Normoxic Ventilation After Cardiac Arrest Reduces Oxidation of Brain Lipids and Improves Neurological Outcome

Yuanbin Liu, PhD; Robert E. Rosenthal, MD; Yolanda Haywood, MD; Milena Miljkovic-Lolic, MD; Jack Y. Vanderhoek, PhD; Gary Fiskum, PhD

Background and Purpose—Increasing evidence that oxidative stress contributes to delayed neuronal death after global cerebral ischemia has led to reconsideration of the prolonged use of 100% ventilatory O\textsubscript{2} following resuscitation from cardiac arrest. This study determined the temporal course of oxidation of brain fatty acyl groups in a clinically relevant canine model of cardiac arrest and resuscitation and tested the hypothesis that postischemic ventilation with 21% inspired O\textsubscript{2} rather than 100% O\textsubscript{2} results in reduced levels of oxidized brain lipids and decreased neurological impairment.

Methods—Neurological deficit scoring and high performance liquid chromatography measurement of fatty acyl lipid oxidation were used in an established canine model using 10 minutes of cardiac arrest followed by resuscitation with different ventilatory oxygenation protocols and restoration of spontaneous circulation for 30 minutes to 24 hours.

Results—Significant increases in frontal cortex lipid oxidation occurred after 10 minutes of cardiac arrest alone with no reperfusion and after reperfusion for 30 minutes, 2 hours, and 24 hours (relative total 235-nm absorbing peak areas 7.1±0.7 SE, 17.3±2.7, 14.2±3.2, 16.1±1.0, and 14.0±0.8, respectively; n=4, P<0.05). The predominant oxidized lipids were identified by gas chromatography/mass spectrometry as 13- and 9-hydroxyoctadecadienoic acids (13- and 9-HODE). Animals ventilated on 21% to 30% O\textsubscript{2} versus 100% O\textsubscript{2} for the first hour after resuscitation exhibited significantly lower levels of total and specific oxidized lipids in the frontal cortex (13-HODE/ g wet wt cortex., n=4 to 6, P<0.05) and lower neurological deficit scores (45.1±3.6 versus 58.3±3.8, n=9, P<0.05).

Conclusions—With a clinically relevant canine model of 10 minutes of cardiac arrest, resuscitation with 21% versus 100% inspired O\textsubscript{2} resulted in lower levels of oxidized brain lipids and improved neurological outcome measured after 24 hours of reperfusion. This study casts further doubt on the appropriateness of present guidelines that recommend the indiscriminate use of 100% ventilatory O\textsubscript{2} for undefined periods during and after resuscitation from cardiac arrest. (Stroke. 1998;29:1679-1686.)

Key Words: heart arrest ■ reperfusion ■ resuscitation ■ dogs

Free radical–induced brain lipid peroxidation has been reported to occur in many different models of cerebral ischemia and reperfusion,\textsuperscript{1-5} including clinically relevant models of complete global cerebral ischemia and reperfusion induced by cardiac arrest and resuscitation.\textsuperscript{6-10} Lipid peroxidation results in both physiological effects, eg, altered blood flow and neutrophil chemotraction, and toxic effects on cellular activities that have been associated with excitotoxicity and neurodegeneration.\textsuperscript{11-16} Breakdown products of lipid peroxidation, eg, 4-hydroxynonenal, are highly toxic\textsuperscript{17} and can covalently modify proteins,\textsuperscript{18} inactivate enzymes, and inhibit DNA and protein synthesis.\textsuperscript{19} The ability of agents known to inhibit lipid oxidation (eg, free radical scavengers, lipid peroxidation terminators, and iron chelators) and to decrease postischemic neurochemical or neurological alterations suggests that lipid oxidation and/or oxidative modification of other molecules contributes significantly to ischemia/reperfusion brain injury.\textsuperscript{9,11,20,21}

Relatively few studies have attempted to establish a direct relationship between measurements of brain lipid oxidation...
and neurological impairment. The report by Rosenthal et al. indicated that the high molecular weight iron chelator hydroxyethyl starch-conjugated desferoxamine reduced both rat forebrain lipid oxidation, as measured by the UV absorbance of conjugated dienes in Folch lipid extracts and neurological injury and mortality in a rat cardiac arrest/resuscitation model. Exposure of Mongolian gerbils to a 100% O₂ atmosphere after 15 minutes of global brain ischemia has been shown to result in an increased production of expired pentane, a breakdown product of lipid oxidation, and increased mortality compared with animals that were placed in an atmosphere of room air.22

The proper method for O₂ administration after resuscitation from cardiac arrest remains controversial. Current American Heart Association guidelines suggest that “...it is recommended that 100% inspired O₂ be used during advanced cardiac life support.”21 In addition to the study by Mickel et al.23 in which a gerbil carotid arterial occlusion model was used, Zwemer et al.24 have provided compelling evidence that resuscitative and postresuscitative ventilation on room air (21% O₂) results in better neurological outcome than ventilation on 100% O₂ in a canine cardiac arrest model. The present study was performed in an attempt to confirm these provocative observations and to test the hypothesis that differences in neurological outcome elicited with different postischemic inspired O₂ concentrations are related to differences in brain lipid oxidation. Additional goals of this study were to identify the species of oxidized lipids and to determine the temporal course of brain lipid oxidation.

Materials and Methods

All animal experiments were conducted in accordance with guidelines established by the Institutional Animal Care and Use Committee of the George Washington University Medical Center. The use of cardiac arrest and resuscitation as a model for complete global cerebral ischemia and reperfusion in dogs has been described in detail by Rosenthal et al.25 Adult female beagles (8 to 15 kg) were initially anesthetized with either 17.6 mg/kg Bio-Tal (sodium thiamylal for injection, USP) or 15 mg/kg sodium pentothal (used because Bio-Tal became commercially unavailable during the course of this study). Prolonged anesthesia was maintained by infusion of 75 mg/kg o-chloralose. Animals were endotracheally intubated and ventilated with room air (21% O₂) before induction of cardiac arrest. Muscle paralysis was maintained with intravenous pancuronium bromide, and antibiotic prophylaxis was administered with ceftriaxone. Resuscitative drugs were administered via a venous catheter advanced to the level of the right atrium. Arterial pressure was continuously monitored through a femoral arterial catheter. Pulse, ECG, and rectal temperature were also continuously monitored and the temperature maintained at >37°C and <39°C using lights and heating blankets. A thoracotomy through the fourth left lateral intercostal space was performed on all animals, including nonarrested control animals. Ventricular fibrillation cardiac arrest was induced with a train of electric current applied directly to the epicardium of the right ventricle following incision and reflection of the pericardium. Artificial respiration was discontinued at the onset of fibrillation. After 10 minutes of cardiac arrest, animals were either euthanatized or CPR was initiated to allow for periods of reperfusion from 30 minutes to 24 hours. Resuscitation was initiated by open chest cardiac massage at the rate of 50/min, administration of epinephrine and sodium bicarbonate, and ventilation with different concentrations of O₂ as defined by the different oxygenation “protocols.” Open chest CPR was continued for 3 minutes followed by internal defibrillation. Arterial blood gas samples were measured before arrest, 2 minutes after defibrillation, and frequently thereafter. All animals were maintained under intensive care until the end of the experiment. Controlled ventilation was maintained until hour 22 when dogs were weaned from controlled ventilation. After resuscitation, deep postoperative analgesia was maintained with an initial bolus of morphine sulfate (0.1 mg/kg) followed by intravenous infusion (0.1 mg · kg⁻¹ · h⁻¹) for the remainder of the experiment. Intermittent doses of pancuronium bromide (0.1 mg/kg) were administered as necessary to prevent “fighting the ventilator,” only after adequate analgesia was assured.

The ventilatory oxygenation protocols were established for 2 separate studies. In the first study, the temporal course of brain lipid oxidation was determined for tissue samples from animals initially resuscitated with 100% ventilatory O₂ during cardiopulmonary resuscitation (CPR) followed by adjustments of ventilator settings after the initial blood gas determinations (approximately 5 minutes) to maintain P O₂ at >70 and <100 mm Hg and P CO₂ at >25 and <35 mm Hg. In the second study, a comparison of brain lipid oxidation and neurological outcome was made between animals that underwent “normoxic” versus “hyperoxic” oxygenation protocols. The normoxic protocol consisted of ventilation with 21% O₂ during CPR with adjustments initiated soon thereafter, as described above, to maintain P O₂ between 80 and 100 mm Hg. Maintenance of P O₂ within this range never required the administration of greater than 30% inspired O₂ within the first hour after resuscitation. The hyperoxic protocol consisted of continuous ventilation with 100% O₂ during CPR and for 1 hour thereafter, followed by the standard ventilatory adjustments.

Animals in the 24-hour reperfusion group were awakened at 23 hours by an injection of naloxone and then tested for neurological deficit (0%, normal; 100%, brain death) through the use of a standardized neurological deficit scoring (NDS) system previously used in this model22 and similar to that used by Bircher and Safar26 and others.24,27 Testing was performed by 2 persons blinded to the treatment protocols and trained to the criteria of the NDS system; interrater agreement was r = 0.90. A total of 20 animals were randomized into 2 groups designated as “normoxic” and “hyperoxic.” Two animals in the hyperoxic group were excluded because of prolonged postresuscitative hypotension that met the limit of the exclusion criteria (see Rosenthal et al25). A total of 9 dogs in each group completed 24 hours of postresuscitative care. At the end of the neurological deficit testing procedure, the animals were reanesthetized with 1 of the 2 barbiturates and o-chloralose and maintained on the respirator.

At approximately 4 minutes before the end of the experimental period, a craniotomy was performed on the anesthetized animals to expose a large portion of the cerebral cortex. At the end of the experimental period, a wedge of right frontal cortex 2 cm wide × 2 cm long × 1 cm thick was excised and immediately immersed in liquid N₂. The right cerebral hemisphere was then removed and the striatum and hippocampus excised and immersed in liquid N₂. Each sample was stored at –80°C for use in measurements of brain lipid oxidation. Immediately after removal of the biopsy specimens, the animals were euthanatized by intracardiac injection of a pentobarbital-based euthanasia solution.

The method used for sensitive quantification of oxidized fatty acyl groups present in brain lipid extracts was reported previously and uses base-catalyzed transmethylation for preparation of fatty acid methyl esters.28 Briefly, total lipids from 50 mg Folch-washed brain biopsies were treated with triphenylphosphine for 1 hour at 0°C to reduce any lipid hydroperoxides to more stable lipid hydroxides. After evaporation, samples were subjected to sodium methoxide–

<table>
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<th>Selected Abbreviations and Acronyms</th>
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<tr>
<td>CPR = cardiopulmonary resuscitation</td>
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<tr>
<td>5-HETE = 5-hydroxyeicosatetraenoic acid</td>
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<td>HODE = hydroxyoctadecadienoic acid</td>
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<td>HPLC = high-performance liquid chromatography</td>
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<td>NDS = neurological deficit scoring</td>
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catalyzed transmethylation for 1 hour at 25°C. This procedure forms fatty acyl methyl esters from fatty acyl groups present in lipids such as membrane phospholipids. One milliliter of saline was then added and the pH adjusted to 3 with glacial acetic acid. Fatty acid methyl esters and free fatty acids were extracted with chloroform and applied to a silica gel column. Hydroxylated fatty acid methyl esters and oxidized free fatty acids were eluted with 10 mL chloroform:acetone (7:3, vol/vol), dried, and dissolved in the elution solvent mixture for high-performance liquid chromatography (HPLC) and injected into a normal-phase Econosphere silica gel-packed HPLC column (Alltech) using a Perkin-Elmer HPLC system. Elution solvent mixtures were 97.5% A (hexane:isopropanol:acetic acid = 995:4:1) plus 2.5% B (hexane:isopropanol:acetic acid = 899:100:1) with a flow rate of 1 mL/min. More polar lipid hydroxides were eluted with 85% A plus 15% B. In this system, unoxidized fatty acid methyl esters elute early and are undetected at 235 nm since they lack a conjugated diene chromophore. 5-HETE was used as the internal standard in order to calculate relative 235 nm-absorbing peak areas. One tissue sample was exposed to a Fenton-type reagent to verify that oxidized brain lipids could be detected by this procedure. The chloroform phase of the Folch extract was mixed with 0.2 mol/L Tris-HCl (pH 7.4) at a ratio of 5:1. Aliquots of concentrated solutions of H$_2$O$_2$ (in H$_2$O) and FeSO$_4$ (in HCl) were added to bring the final concentrations to 1 mmol/L H$_2$O$_2$ and 100 μmol/L FeSO$_4$. Following 5 minutes of mixing at 25°C, the iron chelator diethylenetriaminepentaacetic acid was added at a concentration of 1 mmol/L to terminate free radical generation. The phases were separated and the chloroform phase used for derivatization as described above.

Identification of the 2 major peaks of 235-nm absorbance eluted from the HPLC column was accomplished through gas chromatography/mass spectrometry. Eluates were collected, evaporated, and treated with 200 mL freshly prepared diazomethane in ether for 5 minutes at room temperature. The hydroxy groups in these methyl esters were converted to trimethylsilyl ester derivatives by treatment with 50 mL bis(trimethylsilyl) trifluoroacetamide plus 1% trimethylchlorosilane for 1 hour at 60°C. Derivatives were dissolved in hexane and analyzed by capillary gas chromatography (OV-1 fused silica capillary column, 12 m length, 0.25 mm inner diameter, 0.5 mm film thickness) on a Hewlett-Packard gas chromatograph interfaced with a Hewlett-Packard quadrupole mass spectrometer. The operating temperature for the source and injector was 250°C. The oven temperature was programmed from 100°C to 200°C at a rate of 5°C/min. Helium was used as the carrier gas. Mass spectra were recorded in the electron-impact ionization mode with an electron energy of 70 eV.

Tissue lactate determinations were performed on brain biopsy samples stored at −80°C. Samples were weighed and homogenized in 3% perchloric acid with a Brinkman Polytron homogenizer. Homogenates were briefly centrifuged to remove precipitated macromolecules, and the supernatants were used for the assay of lactate with a YSI model 2300 Stat glucose and lactate analyzer.

The NDS of dogs in the hyperoxic group were compared with values from the normoxic group through the use of a Wilcoxon rank sum comparison with 2-tailed t considered significant at P<0.05. Differences in brain lipid oxidation and tissue lactate levels among experimental groups were analyzed by 1-way ANOVA and Duncan’s test. Two-way ANOVA or 2-tailed Student’s t test were also used under appropriate circumstances. Physiological parameters were compared between normoxic and hyperoxic treatment groups using Student’s t test. Comparisons with P<0.05 were considered significantly different.

**Results**

The HPLC-based procedure used in this study for quantifying oxidized brain lipids primarily measures fatty acyl hydroxides generated from both free fatty acids and fatty acyl groups present in membrane phospholipids. Figure 1 describes the HPLC elution profiles of oxidized lipids extracted from the frontal cortex of a nonischemic control animal, a dog that underwent 10 minutes of complete cerebral ischemia due to cardiac arrest with no reperfusion, and a dog that was resuscitated after the 10-minute period of ischemia and reperfused for 2 hours using the standard ventilatory oxygenation protocol described in “Materials and Methods.” The Folch extract of a control sample was also exposed to 1 mmol/L H$_2$O$_2$ plus 100 mol/L FeSO$_4$ (pH 7.4) for 5 minutes at 25°C prior to derivatization and separation. 5-HETE was the internal standard and was used to determine relative peak areas for different 235-nm absorbing peaks within and among different brain samples.

It is evident from Figure 1 that very few absorbance peaks that were apparent within the first 10 minutes of elution were because of reagents used in the extraction and derivatization procedure. Known products of lipid oxidation (eg, 11-, 12-, and 15-HETE and 13-hydroxyoctadecadienoic acid) eluted from the HPLC column with retention times ranging from 10 to 20 minutes (authors’ unpublished observations), whereas elution of other oxidation products, eg, 5-HETE, required a second solvent system (apparent as the large peak at approximately 40 minutes). As very few absorbance peaks with retention times as great as that of 5-HETE were observed with tissue extracts, 5-HETE was commonly added to these samples and used as an internal standard for purposes of quantitative comparison.

Figure 1. Chromatograms of hydroxy lipids generated from Folch lipid extracts of canine frontal cortex and separated by HPLC. Conjugated dienes present in hydroxy lipid methyl esters were detected with UV absorbance at 235 nm. Lipids were extracted from representative samples of frontal cortex (50 mg) taken from a sham-operated, nonischemic dog (Control), an animal subjected to complete global cerebral ischemia due to 10 minutes of ventricular fibrillation cardiac arrest (10 min Ischemia), and an animal subjected to cardiac arrest followed by resuscitation and reperfusion for 2 hours (2 hr Reperfusion). Free fatty acids and esterified fatty acyl groups were transmethylated, reduced, and separated with an HPLC system as described in “Materials and Methods.” The Folch extract of a control sample was also exposed to 1 mmol/L H$_2$O$_2$ plus 100 mol/L FeSO$_4$ (pH 7.4) for 5 minutes at 25°C prior to derivatization and separation. 5-HETE was the internal standard and was used to determine relative peak areas for different 235-nm absorbing peaks within and among different brain samples.
The effects of different postischemic ventilatory O2 concentrations on cerebral cortex lipid oxidation are described in the Table. A significant (50%) increase in the relative total HPLC peak area was observed for animals in the hyperoxic treatment protocol compared with the normoxic protocol (P<0.05). Even greater differences were observed between the 2 groups when the areas of the 2 major peaks corresponding to 13- and 9-HODE were compared. The hyperoxic animals exhibited a nearly 100% increase in 13-HODE and a 400% increase in 9-HODE compared with the normoxic animals (P<0.05).

During the course of these experiments, HPLC measurements of the total amount of lipid oxidation present in the striatum and hippocampus of 24-hours’ reperfusion animals in the hyperoxic group were performed. A comparison of the results of these measurements for all 3 areas of the brain between this group and the nonischemic control group is provided in Figure 4. Significant elevation of brain lipid oxidation occurred in the striatum and hippocampus in addition to the frontal cortex for animals that underwent cardiac arrest and hyperoxic reperfusion. The order of the greatest increase in lipid oxidation among these areas was striatum>cortex>hippocampus. As for the cortex, the 2 most prominent 235-nm absorbing peaks for the samples of striatum and hippocampus eluted at approximately 15 and 19 minutes (authors’ unpublished observations).

In addition to measuring brain lipid oxidation, frontal cortex biopsy samples were also used for determinations of tissue lactate levels. Because a previous study using this animal model and our standard resuscitation protocol reported persistent elevation of cortical lactate at 2 hours’ reperfusion,25 a comparison was made between lactate levels present at this time for normoxic and hyperoxic resuscitated animals and for nonischemic controls. Lactate levels in these by hyperoxic ventilation in a similar model, a comparison was made between the effects of postischemic normoxic ventilation with 21% O2 versus postischemic hyperoxic ventilation with 100% O2 on brain lipid oxidation and neurological outcome after 23 to 24 hours of reperfusion. The mean neurological deficit score for the 9 animals in the normoxic group was 45.1±3.6 SE. Most dogs in this group appeared to respond to some external stimuli; several righted themselves and attempted to stand. The mean NDS for the hyperoxic animals (58.3±3.8 SE) was significantly worse than for the normoxic group (P<0.05). Several animals in this group were judged to be totally unaware of their surroundings. Stereotypical purposeless running motions were observed in 6 of the 9 animals examined. It should be noted that no significant differences in preischemic or 2-hour postischemic values for rectal temperature, arterial blood pressure, pulse, blood pH, or PCO2 were observed between the 2 groups of animals (data not shown). The PO2 for both groups was also not significantly different before cardiac arrest or after 2 hours of reperfusion. As would be expected, at 5 minutes following resuscitation, ie, during the first hour of reperfusion when the level of inspired O2 was different between the 2 groups, the values for PO2 were also substantially different (normoxic=83.8±3.7 SE mm Hg; hyperoxic=454 mm±34.2 SE mm Hg; P<0.0001).

The effects of different postischemic ventilatory O2 concentrations on cerebral cortex lipid oxidation in this canine model of cardiac arrest and resuscitation and in view of the results of Zwemer et al24 indicating worsening of neurological outcome
3 groups were 1.8±0.2, 2.5±0.3, and 4.2±0.5 SE μmol/g wet wt (n = 4) for control animals and 2-hour normoxic and hyperoxic reperfused animals, respectively. The normoxic 2-hour reperfusion group was not significantly different from the control group, but the hyperoxic 2-hour reperfusion group was significantly different from the control group and the normoxic reperfused group (P<0.05).

Discussion

The HPLC method used in this study and previously with a neural cell model of chemical hypoxia has several advantages over other commonly used assays of lipid oxidation. This method directly measures the primary products of lipid peroxidation, ie, lipid hydroperoxides and hydroxides, rather than secondary, lower-molecular-weight breakdown products, eg, malondialdehyde, ethane, or pentane. The use of HPLC to separate 235-nm absorbing species also greatly minimizes interference by molecules other than oxidized lipids that also absorb light of this wavelength and can contaminate simple Folch tissue extracts. Other investigators have also shown HPLC separation of oxidized lipids together with alternative methods of detection, eg, chemiluminescence, to be a sensitive and reliable method of quantifying oxidized lipids present in tissue extracts. In addition to using an HPLC procedure to quantify the level of total oxidized fatty acyl groups present in brain biopsies, the present study also used gas chromatography/mass spectrometry to actually identify the primary species of oxidized lipid. These species were identified as 13- and 9-HODE, each being oxidation products of linoleic acid. As the Folch brain lipid extracts were reduced with triphenylphosphine to improve stability, the true products formed in vivo may actually be the respective hydroperoxides. Although these products can be derived from lipoxygenase- or cytochrome P-450–dependent enzymatic peroxidation, they can also arise from nonenzymatic oxidation via attack of fatty acyl groups by hydroxyl radicals or other free radicals thought to be generated at abnormally high levels during ischemia/reperfusion. The significance of the generation of these specific oxidized fatty acids during cerebral ischemia and reperfusion is, at this juncture, speculative. However, oxidation of phospholipid...
Effect of Ventilatory O₂ Concentrations During Reperfusion on Canine Cortex Lipid Peroxidation

<table>
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<tr>
<th>Resuscitation Protocol*</th>
<th>Relative Total Peak Area (per g wet wt)</th>
<th>13-HODE (µg/g wet wt)</th>
<th>9-HODE (µg/g wet wt)</th>
</tr>
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<tbody>
<tr>
<td>Normoxic</td>
<td>10.48 ± 0.66†</td>
<td>1.73 ± 0.12</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>Hyperoxic</td>
<td>16.80 ± 2.60†</td>
<td>3.12 ± 0.78‡</td>
<td>2.03 ± 0.46‡</td>
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*Resuscitation protocols were as described in “Materials and Methods.” Normoxic: 21% O₂ during CPR and adjustments made starting approximately 5 minutes later to maintain P O₂ between 80 and 100 mm Hg and P CO₂ between 25 and 35 mm Hg. Hyperoxic: 100% O₂ during CPR and for 1 hour thereafter followed by standard adjustments.
†Canine frontal cortex samples were obtained 24 hours after 10 minutes of cardiac arrest and resuscitation and processed for measurements as described in “Materials and Methods.” Relative total 235 nm–absorbing peak areas were calculated from HPLC spectra such as those described by Figure 1, with 5-HETE used as an internal standard. Peaks with elution times of 15 and 19 minutes were quantified as 13- and 9-HODE, respectively, based on data obtained from analysis with gas chromatography/mass spectrometry. Data are expressed as mean ± SE for n = 4–6 different animals per group.
‡Significantly different from normoxic group by 1-way ANOVA and Duncan’s test.

Linoleic acid has been demonstrated to be cytotoxic and has been associated with oxidative DNA damage. 13- and 9-HODE also exhibit biological activities strongly implicated in ischemic brain injury, eg, chemotactic activity for polymorphonuclear leukocytes and the ability to activate cellular protein kinase C. Although 13- and 9-HODE were consistently the most predominant oxidized lipids found among different animal groups and throughout different areas of the brain, they only represented 20% to 35% of the area typically observed for at least 10 different 235 nm–absorbing peaks eluted from the HPLC column. Further effort is being made to identify these other species of oxidized lipid.

The increase in oxidized brain lipids observed after global cerebral ischemia and reperfusion in this study is consistent with the results of other studies with other animal models and consistent with our previous results with the canine model in which cerebral cortex protein oxidation was demonstrated. In contrast to our observations for protein oxidation, the level of oxidized lipids did not increase with increasing periods of reperfusion (Figure 2). This finding suggests that oxidized fatty acyl groups do not accumulate but rather exist in a dynamic state in which degradation to smaller products, eg, malondialdehyde, is balanced by ongoing production of new lipid peroxides and hydroxides. One of the most intriguing observations made in this study was that significant lipid oxidation occurred during the 10-minute period of ischemia in the absence of reperfusion. The fact that this result was obtained with a cardiac arrest model of global cerebral ischemia is significant since a complete lack of blood flow to all parts of the brain unquestionably occurs within seconds after the induction of ventricular fibrillation, whereas some flow of blood to various regions of the brain can occur in many vascular occlusion models of “complete” cerebral ischemia, including those where electron spin resonance/spin trapping measurements have indicated the formation of free radicals during the period of ischemia. Although direct evidence of lipid oxidation during complete cerebral ischemia is scarce, elevated levels of malondialdehyde have been reported in forebrain mitochondria after 30 minutes of ischemia in a standard rat 4-vessel occlusion model and in canine parietal cortex after 15 minutes of cardiac arrest. Taken together the results of these studies suggest that free radical–induced molecular alterations may contribute to tissue injury during complete ischemia and during various phases of reperfusion.

Although significant brain lipid oxidation can occur following ischemia alone, the fact that it is an ongoing, dynamic process makes this form of molecular injury susceptible to postischemic intervention. The present study provides new, direct evidence that hyperoxic resuscitation and reperfusion exacerbates postischemic lipid oxidation. Under these conditions a significant increase in lipid oxidation occurred in the striatum and hippocampus as well as in the frontal cortex. Although the degree of reperfusion-dependent lipid oxidation was not significantly different among these areas, the observation of a trend toward the greatest increase in the striatum is consistent with the findings of Zhang et al that indicated that the increase in phospholipid hydroperoxides during aging in gerbils is greatest in the striatum. Zwemer et al previously provided evidence with a similar canine cardiac arrest model that hyperoxic, postischemic ventilation results in significantly worse neurological outcome when compared with normoxic ventilation (21% O₂). The finding that pretreatment of animals in their hyperoxic group with the antioxidant tirilizad mesylate improved neurological outcome suggested that increased oxidative molecular alterations, eg, lipid peroxidation, may contribute to the deleterious effects of hyperoxic ventilation; however, no direct measurements of such alterations were provided. In another recent study in which an intracranial fluid compression model of global cerebral ischemia in rabbits was used, immediate postischemic treatment with hyperbaric oxygen appeared to increase the production of free radicals, as reflected by an increase in the ratio of brain oxidized/reduced glutathione; however, no increase in the oxidized lipid breakdown product malondialdehyde was observed after 75 minutes of reperfusion. These findings...
suggest that either immediate postischemic hyperbaric oxygen is not as neurotoxic as normobaric hyperoxygenation or that brain malondialdehyde measured within the first 1 to 2 hours of reperfusion is not as sensitive an indicator of lipid oxidation as our HPLC measurements of discrete 235-nm absorbing species of fatty acyl groups performed after 24 hours of reperfusion. The results of the present study not only confirm that normobaric, hyperoxic postcardiac arrest ventilation can be neurologically detrimental but they also demonstrate a close, albeit correlative, relationship between increased neurological impairment and increased frontal cortex lipid oxidation. The additional present finding that hyperoxic reperfusion actually exacerbates rather than ameliorates brain lactic acidosis also challenges the notion that the prolonged use of 100% ventilatory O₂ after cardiac arrest may be beneficial through stimulation of aerobic and inhibition of anaerobic cerebral energy metabolism.

The neurological and neurochemical results of this study taken together with those of the study by Mickel et al.\(^\text{22}\) and the neurological results of Zwemer et al.\(^\text{23}\) cast serious doubt on the appropriateness of the present Advanced Cardiac Life Support guidelines that recommend the use of 100% ventilatory O₂ for undefined periods during and after resuscitation from cardiac arrest.\(^\text{24}\) However, the present study used only one 10-minute period of cardiac arrest in young healthy animals. Humans are resuscitated after widely variable periods of cardiac arrest and are often elderly with impaired respiratory and cardiovascular systems. Clearly, clinical trials will be necessary to resolve this issue.

**Acknowledgments**

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**References**

In this very interesting study, the authors determined the temporal course of oxidation of brain fatty acyl groups in a dog model of cardiac arrest and resuscitation and tested the hypothesis that postischemic ventilation with 21% inspired O₂ versus 100% O₂ produced a reduction in levels of oxidized brain lipids and a decreased neurological impairment. The authors found that in this canine model of 10 minutes of cardiac arrest, resuscitation with 21% versus 100% inspired O₂ resulted in lower levels of oxidized brain lipids and improved neurological outcome measured after 24 hours of reperfusion. This outcome, in a certain sense, is reasonable if one subscribes to the oxidant radical damage hypothesis, ie, more O₂ available would produce increased O₂ radicals and would increase oxidant injury, whereas less O₂ would produce less injury. The authors used neurological deficit scoring and high-performance liquid chromatography (HPLC) measurements of fatty acyl lipid oxidation as the key outcome measurements. For those who have espoused the O₂ radical injury hypothesis, it has always been a question of why one subscribes to the oxidant radical damage hypothesis, ie, from cardiac arrest. One would think, perhaps, that even the use of a mild hypoxic mixture might result in a better outcome, because even less oxidant injury would be produced with the hypoxic gas mixture than with the hyperoxic gas mixture. In this study, the lowered O₂ (21% versus 100%) was administered at reperfusion, the time when O₂ radical production may be at its peak. The HPLC method for assaying lipid oxidation is a good one, because this method directly measures primary products of lipid peroxidation (lipid hydroperoxide and hydroxides). This technique is a sensitive and reliable method to quantify oxidized lipids in tissue extracts. The authors then used gas chromatography/mass spectrometry to identify the primary species of oxidized lipids. These substances have been strongly implicated in ischemic brain injury over the years, and the increase in oxidized brain lipids observed in this study after global cerebral ischemia and reperfusion is consistent with results of other previous studies in other animal models.

One of the more interesting observations made in this study was that significant lipid oxidation occurred during the 10-minute period of ischemia in the absence of reperfusion. So the question becomes, how are radicals produced without reperfusion in these circumstances? The importance of the production of oxidants during ischemia remains unclear, and further work is required to evaluate this issue. Nevertheless, the authors clearly show that upon reperfusion, with the animal ventilated with 21% O₂ versus 100% O₂, hyperoxic reperfusion actually exacerbates rather than ameliorates the injury that occurs. However, in another study which tested the hypothesis that limiting the O₂ content of the blood which reperfuses ischemic tissue would decrease posts ischemic injury by allowing less substrate for the formation of O₂ radicals, the results are not positive. In this article, Ulatowski et al tested the hypothesis that transient hypoxic reperfusion after 15 minutes of global cerebral ischemia in piglets would improve acute recovery of electrical function. However, the authors found that hypoxemia during reperfusion after cerebral ischemia in this model did not improve acute brain electrical function and in fact prolonged posts ischemic hyperemia. What accounts for the differences in these studies remains unclear. The authors of the accompanying article would cast doubt on the advanced cardiac life support guidelines that recommend the use of 100% ventilatory O₂ for undefined periods during and after resuscitation from cardiac arrest. However, it is clear that further studies must be performed to evaluate this issue, and certainly clinical trials will be necessary to resolve this issue in the final analysis.

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Reference
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