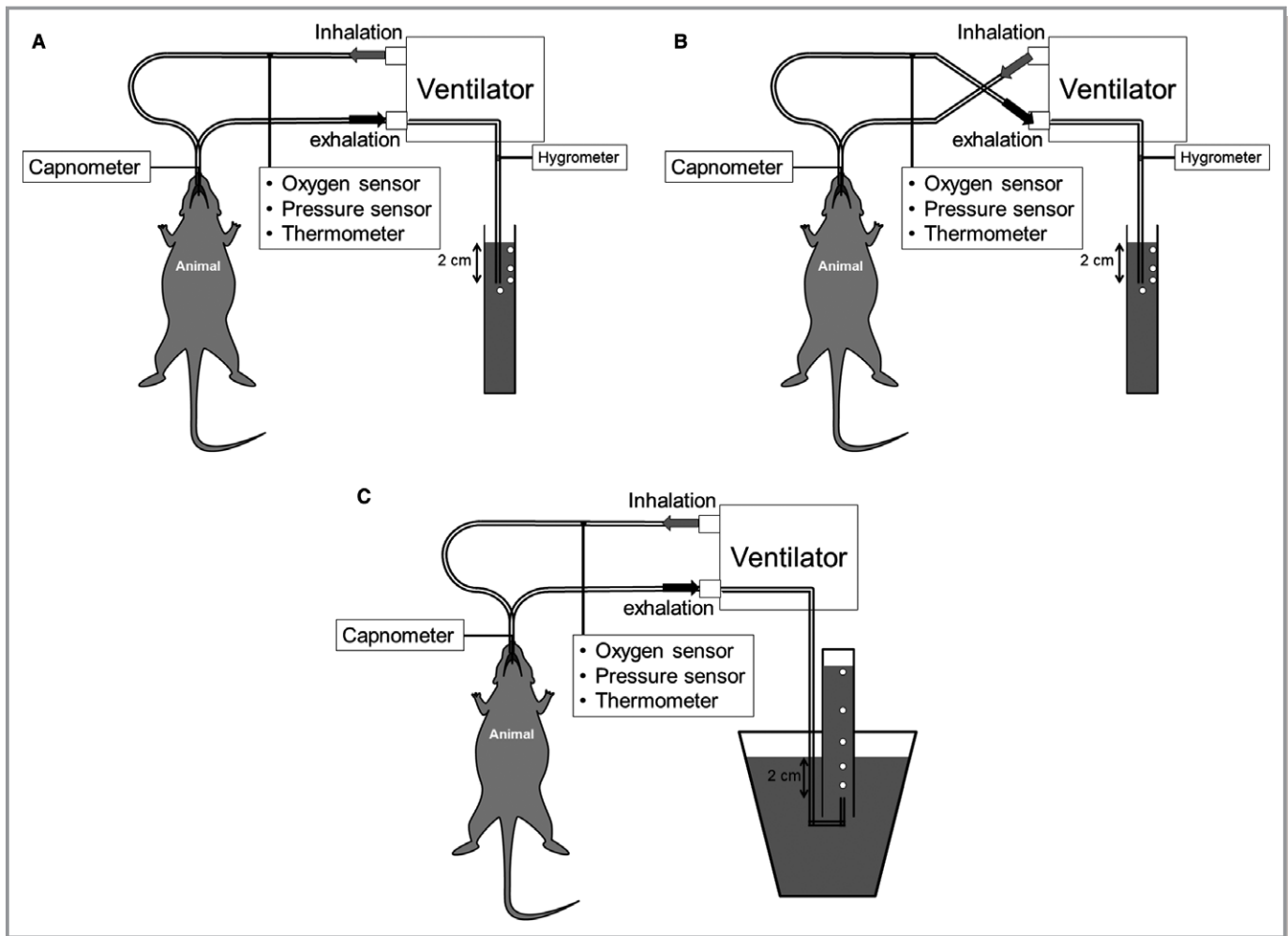
were consistently anesthetized with 2% inhaled isoflurane/30% O<sub>2</sub> during the entire series of measurements (Figure 1). All surgical procedures including resuscitation were performed by 1 investigator and therefore blinding procedures were not applied. Blood gas analysis was performed at 10, 20, 30, 45, 60, and 120 minutes after starting CPR. Mechanical ventilation was discontinued 120 minutes after CPR and animal survival was monitored up to 72 hours. Surviving animals were euthanized 72 hours after CPR. Postsurgical care including animal housing and observation were provided by facility in a blinded manner.

## Measurement Preparation

A photoluminescence-quenching sensor (FOXY AL300 Oxygen Sensor Probe, Ocean Optics) and a fluorometer (NEOFOX-GT, Ocean Optics) were used to measure the gas concentration of O<sub>2</sub>. O<sub>2</sub> was measured inline in the exhalation or inhalation branch of the ventilator circuit (Figure 2A and 2B). A micro-capnograph (Micro-Capnograph CI240, Columbus Instruments) was used to measure CO<sub>2</sub>. CO<sub>2</sub> was measured using a continuous side-stream sample taken from the endotracheal tube of the intubated animal (Figure 2A and 2B). The sampling rate was set at 10 mL/min. The mechanical ventilation duty cycle was 50%, meaning that 5 mL/min was taken from both the inspiration and the expiration. Under a constant temperature condition, we manually calibrated the O<sub>2</sub> and CO<sub>2</sub> sensors before each experiment. Two-point calibration was performed and medical air and medical O<sub>2</sub> (General Welding Supply Corp.) were used for calibrating the O<sub>2</sub>



**Figure 1.** Study protocol. Animals were randomized into 3 groups at the end of surgery: (1) successful resuscitation from 10-minute asphyxia arrest treated with inhaled 100% O<sub>2</sub>: cardiac arrest (CA)-fraction of inspired O<sub>2</sub> (FIO<sub>2</sub>) 1.0 group (n=12); (2) successful resuscitation from 10-minute asphyxia arrest treated with inhaled 30% O<sub>2</sub>: CA-FIO<sub>2</sub> 0.3 group (n=10); and (3) surgical sham control treated with inhaled 30% O<sub>2</sub>: sham-FIO<sub>2</sub> 0.3 group (n=12). CPR indicates cardiopulmonary resuscitation; ROSC, return of spontaneous circulation.



**Figure 2.** Illustration of the mechanical ventilation circuit with 2 sensors for measurements of  $O_2$  and  $CO_2$  concentrations. A, Before and after  $O_2$  consumption ( $VO_2$ ) measurements, fraction of inspired  $O_2$  ( $FIO_2$ ) is measured for at least 10 minutes. B, During measurements of  $VO_2$ , fraction of expired  $O_2$  ( $FEO_2$ ) is measured after the ventilator circuit has been switched.  $O_2$  extraction is calculated by a subtraction between  $FIO_2$  and  $FEO_2$ . C, Volume of exhaled gas ( $V_E$ ) was measured using a water bath and a 500 mL cylinder. For each measurement, expired gas was collected for 2 minutes and minute ventilation of  $V_E$  was calculated.

sensor. We used 20.9% as a low reference and 100.0% as a high reference. We used medical air (0%  $CO_2$ ) and industrial  $CO_2$  (10.4%  $CO_2$ , General Welding Supply Corp.) for calibrating the  $CO_2$  sensor. Gas humidity was measured inline with a hygrometer (TFH 620, ebros). The calibration was completed at a humidity of 0%. A temperature probe (T-type thermocouple probes, ADInstruments) and a pressure probe (MLT844, ADInstruments; Bridge Amplifier ML221, ADInstruments) were placed inline (Figure 2A and 2B). Ambient temperature and pressure were also monitored outside of the ventilator system (T-type thermocouple probes, ADInstruments; Traceable Workstation Digital Barometer, Fisher Scientific).

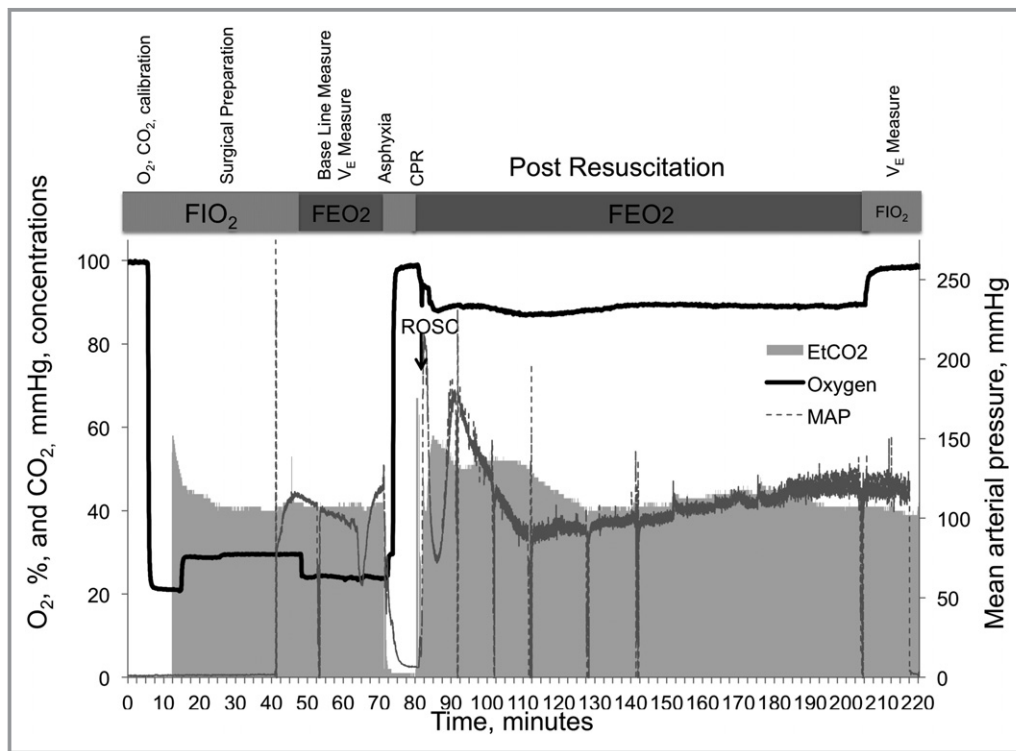
### $VO_2$ and $VCO_2$ Measures in Intubated Rats

The fluorometer measures  $O_2$  concentration every 100 ms. The fluorometer performed a windowed average of these

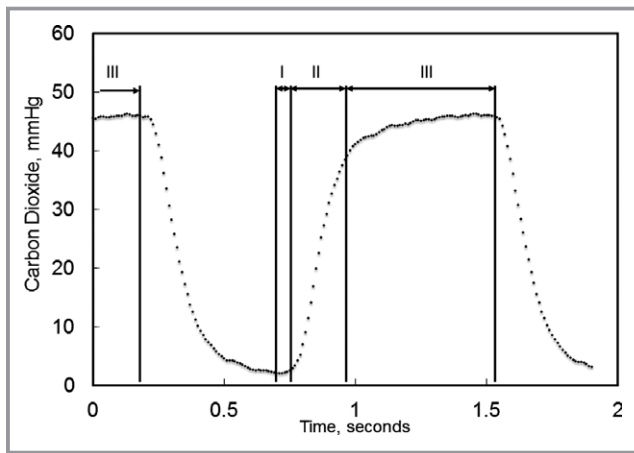
measurements, and exported a value every second. The time constant for the fluorometer is about 300 ms. Given the ventilation rate, this sensor is too slow to perform mainstream  $O_2$  concentration measurements in the endotracheal tube. For this reason, the  $O_2$  sensor was placed in the middle of a ventilator tubing arm  $\approx 100$  cm from the endotracheal tube (Figure 2).  $O_2$  concentrations were measured in the inspiratory arm ( $FIO_2$ ) or in the fraction of expired  $O_2$  arm ( $FEO_2$ ) by swapping which ventilator circuit arm was connected to the in and out port of the ventilator. Given enough equilibration time after switching ventilator port connections, this setup provided relatively constant  $O_2$  concentrations for measurement. The differences between the alveolar and tracheal  $O_2$  concentrations lead to a concentration gradient in the expiratory arm of the ventilator circuit. However, the relatively large volume of our ventilator circuit ( $\approx 15$  mL) compared with our tidal volume ( $\approx 4$  mL) allowed sufficient time for gas

diffusion, which significantly decreased the  $O_2$  concentration gradients in the expiratory arm of the circuit. After the experiments, the exported  $O_2$  concentrations were averaged every minute, and measurements made in the inspiratory arm are reported as  $FIO_2$  and measurements made in the expiratory arm are reported as  $FEO_2$ . Figure 3 depicts a typical experimental course of an animal in the CA- $FIO_2$  1.0 group. Sensors were calibrated before each experiment. Animal preparation was completed within 40 minutes after successful intubation. Baseline  $FIO_2$  was measured during surgical preparation.  $FEO_2$  was then measured by swapping the in/out ports in the ventilator circuit (Figure 2A and 2B).  $FIO_2$  was measured twice in each  $VO_2$  measurement for a period of at least 10 minutes, before and after the 2-hour period of  $FEO_2$  measurement. From asphyxia to starting CPR, the rats were disconnected from the ventilator circuit and so a test lung (small rubber balloon) was connected to the circuit.  $FIO_2$  was measured with the test lung while the animal was in asphyxia. After 10 minutes of asphyxia, the animal was reconnected to the ventilator circuit, the ventilator circuit was adjusted to measure  $FEO_2$ , and CPR was initiated.

Because we measured  $CO_2$  concentration using a side-stream breath-by-breath method, we used a slightly different calculation for fractional concentration of  $CO_2$  in exhaled gas ( $FECO_2$ ). To estimate  $FECO_2$ , we quantitated % concentration of  $CO_2$  from the capnograph waveform (Figure 4).<sup>27</sup> The small increase in partial pressure of carbon dioxide ( $PCO_2$ ), noted as phase I in Figure 4, marks the transition from inspiration to expiration. This is followed by a significant increase in  $PCO_2$ , labeled phase II. The end of expiration at phase III is defined as end-tidal  $PCO_2$ . Capnograph data were extracted every 10 ms. We calculated the first derivative of the capnograph to detect the beginning of phase I and the end of phase III. The interval starting from the beginning of phase I to the end of phase III is considered the expiratory phase of the ventilation. Using this method, we segmented the capnograph into inspiratory and expiratory phases. Using only the expiratory phases of the capnograph, we averaged 1 minute worth of  $FECO_2$  measurements. The  $CO_2$  sensor is affected by changes in the  $O_2$  concentration in the sampled gas. Therefore, we corrected  $CO_2$  measurements empirically based on our sensor calibration experiments, described below.



**Figure 3.** Experimental course of an animal. The figure depicts a typical experimental course of an animal in the cardiac arrest (CA)-fraction of inspired  $O_2$  ( $FIO_2$ ) 1.0 group. We firstly performed sensor calibrations for each experiment. Animal preparation was completed within 40 minutes after successful intubation. Baseline  $FIO_2$  was measured during surgical preparation. Fraction of expired  $O_2$  ( $FEO_2$ ) was then measured by swapping the in/out ports in the ventilator circuit. We measured  $FEO_2$  and monitored animal physiology,  $O_2$  consumption ( $VO_2$ ), and  $CO_2$  production ( $VCO_2$ ) for 2 hours after resuscitation.  $FIO_2$  was measured twice in each  $VO_2$  measurement, and before and after a 2-hour period of  $FEO_2$  measurement. CPR indicates cardiopulmonary resuscitation.



**Figure 4.** Volumetric capnograph. Phase I is a starting point of an expiration with a tiny elevation of the curve of partial pressure of carbon dioxide (PCO<sub>2</sub>) followed by a steep slope of phase II. The end of expiration at phase III is defined as end-tidal PCO<sub>2</sub>.

To measure volume of exhaled gas ( $V_E$ ), we used an inverted, water-sealed 500 mL cylinder that was filled with water. Exhaled gas displaced the water in the cylinder during volume measurements. Exhaled gas was collected for 2 minutes and the MV volume was calculated. The resolution of the measurement was 2.5 mL/min.  $V_E$  measurements were performed twice in each experiment before and after a 2-hour period of metabolic measurement (Figure 2C). Ventilator airway pressure was measured through our experiment so we could verify that the pressure inside was kept positive by a positive end-expiratory pressure of 2 cm (Figure 5). The positive pressure

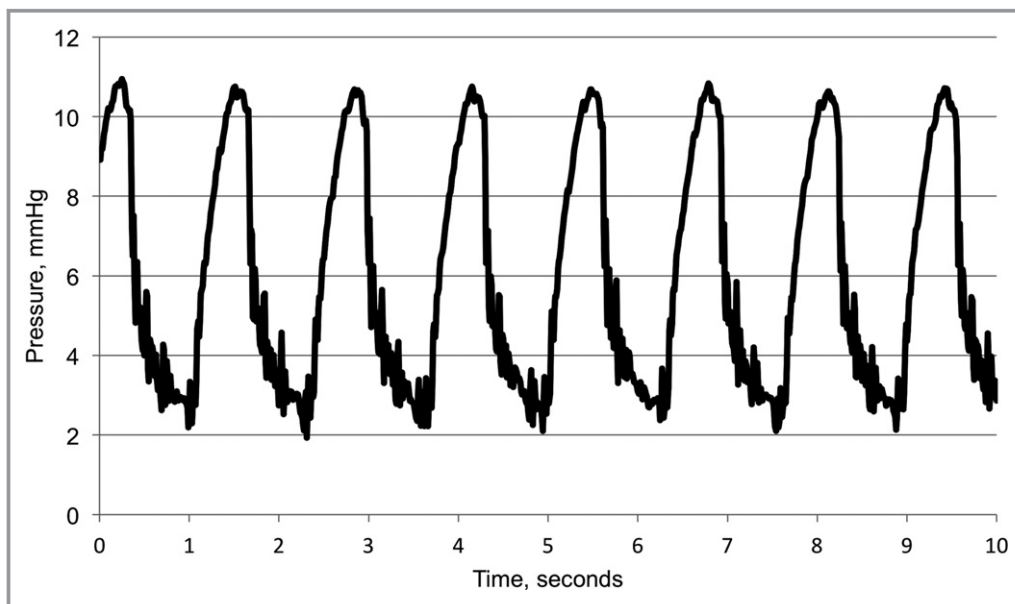
inside the circuits ensures that the circuit is not contaminated by ambient air. We regularly inspected the rat oropharynx visually to eliminate the possibility of a major leak around the endotracheal tube. Since the airway pressure was positive, gas leak through the space between the endotracheal tube and the vocal cords would create bubbles in the oropharynx. In addition, we compared the  $V_E$  measurements before and after the metabolic measurement. The comparison of post- $V_E$  to pre- $V_E$  was made for each experiment and the average ratio of post- $V_E$ /pre- $V_E$  was 1.011 (95% confidence interval, 0.994–1.028 [n=34]). This shows that the CA injury model had no observable effect on our  $V_E$  measurements.

### Gas Concentration Measurements: Temperature Control

Gas concentration measurements are temperature dependent. The O<sub>2</sub> and CO<sub>2</sub> measurements used different temperature control techniques. The micro-capnograph uses a warming chamber to warm the sampled gas to 40°C. We used a water bath and copper tubing to control the temperature for the O<sub>2</sub> sensor. The fluorometer simultaneously measures fluorescence and sample temperature in order to correct for temperature effects. The water bath was kept in a narrow temperature range of 19 to 21°C.

### Measurement Validation

Four different 4% CO<sub>2</sub> gas mixtures (4% CO<sub>2</sub> contained in 10%, 45%, 80%, and 96% O<sub>2</sub>; MESA International Technologies, Inc)



**Figure 5.** Pressure curve inside the mechanical ventilator circuit. Ventilator airway pressure was measured through our experiment so we could verify that the pressure inside was kept positive by a positive end-expiratory pressure of 2 cm.

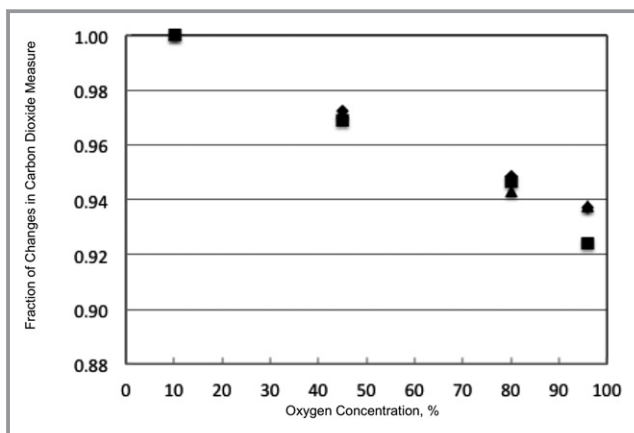
were prepared. The accuracy of O<sub>2</sub> and CO<sub>2</sub> measurements was tested using these mixtures. The error of our O<sub>2</sub> measurements was ±0.7% at a range from 20.9% to 100%. Because we swapped the in/out ports and used 1 sensor for measuring FIO<sub>2</sub> and FEO<sub>2</sub>, the error (uncertainty) of FIO<sub>2</sub> and FEO<sub>2</sub> were dependent. Since the propagation of uncertainty for subtraction of FIO<sub>2</sub>–FEO<sub>2</sub> keeps the covariance, σ<sub>FIO<sub>2</sub>-FEO<sub>2</sub></sub>, in its calculation the error of O<sub>2</sub> extraction rate, FIO<sub>2</sub>–FEO<sub>2</sub>, is calculated as ±0.7% at a range from 20.9% to 100%.

We tested the effect of different O<sub>2</sub> concentrations on our CO<sub>2</sub> sensor under humid conditions. Deionized pure water was poured into Tygon tubing (Saint-Gobain) and it was warmed to 50°C by a hot water bath. The gas mixture was passed through the tubing and the CO<sub>2</sub> concentration was measured for each mixture. In order to reduce CO<sub>2</sub> measurement errors caused by changes in O<sub>2</sub> concentrations, we measured the effect of different O<sub>2</sub> concentrations on the CO<sub>2</sub> sensor and created an equation to correct the CO<sub>2</sub> errors, as shown in Figure 6. The CO<sub>2</sub> concentration measurement decreased linearly as the O<sub>2</sub> concentration increased. Humidity was 92.8±3.5% at a gas temperature of 21.3±0.6°C. Based on this result, we derived the following empirical equation:

$$CO_2 = \frac{mCO_2}{-0.00077 \times O_2 + 1.0077} \quad (1)$$

where, CO<sub>2</sub> is the estimated concentration, mCO<sub>2</sub> is the measured value, O<sub>2</sub> is the concentration of O<sub>2</sub> (%).

Temperature and pressure are the major components that affect the volume measure of a gas. Therefore, we tested whether there were differences in temperature and/or pressure between inhaled and exhaled gases before and after swapping the in/out ports. Data were obtained 1 minute



**Figure 6.** Effect of O<sub>2</sub> concentration on the CO<sub>2</sub> sensor. Four different mixture gases (O<sub>2</sub> 10% and CO<sub>2</sub> 4%, O<sub>2</sub> 45% and CO<sub>2</sub> 4%, O<sub>2</sub> 80% and CO<sub>2</sub> 4%, and O<sub>2</sub> 96% and CO<sub>2</sub> 4%) were used. The experiment was performed under humid conditions at a relative humidity of 92.8±3.5% at a gas temperature of 21.3±0.6°C.

before and after swapping the in/out ports of the ventilator circuit. As can be seen in Table 1, the gas temperature was 19.69±0.46°C when FIO<sub>2</sub> was measured and it increased by 0.01°C when FEO<sub>2</sub> was measured. Even though exhaled gas from animals is warmer than inhaled gas supplied from a mechanical ventilator, it did not make a significant impact on our measurements because the water bath system controls the temperature. The pressure inside the circuit was 5.62±0.42 mm Hg when FIO<sub>2</sub> was measured and it decreased by 0.19 mm Hg when FEO<sub>2</sub> was measured.

The estimation of inspiration volume by the Haldane transformation<sup>28</sup> is limited to FIO<sub>2</sub> <0.6.<sup>29</sup> We needed a method that was applicable to FIO<sub>2</sub> of 1.0. Therefore, we performed an experiment to measure the effect of CO<sub>2</sub> production and O<sub>2</sub> consumption on the difference between V<sub>I</sub> and V<sub>E</sub>, where V<sub>I</sub> and V<sub>E</sub> are MV volumes of the mechanical ventilation in the inhaled and exhaled gases, respectively. Before conducting the experiment, the ventilator and ventilator circuit were leak tested. The first part of the experiment was the determination of V<sub>I</sub>. The volume of gas exiting the ventilator per minute was measured in the presence of a test lung. Because the system was leak tested, and there was no gas exchange in the test lung, the volume of gas exiting the ventilator is equivalent to the volume of gas entering the test lung, which is V<sub>I</sub>. This measurement was made in triplicate. Then, an animal was anesthetized and intubated. The side-stream capnography measurement was omitted and the port sealed so that no ventilation volume was lost. The volume of gas exiting the ventilator was then measured in triplicate. In this setting, the measured volume is V<sub>E</sub>. Next, the animal was euthanized by stopping mechanical ventilation under a surgical plane of anesthesia and neuromuscular blockade. This resulted in asphyxia and CA. V<sub>E</sub> was measured in triplicate 15 and 45 minutes after euthanasia. Because the animal was euthanized, the ventilations also resulted in no gas exchange with the animal. The results of these experiments are reported in Table 2. V<sub>I</sub> measured using the rubber-test

**Table 1.** Gas Conditions for FIO<sub>2</sub> and FEO<sub>2</sub> Measurements of Experimental Animals

	FIO <sub>2</sub> (n=5)	FEO <sub>2</sub> (n=5)	P Value
Gas temperature inside ventilator circuit, °C	19.69±0.46	19.70±0.46	<0.01
Gas pressure inside ventilator circuit, mm Hg	5.62±0.42	5.43±0.44	<0.01
Ambient temperature, °C	21.69±0.26	21.67±0.15	0.886

Values are expressed as mean±SD. The data were collected 1 minute before and after swapping the in/out ports in the ventilator circuit. The results are shown as the average and SD of data obtained from 5 animals. Paired-sample *t* test was used to compare the values between fraction of inspired O<sub>2</sub> (FIO<sub>2</sub>) and fraction of expired O<sub>2</sub> (FEO<sub>2</sub>).

lung was 177.5 mL/min. As can be seen in the Table 2, there were no detectable differences between  $V_E$  and  $V_I$  in the animal. The data support discarding the Haldane transformation in favor of the assumption that  $V_I$  equals  $V_E$  in animals, particularly for high  $FIO_2$  values. However, it is possible that the difference between  $V_I$  and  $V_E$  is smaller than the resolution of our volume measurement. The resolution of our volume measurement was 2.5 mL/min and so it was  $\approx 1.4\%$  of an MV of 180 mL/min. This number indicates that  $V_I$  can be 1.4% more or less than  $V_E$ .

## Calculations

$VO_2$  and  $VCO_2$  are calculated by the following equations<sup>29,30</sup>:

$$VO_2 = V_I \times FIO_2 - V_E \times FEO_2 \quad (2)$$

$$VCO_2 = V_I \times FICO_2 - V_E \times FECO_2 \quad (3)$$

In our calculation,  $FICO_2$  is assumed to be zero since the inhaled gas did not contain  $CO_2$ . It was not appropriate to use the Haldane transformation<sup>28</sup> to estimate  $V_I$  from  $V_E$  because we used inhaled  $O_2$  concentrations of 100% in the CA- $FIO_2$  1.0 group.<sup>29</sup> Based on our validation study, we determined that it was reasonable to assume that  $V_I$  equals  $V_E$  for this experimental setup; this assumption results in the following calculation of  $VO_2$ :

$$VO_2 = V_E \times (FIO_2 - FEO_2) \quad (4)$$

Room temperature and atmospheric pressure were measured in each experiment so that  $VO_2$  and  $VCO_2$  could be calculated at standard temperature and pressure. The RQ was calculated by the following equation:

$$RQ = VCO_2/VO_2 \quad (5)$$

$$RQ = \frac{FECO_2}{FIO_2 - FEO_2} \quad (6)$$

Different  $FIO_2$  settings affect errors in  $VO_2$  caused by the assumption that  $V_I$  equals  $V_E$ . In order to evaluate errors in

**Table 2.** Metabolic Effect on  $V_E$  of an Animal

Test Lung $V_I=V_E$ , mL/min	Animal—Alive $V_E$ , mL/min	Animal—Dead	
		15 min $V_I=V_E$ , mL/min	45 min $V_I=V_E$ , mL/min
177.5 $\pm$ 0	177.5 $\pm$ 0	176.7 $\pm$ 1.4	177.5 $\pm$ 0

One animal was used in this experiment. The volume measurements were repeated 3 times at each condition. Values are expressed as mean $\pm$ SD.  $V_E$  indicates volume of exhaled gas;  $V_I$ , volume of inspired gas.

$VO_2$  of our study, we calculated %error from the following equation:

$$\%error = \frac{\text{Exact} - \text{Approximate}}{\text{Approximate}} \times 100 \quad (7)$$

Exact  $VO_2$  was calculated by equation (2), approximate  $VO_2$  was calculated by equation (4), and equation (6) is transformed to:

$$\%error = \frac{(V_I \times FIO_2 - V_E \times FEO_2) - V_E \times (FIO_2 - FEO_2)}{V_E \times (FIO_2 - FEO_2)} \times 100 \quad (8)$$

$$\%error = \left( \frac{V_I}{V_E} - 1 \right) \times \left( \frac{FIO_2}{FIO_2 - FEO_2} \right) \times 100 \quad (9)$$

The resolution of our  $V_E$  measurement was 2.5 mL/min and it was 1.4% of the MV volume (180 mL/min). We assumed that  $V_I$  equals  $V_E$  but exact  $V_I$  might be either more or less than  $V_E$  ( $\pm 1.4\%$ ). Therefore, the %error of our measurements is:

$$\%error = \pm 1.4 \times \left( \frac{FIO_2}{FIO_2 - FEO_2} \right) \quad (10)$$

The %error becomes greater when higher inhaled  $O_2$  is used and it becomes smaller when the animal's  $O_2$  extraction rate,  $FIO_2 - FEO_2$ , is high. In our injured animal model, the average of  $O_2$  extraction rate was 7.5% when inhaled  $O_2$  was 30%, where it was 9.3% when inhaled  $O_2$  was 100%. Therefore, the %error of our  $VO_2$  measurements was estimated as  $\pm 5.6\%$  in the CA- $FIO_2$  0.3 group and  $\pm 15.1\%$  in the CA- $FIO_2$  1.0 group.

## Statistical Analysis

We reported continuous data as mean and SD. Group comparisons were made with ANOVA, and Scheffé multiple comparison procedure was used for post hoc analyses. The numbers of  $VO_2$ ,  $VCO_2$ , and RQ were compared within the 3 different test groups (CA- $FIO_2$  1.0 group, CA- $FIO_2$  0.3 group, and sham- $FIO_2$  0.3 group) at each time point. We used Fisher exact test for comparison of categorical outcomes and log-rank test for comparison of survival curves among groups. A 2-tailed  $P < 0.05$  was considered statistically significant. All calculations were performed with SPSS Statistics version 22 for Mac (IBM).

## Results

### CA Animal Models

No significant differences in basal characteristics were found between the groups (Table 3). Arterial  $O_2$  pressures for the 3



**Table 3.** Basal Characteristics and Resuscitation Data in the 3 Groups

	CA-FIO <sub>2</sub> 1.0 (n=12)	CA-FIO <sub>2</sub> 0.3 (n=10)	Sham (n=12)
Weight, g	479±32	462±12	480±36
CPR to ROSC time, s	109±37	100±30	...
Tidal volume, mL/kg	7.4±0.6	7.7±0.2	7.2±0.8
FIO <sub>2</sub>	0.31±0.02	0.31±0.01	0.31±0.01
Body temperature, °C	36.6±0.3	36.7±0.4	36.9±0.4
Blood gas analysis			
pH	7.46±0.03	7.44±0.02	7.44±0.02
PO <sub>2</sub> , mm Hg	140±17	146±16	144±18
PCO <sub>2</sub> , mm Hg	40±2	40±4	42±5
Lactate, mmol/L	1.3±0.4	1.3±0.4	1.1±0.5

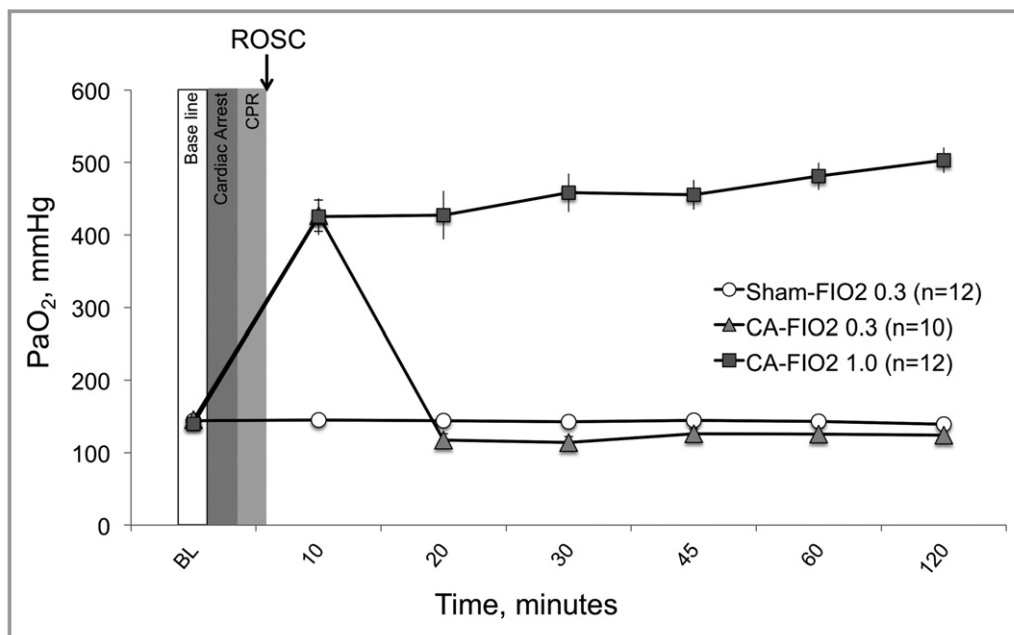
Blood samples were obtained from arterial line before asphyxia (baseline). Return of spontaneous circulation (ROSC) time was defined as systolic blood pressure >60 mm Hg. Values are expressed as mean±SD. CA indicates cardiac arrest; CPR, cardiopulmonary resuscitation; FIO<sub>2</sub>, fraction of inspired O<sub>2</sub>; PCO<sub>2</sub>, partial pressure of carbon dioxide; PO<sub>2</sub>, partial pressure of O<sub>2</sub>.

groups are shown as a function of time in Figure 7. As previously mentioned, we used 1 ventilation setting (respiratory rate: 45 breathes per minute; MV: 180 mL/min) for all animals at all times and did not change the MV or respiratory rate over the experiments. All measured end-tidal CO<sub>2</sub> values were within a range of 35 to 45 mm Hg during surgical preparation. Mean PaO<sub>2</sub> remained significantly higher in the CA-FIO<sub>2</sub> 1.0 group compared with the CA-FIO<sub>2</sub> 0.3 and the

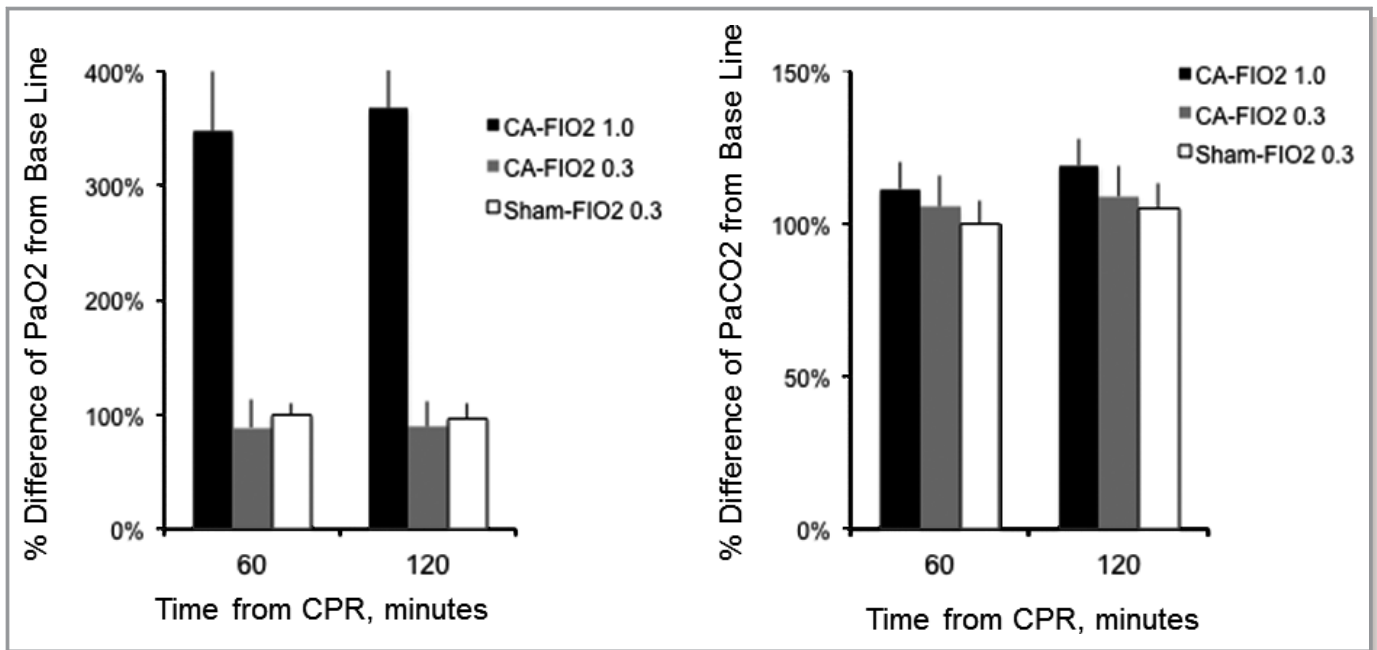
sham-FIO<sub>2</sub> 0.3 groups. The %difference of PaO<sub>2</sub> from baseline decreased by 11% in the CA-FIO<sub>2</sub> 0.3 group at 60 and 120 minutes from CPR. There were no statistical differences between the CA-FIO<sub>2</sub> 0.3 group and the sham-FIO<sub>2</sub> 0.3 group (Figure 8). The %difference of arterial partial pressure of carbon dioxide from baseline increased by 11% at 60 minutes and by 19% at 120 minutes in the CA-FIO<sub>2</sub> 1.0 group, and increased by 5% at 60 minutes and by 9% at 120 minutes in the CA-FIO<sub>2</sub> 0.3 group. There were no statistical differences between the 3 groups at either time point (Figure 8). Seventy-two-hour survival curves for the 3 groups are shown in Figure 9. Survival rates in the CA-FIO<sub>2</sub> 1.0 and the CA-FIO<sub>2</sub> 0.3 groups were not found to be different ( $P=0.164$ ) using log-rank test. Using Fisher exact test, the survival rates of CA-FIO<sub>2</sub> 1.0 versus CA-FIO<sub>2</sub> 0.3 at each time point (24, 36, 48, and 72 hours after CA) were 67% (8/12) versus 100% (10/10,  $P=0.096$ ), 42% (5/12) versus 80% (8/10,  $P=0.099$ ), 33% (4/12) versus 70% (7/10,  $P=0.198$ ), and 25% (3/12) versus 40% (4/10,  $P=0.652$ ), respectively. No animal died in the sham-FIO<sub>2</sub> 0.3 group.

### Alterations of VCO<sub>2</sub>, VO<sub>2</sub>, and RQ After CA

VCO<sub>2</sub> was significantly greater in both CA groups compared with the sham group 25 minutes after ROSC (CA-FIO<sub>2</sub> 1.0: 16.7±2.2,  $P<0.01$ ; CA-FIO<sub>2</sub> 0.3: 17.4±1.4,  $P<0.01$ ; versus sham-FIO<sub>2</sub> 0.3: 13.6±1.1 mL/kg per minute at standard temperature and pressure, respectively). VCO<sub>2</sub> in the CA groups returned to normal 45 minutes after ROSC



**Figure 7.** Arterial O<sub>2</sub> pressure (arterial partial pressure of O<sub>2</sub> [PaO<sub>2</sub>], mm Hg) among the 3 groups. Values are expressed as mean±SE. CA indicates cardiac arrest; CPR, cardiopulmonary resuscitation; FIO<sub>2</sub>, fraction of inspired O<sub>2</sub>; ROSC, return of spontaneous circulation.

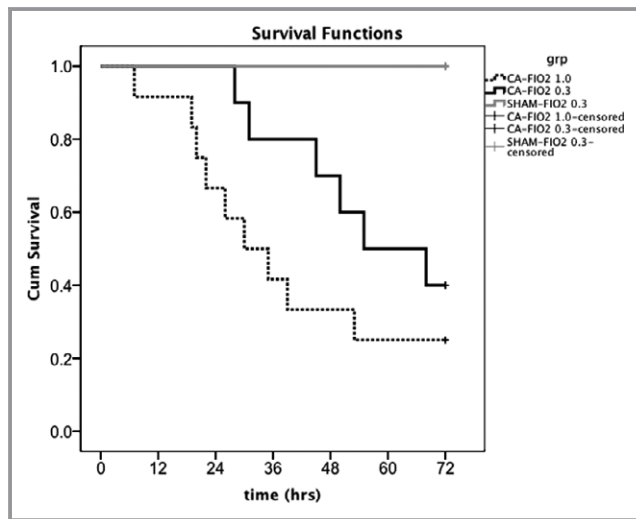


**Figure 8.** Arterial partial pressure of oxygen ( $\text{PaO}_2$ ) and arterial partial pressure of carbon dioxide ( $\text{PaCO}_2$ ) differences from baseline. The % difference was compared from baseline in a value of  $\text{PaO}_2$  at 60 minutes after cardiopulmonary resuscitation (CPR) and 120 minutes after CPR. The % difference was compared from baseline in a value of  $\text{PaCO}_2$  at 60 minutes after CPR and 120 minutes after CPR. There were no statistical differences at any time points.

(Figure 10A). As can be seen in Figure 10B, the  $\text{VO}_2$  value was greater in the CA groups than in the sham group. Values measured 115 to 120 minutes after ROSC were significantly different (CA-FIO<sub>2</sub> 1.0:  $28.7 \pm 6.7$ ,  $P < 0.01$ ; CA-FIO<sub>2</sub> 0.3:  $24.4 \pm 2.3$ ,  $P < 0.01$ ; versus sham-FIO<sub>2</sub> 0.3:  $15.8 \pm 2.4$  mL/kg per minute at standard temperature and pressure). As a result, the RQ was significantly and persistently lower in the CA groups than in the sham group over the 2-hour

measurement period (CA-FIO<sub>2</sub> 1.0:  $0.54 \pm 0.12$  versus CA-FIO<sub>2</sub> 0.3:  $0.68 \pm 0.05$  versus sham-FIO<sub>2</sub> 0.3:  $0.93 \pm 0.11$  in minutes 115 to 120 of ROSC,  $P < 0.01$  [Figure 10C]). The proportion (numbers) of animals that had an RQ  $< 0.7$  at the minimum was: 92% (11/12) in the CA-FIO<sub>2</sub> 1.0 group, 80% (8/10) in the CA-FIO<sub>2</sub> 0.3 group, and 0% (0/12) in the sham-FIO<sub>2</sub> 0.3 group.

In our CA injury model, the  $\text{VO}_2$  value increased by 50% when the FIO<sub>2</sub> was 0.3. When the FIO<sub>2</sub> value was 1.0, the  $\text{VO}_2$  increased by 80%.

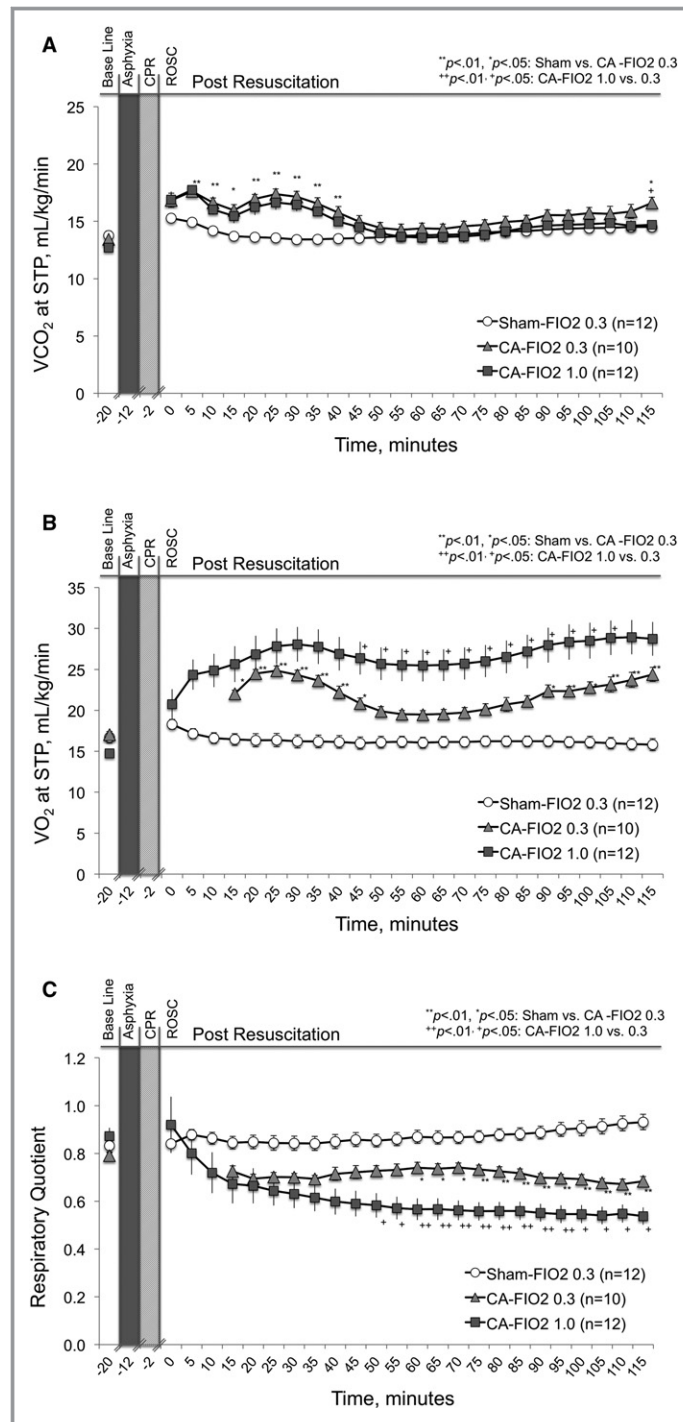


**Figure 9.** Kaplan–Meier survival curves among the 3 groups. Log-rank test shows no significant differences between the cardiac arrest (CA)–fraction of inspired O<sub>2</sub> (FIO<sub>2</sub>) 1.0 and CA-FIO<sub>2</sub> 0.3 groups ( $P = 0.164$ ).

## Discussion

The experiments reported here demonstrate that animals resuscitated from CA have reduced RQs, increased  $\text{VO}_2$  values, and similar  $\text{VCO}_2$  values, relative to their pre-arrest values. The amount of O<sub>2</sub> consumed in the post-arrest period was dependent on the inhaled O<sub>2</sub> concentration. These results confirm our contention that resuscitation from CA causes significant metabolic injury to the animal. What was not expected, and what has previously been considered impossible, is that rats resuscitated from CA could exhibit RQs significantly lower than 0.7. Our expectations had been that resuscitated animals would exhibit classical hypermetabolism or hypometabolism, eg, their RQ would remain unchanged, but they would exhibit either increased or decreased metabolic rate.

This study substantially improves our understanding of the use of indirect calorimetry as a real-time metabolic monitor to



**Figure 10.** Alterations of CO<sub>2</sub> production (VCO<sub>2</sub>), O<sub>2</sub> consumption (VO<sub>2</sub>), and RQ after cardiac arrest (CA). A, VCO<sub>2</sub> over time in the CA groups compared with the sham group; (B) VO<sub>2</sub> over time in the CA groups compared with the sham group; and (C) respiratory quotient (RQ) over time in the CA groups compared with the sham group. CPR indicates cardiopulmonary resuscitation; ROSC, return of spontaneous circulation. Values are expressed as mean±SE. The numbers of VO<sub>2</sub>, VCO<sub>2</sub>, and RQ were compared within the 3 different test groups (CA-fraction of inspired O<sub>2</sub> [FIO<sub>2</sub>] 1.0 group, CA-FIO<sub>2</sub> 0.3 group, and sham-FIO<sub>2</sub> 0.3 group) at each time point. Significant differences between the groups with a *P*<0.05 is indicated as \*, *P*<0.01 \*\* (sham-FIO<sub>2</sub> 0.3 vs CA-FIO<sub>2</sub> 0.3), *P*<0.05 +, and *P*<0.01 ++ (CA-FIO<sub>2</sub> 0.3 vs CA-FIO<sub>2</sub> 1.0).

track injury severity after resuscitation. One of our most important findings is that a resuscitated rat can sustain RQs outside of the commonly cited range of 0.7 to 1.0. RQ values <0.7 are often considered to be in error.<sup>30–33</sup> Oshima et al<sup>32</sup> reported 7 cases of CA in which energy expenditures were found to be unexpectedly high in the post-ROSC period, but excluded measurements when the RQ was lower than 0.7. Black et al<sup>34</sup> reported the results of a careful study of mechanically ventilated patients in the intensive care unit with  $\text{VO}_2$  measured using 3 distinct methods. In that article, nearly 25% of collected data were excluded from analysis because the measured RQ was <0.6 or >1.2. An increase in the metabolic rate, eg, hypermetabolism, has been reported in patients with burns,<sup>35,36</sup> sepsis,<sup>37</sup> and other diseases.<sup>38,39</sup> However, these reports did not include measurements of  $\text{VCO}_2$  and therefore it is possible that a shift in  $\text{O}_2$  metabolism, such as the one described here, was interpreted as hypermetabolism. In all, our results suggest that our finding of an RQ <0.7 may be more prevalent than previously suspected. Furthermore, our finding that changes in  $\text{VO}_2$  are not always linked to changes in  $\text{VCO}_2$  clarifies that claims of increased or decreased metabolic rate cannot be supported without measuring both  $\text{VO}_2$  and  $\text{VCO}_2$ .

There are some studies that report  $\text{VO}_2$  after CA using the Fick method in rats,<sup>40,41</sup> but the reported post-CA  $\text{VO}_2$  was either comparable to or lower than the baseline measurements or reference values in those studies. However, none of these studies measured  $\text{VCO}_2$ , thus the RQ values remain unknown. Hensel and Kox<sup>42</sup> reported that the number of  $\text{VO}_2$  measured by indirect calorimetry was higher than that by the Fick method in patients with lung infection. It was inferred that the injured lung itself might have measurable  $\text{VO}_2$ .

We currently lack a biochemical explanation for the change in  $\text{O}_2$  utilization manifested in these experiments. Under normal aerobic respiration, nearly 95% of  $\text{O}_2$  is consumed as the terminal electron acceptor within the mitochondria's electron transport chain (Figure 11).<sup>43–45</sup> The electrons that are used to reduce  $\text{O}_2$  to water are supplied by the Krebs cycle. The mitochondrial oxidation of glucose consumes 6  $\text{O}_2$  molecules for each 6-carbon glucose molecule and generates 6  $\text{CO}_2$  molecules to yield an RQ of 1, while the typical  $\beta$ -oxidation of a fatty acid consumes 23 molecules of  $\text{O}_2$  for each 16-carbon fatty acid, which will generate 16  $\text{CO}_2$  molecules, yielding an RQ of 0.7. This stoichiometry defines the “normal” range for RQ. In our experiments, the production of  $\text{CO}_2$ , and therefore the production of reducing equivalents by the Krebs cycle, returned to baseline after 45 minutes, but our  $\text{VO}_2$  increased to 150 or 200% of baseline, depending on the inspired  $\text{O}_2$  fraction. This change represents a fundamental shift in the cellular utilization of  $\text{O}_2$ .

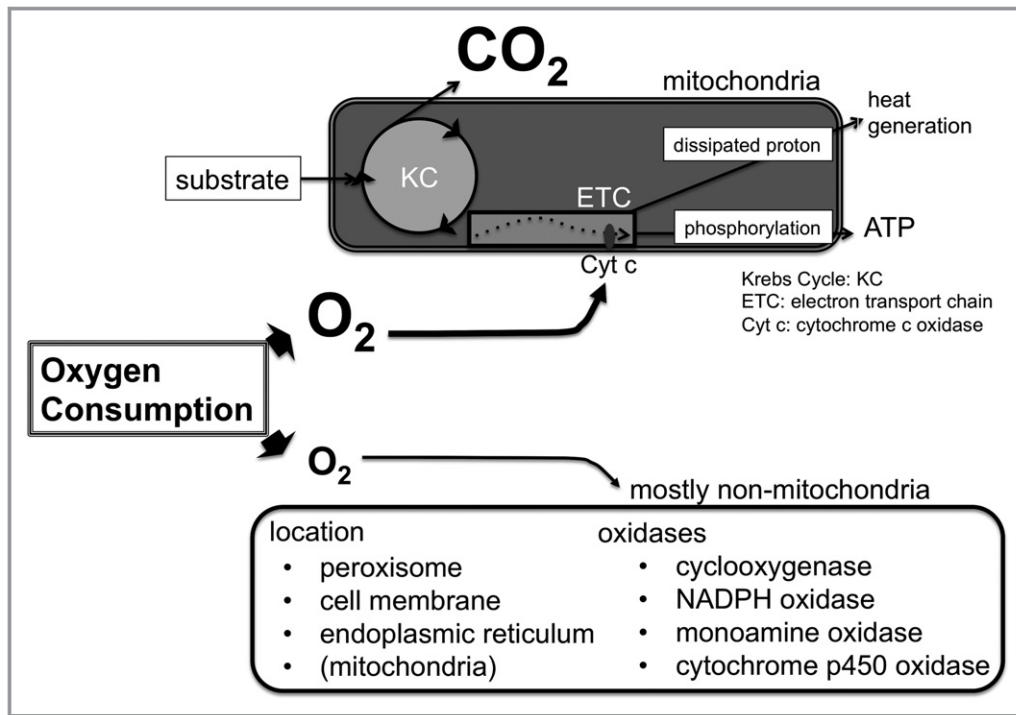
There is an ongoing debate about the balance between the potential benefits and dangers of supplemental  $\text{O}_2$ . It is

standard clinical practice to provide supplemental  $\text{O}_2$  when the  $\text{O}_2$  saturation is low.<sup>38</sup> However, it has been shown that supplemental  $\text{O}_2$  increases mortality in some critical illness injury models.<sup>25</sup> In our CA injury model, the  $\text{VO}_2$  increased by 50% when the  $\text{FIO}_2$  was 0.3. When the  $\text{FIO}_2$  was 1.0, the  $\text{VO}_2$  increased by 80%. Previous studies have shown that  $\text{VO}_2$  is not dependent on the  $\text{FIO}_2$  in healthy animals and humans.<sup>28,46</sup> This is consistent with our biochemical understanding of mitochondrial respiration, which suggests that the electron transport chain is nicotinamide adenine dinucleotide limited.<sup>43,47</sup> Therefore, we interpret our finding that  $\text{VO}_2$  is dependent on the inspired  $\text{O}_2$  fraction in the CA animal model as an indication that resuscitation may lead to metabolic processes or states that are  $\text{O}_2$  limited. In our injury model, the  $\text{FIO}_2$  1.0 group trended toward higher mortality than the  $\text{FIO}_2$  0.3 group. While this was not statistically significant with our numbers, it appears consistent with previously published results.<sup>25</sup>

The idea that resuscitation from CA results in “metabolic injury” has become resuscitation science dogma. Alterations in mitochondrial function have been detected in a variety of CA models in a variety of animal species.<sup>22,24,48</sup> Postischemic conditioning has been shown to improve survival in swine resuscitated from CA.<sup>49</sup> The American Heart Association's guidelines highlight reperfusion injury as a problem resuscitated patients will face.<sup>19,50</sup> While it remains unclear how our findings relate to this spectrum of metabolic injuries, we have demonstrated that significant differences in the metabolisms of resuscitated rats can be detected using indirect calorimetry. Interestingly, the global metabolic rate in the resuscitated rat did not appear to be increased or decreased. Instead, it appears that additional  $\text{O}_2$  consuming processes are making a significant contribution to the total  $\text{VO}_2$  of the animal.

We believe this novel metabolic phenotype in rats resuscitated from prolonged CA is in vivo evidence of postresuscitation metabolic injury, and might be used to estimate severity of postresuscitation injury. Measuring  $\text{VO}_2$ ,  $\text{VCO}_2$ , and RQ is noninvasive and can be translated into larger animal and human studies. However, currently available medical devices are associated with measurement limitations that undermine their accuracy.<sup>44,51,52</sup> However, a reliable device could be easily designed and constructed using existing technology, and we anticipate validating our finding in human survivors of CA.

There are no commercially available methods to measure  $\text{VO}_2$  and  $\text{VCO}_2$  in mechanically ventilated rodents. However, we have confidence in our  $\text{O}_2$  and  $\text{CO}_2$  measurements because our baseline  $\text{VO}_2$  and  $\text{VCO}_2$  values are consistent with reports from other rodent laboratories. For example, Barrow<sup>36</sup> reported  $\text{VO}_2$  in the range of 13 to 18 mL/kg per minute (800–1100 mL/kg per hour) in rats with sham surgery, which matches our measured  $\text{VO}_2$  range of 16 to



**Figure 11.** Pathways of cellular energy metabolism and  $O_2$  consumption ( $VO_2$ ). ATP indicates adenosine triphosphate; Cyt c, cytochrome c oxidase; ETC, electron transport chain; KC, Krebs cycle; NADPH, nicotinamide adenine dinucleotide phosphate.

18 mL/kg per hour in our sham group animals. Yamaoka et al<sup>53</sup> used a commercially available metabolic chamber for rats and reported that  $VCO_2$  was 14 to 20 mL/min per kg (10–14 mL/min per kg $0.75$ ) at sleep and 25 to 31 mL/min per kg (18–22 mL/min per kg $0.75$ ) at normal activity. Musch et al<sup>54</sup> reported that  $VCO_2$  was 20 mL/min per kg at rest and 67 to 72 mL/min per kg at maximal exercise.  $VCO_2$  in our sham group animals was 14 to 16 mL/min per kg. This number is close to that of sleeping animals but less than animals at normal activity or rest.  $VCO_2$  of the baseline and the sham surgery animals were measured under anesthesia and it is reasonable that the number we reported was close or less than those of nonanesthetized animals. We interpret this congruence with reported values as evidence that our system provides reasonable measurements.

There are at least 3 biochemical pathways that could explain the imbalance between  $VO_2$  and  $VCO_2$ . The first is that cytochrome c oxidase is consuming the additional  $O_2$  with extra reducing equivalents being provided by processes that do not generate  $CO_2$ , eg, processes other than the Krebs cycle such as glycerol-phosphate shuttle and/or the malate-aspartate shuttle pathways providing the electron transport chain with  $FADH_2$  and nicotinamide adenine dinucleotide, respectively.<sup>55,56</sup> The second is that the amount of  $O_2$  being consumed by oxidases and oxygenases other than cytochrome c oxidase has increased. Nonmitochondrial  $VO_2$  has

been described (Figure 11)<sup>57,58</sup> and these reactions do not involve nicotinamide adenine dinucleotide or  $FADH_2$  in mitochondria, and therefore are not coupled to  $CO_2$  created by the Krebs cycle. Enzymes such as nicotinamide adenine dinucleotide phosphate oxidases, cytochrome p450 oxidases, monoamine oxidases, and xanthine oxidases<sup>59</sup> primarily oxidize water without a concomitant production of  $CO_2$ . The third is the prolonged production of reactive  $O_2$  species within the electron transport chain enzymes. The creation of superoxide requires a single electron to reduce molecular  $O_2$ , where normal electron transfer requires 4 electrons to reduce molecular  $O_2$ .<sup>60</sup> This would shift the balance of  $VO_2$  and  $VCO_2$  in the direction we have observed. All these provisions could create a situation where  $O_2$  is the rate-limiting substrate for the electron transport chain, which could explain our observation that post-arrest  $VO_2$  is  $O_2$  concentration dependent.

### Study Limitations

This study is subject to several limitations. The first is that we studied post-arrest metabolism in rats. The novel findings reported in this article may not be found in humans.<sup>61</sup> We are currently conducting human studies to seek this newly found phenotype in survivors of CA. Second, the control group, the sham- $FIO_2$  0.3 group, has 2 differences relative to the CA

groups: it is missing the injury and it includes isoflurane, which is absent in the other groups after successful resuscitation. It is plausible that isoflurane alters the systemic metabolism in rodents. However, our  $VO_2$ ,  $VCO_2$ , and RQ values in the sham-FIO<sub>2</sub> 0.3 group are within a range reported for other rat models in the absence of isoflurane.<sup>36,62</sup> This suggests that the CA injury is solely responsible for the observed changes in RQ values. Finally, our volume measurements may underestimate the true tidal volume. It is possible that there was a small amount of leakage around the uncuffed endotracheal tube used in these studies. In addition, we used a side-stream capnograph and its sampling rate was set at 10 mL/min. However, our baseline  $VO_2$  and  $VCO_2$  values fall into the previously reported range, and we see no evidence that the CA injury had any additional effect on tidal volumes or airway pressures.

## Conclusions

We provide real-time, in vivo evidence of a novel postresuscitation metabolic phenotype in resuscitated rats measured by indirect calorimetry. The phenotype is characterized by a 50% to 100% increase in  $VO_2$  without a concomitant increase in  $VCO_2$ . As such, the metabolic phenotype is not a change in metabolic rate but a fundamental shift in cellular O<sub>2</sub> utilization. There are several possible biochemical explanations for this phenomenon, but they remain untested. Our findings may be relevant to basic scientists who study O<sub>2</sub> metabolism and cellular biochemistry, as well as to clinicians who seek better monitoring technologies and therapies for the treatment of CA.

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## Disclosures

Saeki is an employee of Nihon Kohden Innovation Center, INC. There are no products in development or marketed products to declare. This does not alter the authors' adherence to all of the Journal's policies on sharing data and materials. Shinozaki, Lampe, and Becker own intellectual property in metabolic measurements in critically ill patients. Shinozaki has grant/research support from Nihon Kohden Corp. Lampe has grant/research support from Zoll Medical Corp., Philips Healthcare,

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