Objectives:
1. Discuss the role of oxygen toxicity in the neurologic and pulmonary development of term and preterm infants.
2. Discuss the toxic implications of the pre & postnatal use of caffeine.
3. Discuss the toxic implications of steroid administration in newborns.

History

Joseph Priestly is usually credited with the discovery of oxygen after he was first to publish his experiments on “different kinds of air” in 1774 despite the fact that the Polish alchemist Michael Sedziwoj wrote of the existence of a “food of life” in air (oxygen) nearly 170 years earlier in his book entitled “A New Light of Alchemy”. (1) Priestly, an English clergyman and amateur chemist noticed that mice lived longer in a jar filled with the gas he created from heated mercuric oxide as opposed to the ordinary atmosphere. The gas was named ‘oxygen’, from the Greek word acid former, by Antoine Lavoisier in 1775. (2) Remarkably, it was just seven years later that Francis Chaussier initiated oxygen use in clinical medicine when he invented a pressure – limited ventilator device that was used for newborn resuscitation. (3) Within a few years the use of oxygen in neonatal resuscitation became routine throughout Europe and was expanded to neonatal specialty units around 1891 after Tarnier noticed improved survival of premature infants with inhaled oxygen. (4) However the era of continuous oxygen therapy really began in earnest after J S Haldane identified that a frequent cause of hypoxemia was diffusion impairment and ventilation-perfusion mismatch in the lung. He also understood that high oxygen concentration in inspired air could correct hypoxemia and speed recovery of patients. (5,6) The development of compressed gas in mobile units allowed easier access to oxygen and increased usage of it in the delivery rooms. (7) In 1953, New York anesthesiologist Virginia Apgar introduced an evaluation and assessment score quantifying the degree of asphyxia of the neonate. (8) In her assessment tool oxygen therapy alone could quantifiably render an improved score because a “pink” infant was evaluated as a “healthier” infant. Two of the pioneers in neonatology, Klaus and Myer, went on to make the following recommendation in 1966:
“There is no contraindication to the use of warm 100% oxygen during resuscitation. The birth process is an asphyxial episode, and high concentrations of oxygen during the first few minutes of life can only be helpful.”(9)

Longer periods of exposure to oxygen became more readily accepted for the therapeutic support of the preterm infant’s immature respiratory function. This simple therapy proved to have devastating consequences when the philosophy of “if a little is good a lot should be better” was espoused and many of these infants developed retrolental fibroplasia, now called retinopathy of prematurity (ROP). (10) The adverse clinical outcomes of ROP somewhat curtailed the use of 100% oxygen in the neonatal special care units but it continued to prevail in the delivery rooms. Clinicians and researchers began to think in terms of the “oxygen paradox” – the fact that cell and tissue injury was increased if hypoxic tissue was exposed to high concentrations of oxygen. (11) It appeared that the uncritical use of 100% oxygen was introduced and used in newborn care for nearly 200 years without the evidence-based studies that would be required today.

Over the past several decades a more discerning view of oxygen in clinical medicine has been established. The role of free radical formation, antioxidants and their link to oxidative stress as a cause of apoptosis and reperfusion injury has led to studies at the cellular, subcellular, and molecular level in both animals and humans. The International Liaison Committee on Resuscitation (ILCOR) 2005 Consensus on Science and Treatment reviewed sixteen animal studies that looked for possible advantages of using oxygen as opposed to air for the resuscitation of immature animals exposed to hypoxia. In virtually all of these studies there was no apparent advantage to using oxygen except for three studies by Solas. (12) In 2007, a summary of the results of three systemic reviews (13-15) of five trials and seven individual studies, including 2133 newborn infants, indicated that resuscitation with room air reduced mortality by 31%. Based on this analysis for every 25 newborns resuscitated in room air there would be one more surviving infant. (16) Ambient air resuscitation also reduced the time to first breath by 30 seconds, increased heart rate at 90 seconds of age as well as the 5-minute Apgar score. (13) Regarding premature infants < 32 weeks three small randomized trials have been published. (17-19) Wang (17) randomized babies <29 weeks to room air or 100% oxygen. Approximately half of the babies in the room air group required oxygen supplementation however no difference in outcome was recorded for the low versus high oxygen group. In the other two studies infants <30 weeks were randomized to 30% or 90% oxygen. The end target was an oxygen saturation of 85% after 10 minutes. These studies documented that room air was insufficient for extremely low birth weight babies (ELGANs) to achieve targeted SpO2 values in the delivery room but the use of FIO2 of 30% was effective, thus avoiding the need for rescue therapy with pure oxygen. Infants in the 30% oxygen group were also found to have decreased mechanical ventilation and CPAP time, as well as the development of bronchopulmonary dysplasia. (18-19) Vento et al (20) looked at reduced –to-oxidized glutathione ratio, a biochemical marker in which a low value indicates oxidative stress. At 28 days the ratio remained significantly lower in the 100% oxygen resuscitated infants indicating persistent oxidative stress, which was not seen in the room air or control group. Several epidemiologic studies have reported that a brief
exposure to high oxygen concentration at birth of 3 minutes or longer constituted an increased risk of developing childhood leukemia. (21-23)

The accumulation of data over the last several decades has led to the re-examination of the role of oxidant injury resulting from the use of 100% oxygen. Oxidative stress has been implicated in several newborn conditions leading Saugstad in 1988 to coin the phrase “oxygen radical diseases of neonatology”. (24) He included bronchopulmonary dysplasia (BPD), chronic lung disease (CLD), pulmonary circulation, periventricular leukomalacia (PVL), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), and patent ductus arteriosus (PDA) in this category. If his concept was correct it meant that the above-mentioned conditions were not different disease entities but were simply different organ manifestations of the same complex processes of oxidative stress and metabolism.

**Oxidative Metabolism and Oxidative Stress**

The anaerobic metabolism of one molecule of glucose (C6) produces four ATP molecules. With the evolution of oxygen as an electron acceptor for the electron transport chain in oxidative phosphorylation 36-38 molecules of ATP could be synthesized from a single glucose molecule. Cellular energy metabolism increased ATP formation through the electron-transport reaction in which oxygen accepted electrons and H+ and was eventually reduced to water. The price for this evolutionary step was the generation of oxygen free radicals which are highly reactive molecules containing one or more unpaired electrons. They donate or abstract electrons from other molecules in an attempt to pair their electrons and generate a more stable species. Oxygen-derived reactants collectively termed reactive oxygen species (ROS) and reactive nitrogen species (RNS) are normally produced in living organisms. (See Table I) When produced in excess they are important mediators of cell and tissue injury with the resulting damage referred to as oxidative stress. (25, 26) The hydroxyl radical and the peroxynitrite anion are the most reactive and highly destructive of these products. (27) If these toxic products are not inactivated, their high chemical reactivity leads to damage to a variety of cellular macromolecules including proteins, carbohydrates, lipids and nucleic acid (DNA fragmentation, base modifications, and strand breaks.) (28, 29) There are a number of antioxidant enzymes that convert reactive species to less reactive products. These endogenous antioxidants may be classified into primary, secondary, and tertiary antioxidant defenses. (30) Primary defenses are those which prevent radical formation. The iron binding properties of transferrin and lactoferrin have this role in extracellular fluids. Secondary defenses remove or inactivate formed ROS/RNS. These may be enzyme systems such as superoxide dismutase (SOD), catalase, and glutathione peroxidase as well as non-enzymatic molecules including reduced glutathione, vitamins E, C and A, albumin, mucin, glucose, bilirubin and uric acid. Tertiary defenses operate to remove and/or repair oxidatively damaged molecules and are particularly important for proteins and deoxyribonucleic acid. (31) (See figure 1)
Table I Reactive oxygen and nitrogen species of biological interest [36]

<table>
<thead>
<tr>
<th>Free Radicals</th>
<th>Non-Radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Oxygen Species</td>
<td></td>
</tr>
<tr>
<td>Superoxide (O$_2^-$)</td>
<td>Hydrogen peroxide (H$_2$O$_2$)</td>
</tr>
<tr>
<td>Hydroxyl (·OH)</td>
<td>Hypochlorous acid (HOCl)</td>
</tr>
<tr>
<td>Peroxyl (R-O$_2^-$)</td>
<td>Ozone (O$_3$)</td>
</tr>
<tr>
<td>Alkoxyl (R-O$^-$)</td>
<td>Singlet oxygen (O$_2^*$)</td>
</tr>
<tr>
<td>Hydroperoxyl (HO$_2^*$)</td>
<td>Hydroperoxide (R-OOH)</td>
</tr>
<tr>
<td>Hydroperoxide (R-OO$^*$)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reactive Nitrogen Species</th>
<th>Non-Radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide (NO$^+$)</td>
<td>Nitric acid (NO$_2$)</td>
</tr>
<tr>
<td>Nitrogen dioxide (NO$_2^*$)</td>
<td>Nitrogen cation (NO$_2^+$)</td>
</tr>
<tr>
<td>Nitrosyl or nitrosyl cation (NO)</td>
<td>Nitrosonium cation (NO$_2^+$)</td>
</tr>
<tr>
<td>Alkyl peroxynitrite (R-OONO)</td>
<td>Nitroxyl anion (NO$^-$)</td>
</tr>
</tbody>
</table>

![Figure 1 Common pathway production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)](image)

Oxygen Toxicity in Pulmonary and Neurologic Development

Labor, vaginal or caesarean delivery and the overall transition from an intrauterine hypoxic environment to an extrauterine normoxic environment induce an elevated production of ROS and RNS in the term newborn. (32, 33) Pre-term birth occurring during the late canalicular or early saccular stage of lung development is very likely to lead to severe respiratory distress syndrome RDS. The poorly developed peripheral airways and immaturity of the lung maturation cells are the major cause of decreased surfactant production by type II cells and inadequate antioxidant responses to increased oxygen. For example, the antioxidant superoxide dismutase (SOD) activity appears at the same time as surfactant synthesis by type II pneumocytes in the developing lung. Other causes of increased oxidative stress in the premature infants with RDS are pro-oxidant drugs, systemic infections, extrapulmonary inflammation and high inspiratory concentrations of oxygen required to achieve adequate arterial oxygenation. (34)

High oxygen therapy, often thought to be essential for survival in preterm infants with respiratory distress syndrome, also produces excessive ROS/RNS in the respiratory system of these infants. (35,36) If these toxic products are not inactivated by the antioxidant defense system structural changes occur in the pulmonary vascular endothelial cells resulting in focal hypertrophy and altered metabolic activity. The molecular damage of proteins, lipids, carbohydrates and nucleic acid caused by the highly reactive ROS/RNS compounds result in cell injury and may induce respiratory cell death. (37,38) When groups of term and preterm rat pups were placed in more than 95% oxygen for 6 days there was histological evidence of alveolar hemorrhage, immune-cell infiltration, edema, and collapse. The T1- and T2-weighted MRI images for the hyperoxic group revealed greater signal intensities when compared to the room air group. (39) Reactive oxygen species may also act in the lung as upstream signaling molecules favoring alveolar epithelial cell apoptosis. (40) Biochemical evidence of molecular damage from ROS/RNS and antioxidant activity has been demonstrated in the serum and bronchoalveolar lavage (BAL) collected from RDS patients. (41-45) Unfortunately, the antioxidant defense mechanism develops late in gestation rendering the premature infant poorly equipped to neutralize and therefore highly susceptible to the toxic derivatives of oxygen. It would be especially prudent to avoid hyperoxia in the preterm population because of their vulnerability to oxygen. (46, 47) A recent study demonstrated that the use of lower oxygen concentrations during resuscitation for extremely premature infants reduced oxidative stress, proinflammatory cytokines and the incidence of BPD. (19)

Animal studies have shown that hyperoxia also causes histological changes of brain injury. Hoehn et al revealed evidence of apoptotic cell death in the frontal cortex, thalamus, and hippocampus of 7-day-old rat pups exposed to 80% oxygen for 24 hours. (48) Experiments in young animals have confirmed evidence of significant oxygen stress in the group exposed to oxygen during resuscitation as opposed to controls in room air. (49, 50) Felderhoff-Mueser exposed different age rat pups to increasing levels of oxygen over prolonged periods of time. The amount
and distribution of damage was age dependent. The greatest overall vulnerability was seen at 3 and 7 days and older rats had much less damage. There was also significantly less brain damage in the 7-day-old rats treated with N-acetylcysteine (a precursor of the antioxidant glutathione) before and after 12-hour exposure to 80% oxygen when compared to controls suggesting that the effect was mediated by hyperoxia-induced stress. (51). Kaindl looked at acute and long term proteome changes induced by oxidative stress in the developing rodent brain. Their results suggested that exposing infant rodents to hyperoxia not only caused an increased rate of apoptosis in the brain but also elicited long-term alterations in cell growth and differentiation, synaptic function, neuronal migration, and axonal arborization. The period of vulnerability of these rats appeared to be in the first two weeks of life, which was the equivalent of their brain growth spurt. This time frame would be similar in humans from the third trimester of pregnancy to several years after birth. (52) Lundstrom et al (53) randomly assigned preterm infants to receive room air or 100% oxygen during stabilization in the delivery room. Global cerebral blood flow measurements continued to be decreased at 2 hours of age in the oxygen group as compared to the room air group suggesting prolonged cerebral vasoconstriction.

Much of the published literature regarding the role of oxygen free radicals and oxidative stress in the pathogenesis of newborn conditions has been published in the neonatology, pediatric and basic science journals. Short and Van Der Walt (54) recently published the results of a survey of pediatric anesthetists in the UK regarding oxygen use in neonatal and infant anesthesia. They found considerable variation in the use of oxygen during anesthesia in preterm, newborn and infant patients. Van Der Walt (55,56) also published two editorials regarding the deleterious effects of high concentrations of oxygen in the practice of neonatal resuscitation and anesthesia. Questions need to be answered and strategies developed to minimize the oxidative stress in the smallest and most vulnerable pediatric patients that require anesthesia.

**Steroids use in Newborns**

The use of postnatal steroids (PNS) became widespread in neonatal care by the 1990’s to facilitate extubation, and reduce BPD by modifying lung inflammation, despite properly conducted clinical trials for safety and efficacy. (57) In fact two large randomized controlled studies of PNS were halted prematurely because of serious short-term complications, such as intestinal perforations, growth retardation, PVL, hyperglycemia, hypertension and infection. (58, 59) Long-term complications have included cardiovascular (60, 61), immune system disorders (62), renal calcifications (63), and neurological and behavioral disorders (64, 65).

Initial trials of corticosteroids for BPD used dexamethasone at high doses for 42 days and subsequent trials over the next 20 years gradually lowered the doses and shortened the duration of treatment in an attempt to determine the optimal dose and time of exposure to improve clinical outcomes. (66). Halliday et al. grouped the trials into three meta-analyses on the basis of time at which corticosteroid therapy
was started and determined that treatment begun beyond seven days decreased mortality and BPD but not the short-term issues. (67-69) A major conclusion of these studies was further long term followup data was needed. Yeh et al (65) reported the outcomes at school age of infants who had received dexamethasone or placebo within the first 12 hours of life followed by a three week tapering regimen. They reported clear adverse effects on growth, IQ, and motor performance and recommended that early high dose steroids should not be continued. Despite the decreased mortality with later onset dexamethasone treatment as evidenced from the Halliday analyses the American Academy of Pediatrics recommended against the routine use of dexamethasone in very low birth weight premature infants in 2002 (70) and reaffirmed this position in 2006 (71). The recommendation was based on the possible negative effects of dexamethasone on neurodevelopment and resulted in a dramatic reduction in the use of the therapy (72).

Controversy still exists among neonatologists regarding the beneficial effects of lower doses of steroids over shorter periods of time. A recent study by Doyle et al (73), suggested that low- dose dexamethasone after the first week of life shortened the duration of intubation in extremely low birth weight infants, without any short-term complications. Also, a metaregression published by the same author reported a significant effect modification by risk for BPD. (74) Corticosteroid treatment increased the chance of death or cerebral palsy when the risk for BPD was less than 35%, whereas when the risk for BPD exceeded 65% it reduced this risk. That is to say the detrimental effects of deteriorating pulmonary status offset the risk of PNS treatment. Wilson-Costello et al (75) confirmed that the risk of the composite death or NDI was modified by the predicted risk of BPD. The study also showed that there is no safe window of development where PNS exposure is less detrimental to neurodevelopmental outcomes. They also documented that for every 1 mg/kg dose increase of corticosteroid there was a 2.0 reduction on the Mental Developmental Index (MDI) and a 40% increase in for disabling cerebral palsy.

The question of the optimal steroid type to be used is unanswered. Hydrocortisone and dexamethasone were both found to reduce ventilator days and oxygen use but hydrocortisone had less adverse effects with hyperglycemia, hypertension and growth (76, 77). Hydrocortisone may have less impact on neurodevelopment and brain growth. A study involving quantitative MRI evaluations of brain development and neurocognitive assessments of preterm and term-born children previously treated with hydrocortisone showed no long term adverse effects. (78).

**Caffeine and Apnea of Prematurity**

More than three decades ago it was demonstrated that methylxanthines (caffeine, theophylline and aminophylline) reduce the frequency of apneic episodes in prematurity. (79) Because apneas occur in up to 90% of preterm infants caffeine has become one of the 10 most frequently prescribed medications in neonatal intensive care commonly used to treat apnea of prematurity. (80) When preterm
infants are treated with caffeine for apnea plasma concentrations are 10-100 times higher than serum levels reached in breast fed babies after mothers consume from moderate intake of coffee. The major effect of caffeine at therapeutic doses is the blockade of adenosine A1 and A2A receptors (A1AR, A2AAR). (81). The maturation of these receptor systems affects how the fetus and infant react to caffeine. Few signaling molecules have the potential to influence the developing mammal as the nucleoside adenosine. In contrast to most neurotransmitters adenosine is released by all cells and is present in all tissues. Adenosine levels rapidly increase with tissue hypoxia and inflammation and have a neuroprotective effect. Given the neuro protective effects of endogenous adenosine, there has been concern over the effects of high doses of caffeine in neonates at risk for apneas and postnatal hypoxic ischemia. (82)

It was thus surprising when Bona et al (83) identified a reduction of brain damage of 50% in rat pups treated with theophylline and exposed to hypoxic ischemia. Recently, Back et al (84) noted that there was reduced cerebral myelination and ventriculomegaly in mice reared in hypoxia from postnatal day 3 through 12 (increased adenosine and A1AR) but these findings were reversed in mice treated with caffeine (adenosine antagonist). The authors speculated that caffeine treatment during periods when premature infants are at risk for periventricular white matter injury may offer a means of protection. A meta-analysis of 21 studies (85) found no significant deleterious effects on cognition or behavior in methylxanthine treated children and that caffeine is unlikely to increase the risk of neurodevelopmental disabilities in the preterm population. Schmidt et al (86) showed that premature infants who were treated with caffeine had improved survival rate, decreased the incidence of BPD, reduced incidences of cerebral palsy and cognitive delay at 18 months. Using a post hoc analysis, about one half of the protective effect was attributed to the profound stimulatory actions of caffeine on respiration effort. The enhanced respiratory drive is believed to be a result of the antagonism of the A1AR in the pons and medulla oblongata by methylxanthines. (87) An expanding body of data shows that adenosine plays an important role during pre and post natal development. While late in the third trimester caffeine has a protective affect administration during embryogenesis may have deleterious effects. Wendler et al (88) showed that exposure to a single dose of caffeine during embryogenesis resulted in both short-term effects on cardiac development and long term effects on cardiac function.

Conclusion

There is convincing evidence that the newborns are highly susceptible to the toxic derivatives of oxygen and are poorly equipped to neutralize its toxic derivatives. Premature infants appear to be at greater risk. Medications administered at this critical period of rapid growth often have short and long term benefits but also risks for short and long term adverse outcomes. Future well designed prospective, randomized, collaborative studies need to be completed to define the population at risk, and accurate measuring tools for short and long term outcomes to ascertain the lowest possible effective dose for the shortest period of time.
References

68. Halliday HL, Ehrenkranz RA, Doyle LW. Moderately early (7–14 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants [Cochrane review]. In: The Cochrane Library. Issue 1. Chichester, United Kingdom: John Wiley and Sons; 2004


