Oxygen Use in Neonatal Care: A Two-edged Sword

Serafina Perrone*, Carlotta Bracciali, Nicola Di Virgilio and Giuseppe Buonocore*

Department of Molecular and Developmental Medicine, General Hospital "Santa Maria alle Scotte", University of Siena, Siena, Italy

In the neonatal period, the clinical use of oxygen should be taken into consideration for its beneficial and toxicity effects. Oxygen toxicity is due to the development of reactive oxygen species (ROS) such as OH• that is one of the strongest oxidants in nature. Of note, generation of ROS is a normal occurrence in human and it is involved in a myriad of physiological reactions. Anyway an imbalance between production of oxidant species and antioxidant defenses, called oxidative stress, could affect various aspect of organisms’ physiology and it could determine pathological consequences to living beings. Neonatal oxidative stress is essentially due to decreased antioxidants, increased ROS, or both. Studies have demonstrated that antioxidant capacity is lower in preterm newborns than term babies. This well-known deficiency of antioxidant factors is only a piece of a cohort of factors, which can be involved in the neonatal oxidative stress and the increased production of ROS may be a main factor. Mechanisms of ROS generation are: mitochondrial respiratory chain, free iron and Fenton reaction, inflammation, hypoxia and/or ischemia, reperfusion, and hyperoxia. Oxidative stress following hyperoxia has been recognized to be responsible for lung, central nervous system, retina, red blood cell injuries, and possibly generalized tissue damage. When supplemental oxygen is needed for care, it would be prudent to avoid changes and fluctuations in SpO2. The definition of the safest level of oxygen saturations in the neonate remains an area of active research. Currently, on the basis of the published evidences, the most suitable approach would be to set alarm limits between 90 and 95%. It should allow to avoid SpO2 values associated with potential hypoxia and/or hyperoxia. Although the usefulness of antioxidant protection in the neonatal period is still under investigation, the risk of tissue damage due to oxidative stress in perinatal period should not be underestimated.

Keywords: oxygen, reactive oxygen species, free iron, mitochondria, oxidative stress, newborn infants

INTRODUCTION

Oxygen is essential for aerobic life, but it can be considered a double-edged sword in perinatal period having both positive biological benefits and toxicity effects (1–3). Oxygen toxicity is due to the development of reactive oxygen species (ROS), such as the superoxide anion (O2•−), hydrogen peroxide (H2O2), lipid peroxide (LOOH), peroxyl radicals (RO•), electron delocalized phenoxy radical (C6H50•), nitric oxide (NO), and the hydroxyl radical (OH•) (4). OH• is a potent oxidant in biological fluids and may damage tissues, through reaction with lipids, proteins, DNA, amino acids, and several other molecules (5).
Despite their well-known harmful effects on cells, ROS reactions are also implicated in a myriad of physiological reactions, cell fate decisions, and signal transduction pathways. They have a key role in various cellular processes such as energy metabolism, gene expression, protein import, or folding, and they are produced in response to a variety of ligands including growth factors, cytokines, and G protein coupled receptors (6, 7).

Shift in redox potential may favor beneficial or detrimental consequences according to various factors. Both high levels of ROS and excessive low levels of ROS can alter the balance between pro-oxidant and antioxidant elements, which is essential for biologic processes (8). An imbalance between oxidants and antioxidants is called oxidative stress and that is a potential cause of damage (9). If oxidative stress is mild, cell defenses may increase by a mechanism, which generally involves enhanced gene expression of ROS scavenging activities (10). On the other hand, severe oxidative stress is generally followed by lipid peroxidation that alters membrane structure, disrupts membrane permeability properties, and alters cellular components. Abnormalities in cell membrane proteins due to high levels of ROS can also induce functional consequences including, for instance, alteration in recognition of cells in the immune response (11), apoptosis, and/or necrosis (12).

Oxidative stress in the newborn may result from decreased antioxidants, increased ROS, or both. Antioxidant capacity is lower in the newborn and particularly the premature infant in comparison to term newborn (13, 14). The level and activity of the most-relevant antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), change dynamically during development and mature in the last weeks of gestation, preparing the fetus for lung respiration (15–17). Non-enzymatic antioxidant factors, such as α-tocopherol and reduced glutathione (GSH), are low in the fetus and newborns (18, 19). Therefore, premature infants are especially prone to oxidant injury, being unprepared for hyperoxic challenge of extrauterine life. It is demonstrated that a 30 min exposure to 100% O₂ at birth can cause a significant increase in lipid peroxidation in live newborn sheep (14).

The deficiency of antioxidant factors, that is characteristic of the neonate, is only a piece of a cohort of factors which can be involved in the neonatal oxidative stress and the increased production of ROS may be an additional factor. Some studies demonstrated that, in the immature lung of preterm newborn, the main sources of ROS could be ischemia, reperfusion, phagocytosis, and hyperoxia (20–22).

The various pathways of ROS generation should be considered and it is necessary to take into account the complexity of redox equilibrium and, therefore, correctly interpreting the origin of oxygen toxicity in newborn.

This review focuses on the mechanisms of ROS production and ROS-induced toxic effects following oxygen administration in newborns, by considering both short- and long-term consequences of oxidative stress exposure. Furthermore, it deals with the recent research on the definition of the safest level of oxygen saturations in the neonatal period and the state of knowledge on oxygen use in clinical practice.

**ROS: BIOCHEMISTRY AND BIOLOGY**

Molecular oxygen (O₂) has two unpaired electrons in separate orbitals in its outer electron shell. This chemical structure enhances ROS generation (23).

In general, the principal endogenous sources of ROS in human and, in particular, in newborn are mitochondrial metabolism, increased free circulating transition metals, inflammation through NADPH oxidase (NOX) reactions, hypoxia–reoxygenation (through hypoxanthine–xanthine oxidase reaction), hyperoxia, and paradoxically hypoxia (24–29). These mechanisms will be discussed in turn.

**Mitochondrial Respiratory Chain**

Mitochondrial respiratory chain is the main source of ROS. Mitochondria play a key role for the ATP production in eukaryotic cells. The sequence of events involved in oxidative phosphorylation, which takes place in mitochondria, leads to ATP formation as a result of the transfer of electrons from NADH to O₂, by a series of electron carriers (30).

Initially, electron donors can convert O₂ to O₂⁻. Dismutation of O₂⁻ by superoxide dismutase (SOD) produces H₂O₂ that in turn may be fully reduced to water (H₂O) by glutathione peroxidase (GSH-Px) and catalase (CAT) or, alternatively, partially reduced to the OH⁻ in the Fenton–Haber Weiss reaction, catalyzed by reduced transition metals, particularly iron, but also copper and zinc (24). Under physiologic conditions, approximately 98% of O₂ undergoes a complete reduction to form H₂O₂, whereas 2% of electrons will leak, causing a partial reduction of the oxygen and producing ROS. ROS generation by mitochondria is mainly dependent on complexes I and III and is highly dependent on metabolic conditions and on the intra-mitochondrial balance between oxidative and antioxidative factors (6, 31).

**Free Iron and Fenton Reaction**

Iron could be considered a two-edged sword for living organisms and, in particular, for newborns (32). It is an essential transition metal for the proper growth and normal neurologic development but it is toxic when unbound. Under conditions of body iron overload, plasma transferrin becomes fully loaded with iron, and chelatable forms of iron escape sequestration in biological systems. They become available to react with reduced oxygen, finally generating the toxic OH⁻ (33). Non-protein bound iron easily enters in the Fenton–Haber Weiss reaction: H₂O₂ generated by dismutation of O₂⁻ can break down, in presence of ferrous ion, to produce the most damaging of the oxygen free radicals, the OH⁻ (25), and to form ferric ion (34).

**Inflammation**

Respiratory burst of phagocytic cells by NOX is a known source of ROS production in mammalian cells (12). While the most relevant generation of ROS by NOX occurs in phagocytes after activation upon exposure microbes, microbial products, or inflammatory mediators (8), ROS are produced via NOX in a variety of cell type and in response to normal physiological signals such as insulin, angiotensin II, growth factors, and various classes of
Receptors, such as formylpeptide receptors and toll-like receptors (35, 36). Furthermore, NOX-dependent ROS generation has been suggested to trigger adaptive response of a variety of stressors (36). Opsonization and activation of phagocytes are also known to occur as consequences of hypoxia, hypoxanthine–xanthine oxidase reaction, and hypoxia–reoxygenation (37). However, NOX-induced ROS generation can activate the NF-E2 related factor 2 pathway, which increases antioxidant protection during inflammation (38).

**Hypoxia and/or Ischemia**

Metabolic conditions and O2 levels modify the rate of ROS generation (39). Hypoxia and/or ischemia results in increased electron leakage, and the interaction of various activated signals with residual oxygen produces superoxide. In animal models, several studies have demonstrated that hypoxia increases lipid peroxidation by peroxynitrite production and decreases Na⁺, K⁺-ATPase activity leading to cellular membrane dysfunction. Moreover, hypoxia induces modification of the N-methyl-D-aspartate receptor-ion channel complex, leading to increased intracellular Ca²⁺. Intracellular calcium activates several enzymes, such as proteases, potentiating free radical generation and resulting in hypoxic cell injury (40).

Furthermore, during hypoxia, redox signals to and from mitochondria are activated. In particular, the respiratory chain increases ROS production stimulating the signaling pathway to induce hypoxia-induced factor (HIF)-dependent gene expression. HIF-1α is an important protein causing a shift from aerobic to anaerobic metabolism and also reducing mitochondrial oxygen consumption. Thus, it seems that the byproducts of oxidative phosphorylation play a role as signaling molecules, conveying cellular oxygen availability (41).

**Reperfusion**

Reperfusion is the second phase of ischemia/reperfusion (I/R) injury, and it is characterized by the generation of ROS when circulation is restored. In this phase, the reestablishment of blood supply to ischemic tissues causes the delivery of blood-borne elements (platelets and leukocytes) that are activated by and release ROS. ROS may induce cell damage and death by interacting with NO, fatty acids, or non-protein bound iron to generate more toxic free radicals such as peroxynitrite, peroxyl radicals, and hydroxyl radicals. Moreover oxygen free radicals facilitate the inflammatory response to reperfusion, by making oxidant-dependent pro-inflammatory mediators (11, 31).

A third stage of I/R injury constitutes the reparative phase. ROS promote angiogenic growth factors, vascular remodeling, activation of matrix metalloproteinases that contribute to fibrosis, and formation of scar tissue (31).

**Hyperoxia**

Hyperoxia could be defined as a state of excess supply of O₂ in tissues and organs. The inhalation of a high level of oxygen, has been reported to be followed by membrane bound NOX activation, free radical generation, and DNA damage with apoptosis (42). At birth, blood oxygen content and oxygen availability sharply increase to their adult values, eliciting the production of a flood of ROS (43, 44), which may act as signaling molecules in specific metabolic pathways, in response to oxidative stress (45, 46).

In animals, exposed to high oxygen concentration, a modification of nuclear membrane function has been reported as consequence of high nuclear Ca²⁺ influx, activation of Ca²⁺/calmodulin-dependent protein kinase pathway, and CREB protein-mediated apoptotic proteins (47).

Furthermore, hyperoxia is involved in activation of a panel of pro-inflammatory cytokines, including IFNγ and macrophage inflammatory protein 2, that, in turn, could finally develop ROS.

**OXYGEN TOXICITY**

In the clinical settings, ROS generation following hyperoxia has been recognized to be responsible for lung, central nervous system, retina, and red blood cell injuries as well as generalized tissue damage, which can be reported both in the neonatal period and in the adult life.

Focusing on neonatal period, the following paragraphs explain the mechanisms of both the short- and the long-term toxic effects of oxygen administration and hyperoxia on various organs and body systems.

**Short-term Adverse Effects**

**Lung**

Hyperoxia is particularly harmful for the lungs and the mechanism of damage is complex. Chronic oxygen toxicity may damage the pulmonary epithelium and inactivate the surfactant, form intra-alveolar edema and interstitial thickening, and later fibrosis, leading to pulmonary atelectasis (48). Lung injury is demonstrated to be caused directly by ROS production in response to hyperoxia and indirectly by ROS due to phagocyte activation and inflammation. The two mechanisms seem to be integrated (49). In vitro and in vivo exposures to hyperoxia result in downregulation of peroxisome proliferators-activated receptor gamma and in increase transdifferentiation of pulmonary protective lipofibroblasts to myofibroblasts (MYFs) (50, 51). Epithelial cell growth and differentiation is not adequately supported by MYFs. This results in a disturbed alveolarization, characterizing bronchopulmonary dysplasia (BPD) (52). High level of neutrophils, IL-8, and leukotrienes in alveolar fluid of BPD infants clearly support the role of inflammation and ROS in the development of this oxidative damage (53).

**Retina**

The exposure to hyperoxia is also associated with higher risk for severe retinopathy of prematurity (ROP), due to susceptibility of the phospholipid-rich retina to ROS (54).

The peripheral temporal portion of the retina is the last to be vascularized, and it is still immature even at term (55). With exposure to excess oxygen, the developing retinal endothelial cells activate various transcription factors, including HIF-1α and vascular endothelial growth factor, which, in turn, cause both cessation of retinal vessel growth and loss of some existing retinal vessels (56). These mechanisms finally lead to abnormal retinal vascular proliferation and the formation of a ridge, which places...
traction on the retina and increases the risk of detachment, as seen in ROP (57).

Red Blood Cells

Newborn erythrocytes are more prone to damaging effects of oxidative stress and to have higher content of free iron than those of adults. In this context, free radical damage is involved in neonatal hemolytic anemia and particularly of the preterm (58, 59). Furthermore, prolonged exposure to hyperbaric oxygen leads to changes of erythrocytes shape, as a consequence of toxic effects of oxygen on the erythrocyte membranes. In an animal model, various forms of abnormal red blood cells are observed after exposure to high oxygen concentration, and in particular echinocyte shape was dominated (60).

Long-term Effects

Exposure to hyperoxia at birth can also be related to long-term pathological effects. Oxygen exposure in the neonatal period has been demonstrated to affect lungs of mice by increasing airway reactivity and persistent inflammation with alteration in the innate immunoregulatory pathways that contribute to "poorer resistance" to respiratory viral infections in adulthood (61, 62). Furthermore, the exposure of newborn mice to hyperoxia may lead to long-term cardiac abnormalities, such as left ventricular dysfunctions (63), and neurodevelopmental impairments in adult life, as demonstrated by abnormal behavior, deficits in spatial and recognition memory, small hippocampal dimensions, in the absence of intracranial pathology such as intraventricular hemorrhage or periventricular leukomalacia in the neonatal period (64).

In conclusion, experimental studies and clinical observations demonstrated high susceptibility of the fetus and newborn to oxidative stress. Increased release and decreased detoxification in the newborn appear to be negatively correlated with the gestational age.

STATE OF KNOWLEDGE OF OXYGEN USE IN NEONATAL CARE

Avoidance of conditions, such as infections, asphyxia, retinal light exposure, iron supplementation, and, in particular, hyperoxia, reduces oxidative stress.

Recent studies, that have been accomplished, have revised the concept of the optimal oxygenation in newborns, children, and adults.

Chow et al. reported the experience of a tertiary neonatal center, where oxygen administration was titrated to optimize neonatal care. To reduce the incidence of ROP, authors recommended to avoid any fluctuation of FiO2 and to maintain oxygen saturation within "acceptable" limits, setting up oxygen alarms below 85% and above 93% in newborns <32 weeks of gestation (65). Tin and Gupta compared two populations of high risk newborns kept at O2 saturations of 88–98% and 70–90%. They found a decrease of incidence of ROP in the group treated with lower O2 saturation without any differences in mortality and morbidity (66). Neonatal outcomes showed that newborns treated with higher level of oxygen had more cognitive disabilities than those treated with lower oxygen, after 10 years (66). A report from the Oxford Vermont Network, in extremely low birth newborns, demonstrates less chronic lung disease and ROP incidence in babies with a target oxygen saturation of <95% than those with oxygen saturation more than 95% (67).

The first two randomized controlled trials (RCT), performed to answer the question of what is the range of optimal saturation by pulse oximetry in preterm infants receiving supplemental oxygen, were the Supplemental Therapeutic Oxygen for Prethreshold Retinopathy Of Prematurity (STOP-ROP) study (68) and the Benefits of Oxygen Saturation Targeting (BOOST) I (69). In the first, the authors concluded that there was no significant difference in the rate of progression to threshold ROP in group of newborns cared for with lower O2 saturation range (89–94%) vs. the higher group (96–99%). But, as secondary outcome, they showed an increased incidence of chronic lung disease and a longer duration of hospitalization, both in the higher group. In the BOOST I, no differences were found in the primary outcomes, defined as growth and neurodevelopmental measures at a corrected age of 12 months, in the two groups (91–94% vs. 95–98%). In the high-saturation group (babies kept at 95–98% of O2 saturation), the newborns required oxygen for a longer period, had a higher dependence on oxygen at 36 weeks of postmenstrual age and need for home oxygen therapy with higher frequency, despite babies of low-saturation group, kept at 91–94% of O2 saturation.

More recently, five large multicenter, masked, RCT were conducted with a similar design and outcome measures to collect data from 5,000 preterm newborns with less than 28 weeks postmenstrual age; they were the Surfactant Positive Pressure and Pulse Oximetry Randomized Trial (SUPPORT) (70), the BOOST II United Kingdom, Australia, and New Zealand (71), and the Canadian Oxygen Trial (COT) (72). Thanks to these data, it was possible to conduct a prospective meta-analysis, NeOProM (73) study, with a primary outcome defined as a composite of death and disability at 18–24 months of corrected age.

The three studies were performed with the same target ranges of oxygen saturation in the two groups: 85–89% in the lower group vs. 91–95% in the higher group.

In the SUPPORT, the primary outcome was a composite of severe ROP, death before discharge from the hospital, or both. The study showed no significant differences in the primary outcome, but the use of a lower range of oxygen saturation results in a decrease of occurrence of severe ROP and an increase of death before the discharge. The SUPPORT was conducted by pulse oximetry systems, with an older software algorithm, despite the other two trials. In the BOOST II and COT, the software algorithm of the oximetry systems changed about at the midpoint of the studies.

The data from the BOOST II showed that a restrictive use of oxygen, with target range of saturation below 90%, is associated with a higher risk of death and necrotizing enterocolitis despite of a reduction of incidence of ROP, significantly increased in the higher group saturation.

The COT study, with a primary outcome defined as death before 18 months of corrected age or survival with one or
more disability, do not showed significant differences in the mortality or other outcome, but only a reduction of duration of O2 therapy. Based on these five RCT, the 2013 European Consensus Guidelines on the Management of Neonatal Respiratory Distress Syndrome in Preterm Infants suggested that SpO2 should be targeted at 90–95%, in infants with gestational age <28 weeks until 36 weeks (74).

However, there are more unanswered questions and the optimal oxygen saturation range for low birth weight preterm infants remains elusive. This is mainly due to the several different clinical conditions of preterm newborns. Some authors indicate that 50 and 70 mmHg (75) is the optimal oxygen tension, but it is noteworthy that pulse oximetry ability remains controversial. Oxygen saturation of more than 90% should be carefully considered because to be found related with an arterial oxygen tension of more than 80 mmHg (76).

In clinical practice, the continuous monitoring of oxygen saturation is mandatory to titrate oxygen therapy as better as possible and the routine use of pulse oximetry systems can be considered a very useful approach for the neonatologists, in order to reach this goal. However, the optimal target range for oxygen saturation in the sick newborns and, above all, in the extremely preterm babies is not clear. The challenge for the clinicians is reaching a balance in the oxygen administration, to avoid the damage and negative outcomes, associated with either hyperoxemia or hypoxemia. Based on all the actually available evidence and considering the lack of evidence about the influence of many factors such as transfusional status, different gestational ages and underlying diseases, the most careful approach is to avoid both hyperoxia and hypoxia in infants requiring oxygen supplementation. In order to maintain an intended optimal range of SpO2 90–95%, it has been suggested to set the acoustic signals at 91 and 96%, with a delay time not longer than 20 s (77). It is essential to control the low limit as well as the upper limit to prevent excessive fluctuations of oxygen saturation (78, 79).

CONCLUSION

Hyperoxia and hypoxia are deeply involved in the development of several neonatal diseases, and the mechanisms are complex and not yet fully understood. However, evidences suggest that both the generation of oxidant species (i.e., free radicals and ROS) and the deficiency of antioxidants may play a role. Hyperoxia and inflammation as well as the episode of hypoxia–re-oxygenation and free iron appear to be sources of increased ROS release, which may cause tissue injury either by direct effect or as consequences of endothelium dysfunction and gene alteration, particularly in preterm newborns. Understanding the effects of O2 administration is important for the management of oxygen therapy in newborns, in order to prevent inadvertent cellular and tissue damage caused by hyperoxia, in the patients requiring supplemental oxygenation.

AUTHOR CONTRIBUTIONS

SP: wrote a draft and supervised the final manuscript; CB: assisted with preparation of manuscript; NV: assisted with preparation of manuscript; GB: conceived the idea and supervised the final manuscript.

ACKNOWLEDGMENTS

The authors are grateful to the “Gruppo di Studio di Biochimica Clinica Neonatale della Società Italiana di Neonatologia” and to EURAIBI Foundation for its support.

REFERENCES

1. Mack WJ, Thimmesch AR, Pierce JT, Pierce JD. Consequences of hyperoxia and the toxicity of oxygen in the lung. Nurs Res Pract (2011) 2011:260482. doi:10.1155/2011/260482

2. Smith JL. The pathological effects due to increase of oxygen tension in the air breathed. J Physiol (1899) 24(1):19–35. doi:10.1113/jphysiol.1899.sp000746

3. Katz A, Hoeck LE, de la Cruz E. Studies on the effect of high oxygen administration in retrolental fibroplasia. I. Nursery observation. Am J Ophthalmol (1952) 35(9):1248–53. doi:10.1016/0002-9394(52)91140-9

4. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med (1991) 91(3C):14S–22S. doi:10.1016/0002-9343(91)90279-7

5. Foote CS, Valentine J, Greenberg A, Liebman JF. Active Oxygen in Chemistry, Vol. 2. Dordrecht: Springer Science & Business Media (2012). 66 p.

6. Lismont C, Nordorem M, Van Veldohoven PP, Fransen M. Redox interplay between mitochondria ad peroxisomes. Front Cell Dev Biol (2015) 3:1–19. doi:10.3389/fcell.2015.00035

7. Holmstrom KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signals. Nat Rev Mol Cell Biol (2014) 15(6):411–21. doi:10.1038/nrm3801

8. Martin KR, Barrett JC. Reactive oxygen species as double-edged swords in cellular processes: low-dose cell signaling versus high-dose toxicity. Hum Exp Toxicol (2002) 21:71–5. doi:10.1191/0960327202ht213oa

9. Buonocore G, Groenendaal F. Anti-oxidant strategies. Semin Fetal Neonatal Med (2007) 12:287–95. doi:10.1016/j.siny.2007.01.020

10. Kaludercic N, Deyrell S, Dilisa F. Reactive oxygen species and compartmentalization. Front Physiol (2014) 5:285. doi:10.3389/fphys.2014.00285

11. Vento M. Oxygen supplementation in the neonatal period: changing the paradigm. Neonatology (2014) 105(4):323–31. doi:10.1159/000360646

12. Orient A, Donko A, Szabo A, Leto TL, Geiszt M. Novel sources of reactive oxygen species in the human body. Nephrol Dial Transplant (2007) 22(5):1281–8. doi:10.1093/ndt/gfn077

13. Patel A, Lakshminrusimha S, Ryan RM, Swartz DD, Wang H, Wynn KA, et al. Exposure to supplemental oxygen downregulates antioxidant enzymes and increases pulmonary arterial contractility in premature lambs. Pediatr Res (2010) 67:66–71. doi:10.1203/PDR.0b013e3181e5f57f

14. Kumar VH, Patel A, Swartz DD, Wang H, Wynn KA, Nielsen LC, et al. Exposure to supplemental oxygen and its effects on oxidative stress and antioxidant enzyme activity in term newborn lambs. Pediatr Res (2010) 67:66–71. doi:10.1203/PDR.0b013e3181e5f57f

15. Frank L, Sosenko IRS. Prenatal development of lung antioxidant enzymes in four species. J Pediatr (1987) 110:106–10. doi:10.1016/S0022-3476(87)80300-1

16. Friel JK, Friesen RW, Harding SV, Roberts LJ. Evidence of oxidative stress in full-term healthy infants. Pediatr Res (2004) 56:878–82. doi:10.1203/01.PDR.0000146032.98120.43

17. Tiina MA, Kari OR, Mika S, Vuokko LK. Expression and development profile of antioxidant enzymes in human lung and liver. Am J Respir Cell Mol Biol (1998) 19:942–9. doi:10.1165/ajrccmb.19.6.3248
| Page | Reference                                                                                          |
|------|---------------------------------------------------------------------------------------------------|
| 39  | Hoffman DL, Brookes PS. Oxygen sensitivity of mitochondrial reactive oxygen species depends on metabolic conditions. *Biochem J* (2009) 284:16236–45. doi:10.1042/BJ20081386 |
| 38  | Lambe TH, Neish AS. New thinking on reactive oxygen: a double-edged sword revised. *Annu Rev Pathol* (2016) 11:57–89. doi:10.1146/annurev-pathol-032015-014049 |
| 37  | Life K. Role of reactive oxygen species in biological processes. *Klin Wochenschr* (2016) 114:1219–28. doi:10.1001/archophthalmol.2016.11833 |
| 36  | Londono J, Garcia-Sala F, Puertes IR, Gascó E, Sastre J, et al. Oxidative stress as a signal to up-regulate gamma-cystathionase in the fetal-to-neonatal transition in rats. *Cell Mol Biol (Noisy-le-grand)* (2007) 53(Suppl):OL1010–7. |
| 35  | Long E, Hornick K, Fritz KL, Mishra OP. Effects of hyperoxia on cortical neuronal nuclear function and programmed cell death mechanism. *Neurochem Res* (2007) 32:1142–9. doi:10.1007/s11064-007-9282-4 |
| 34  | Libel B, Fukuto JM, Miller T, Zhang H, Rinna A, Levy S, et al. The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. *Arch Biochem Biophys* (2008) 477:183–95. doi:10.1016/j.jbmb.2008.06.011 |
| 33  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 32  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 31  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 30  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 29  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 28  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 27  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 26  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 25  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 24  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 23  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 22  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 21  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 20  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 19  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 18  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 17  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 16  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 15  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 14  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 13  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 12  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 11  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 10  | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 9   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 8   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 7   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 6   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 5   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 4   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 3   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 2   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
| 1   | Libya S, Brandt U. Molecular mechanism of superoxide production by the mitochondrial respiratory chain. *Cell Mol Biol* (2002) 120:693–7. doi:10.1039/an9952000693 |
influenza A virus. *Am J Respir Crit Care Med* (2008) 177(10):1103–10. doi:10.1164/rccm.200712-1839OC
63. Ramani M, Bradley WE, Dell'Italia LJ, Ambalavanan N. Early exposure to hyperoxia or hypoxia adversely impacts cardiopulmonary development. *Am J Respir Cell Mol Biol* (2015) 52(5):594–602. doi:10.1165/rcmb.2013-0491OC
64. Ramani M, van Groen T, Kadish I, Bulger A, Ambalavanan N. Neuro-developmental impairment following neonatal hyperoxia in the mouse. *Neurobiol Dis* (2013) 50:69–75. doi:10.1016/j.nbd.2012.10.005
65. Chow LC, Wright KW, Sola A, CSMC Oxygen Administration Study Group. Can changes in clinical practice decrease the incidence of severe retinopathy of prematurity in very low birth weight infants? *Pediatrics* (2003) 111:339–45. doi:10.1542/peds.111.2.339
66. Tin W, Gupta S. Optimum oxygen therapy in preterm babies. *Arch Dis Child Fetal Neonatal Ed* (2007) 92:F143–7. doi:10.1136/adc.2005.092726
67. Sun SC. Relation of target SpO2 levels and clinical outcome in ELBW infants on supplemental oxygen. *Pediatr Res* (2002) 51:A350.
68. The STOP-ROP Multicenter Study Group. Supplemental therapeutic oxygen for prethreshold retinopathy of prematurity (STOP-ROP), a randomized, controlled trial. I: primary outcomes. *Pediatrics* (2000) 105(2):295–310. doi:10.1542/peds.105.2.295
69. Askie LM, Henderson-Smart DJ, Irwing L, Simpson JM. Oxygen-saturation targets and outcomes in extremely preterm infants. *N Engl J Med* (2003) 349(10):959–67. doi:10.1056/NEJMoa03080
70. Carlo WA, Finer NN, Walsh MC, Rich W, Gantz MG, Laptook AR, et al. Target ranges of oxygen saturation in extremely preterm infants. *N Engl J Med* (2010) 362(21):1959–69. doi:10.1056/NEJMoa0911781
71. BOOST II United Kingdom Collaborative Group, BOOST II Australia Collaborative Group, BOOST II New Zealand Collaborative Group, Stenson BJ, Tarnow-Mordi WO, Darlow BA, et al. Oxygen saturation and outcomes in preterm infants. *N Engl J Med* (2013) 368(22):2094–104. doi:10.1056/NEJMoa1302298
72. Schmidt B, Whyte RK, Asztalos EV, Moddemann D, Poets C, Rabì Y, et al. Effects of targeting higher vs lower arterial oxygen saturation on death or disability in extremely preterm infants: a randomized clinical trial. *JAMA* (2013) 309(20):2111–20. doi:10.1001/jama.2013.5555
73. Askie LM, Brocklehurst P, Darlow BA, Finer N, Schmidt B, Tarnow-Mordi W, et al. NeOProM: neonatal oxygenation prospective meta-analysis collaboration study protocol. *BMC Pediatr* (2011) 11(6):6. doi:10.1186/1471-2431-11-6
74. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R. European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants – 2013 update. *Neonatology* (2013) 103:353–68. doi:10.1159/000349928
75. Wolkoff IL, Narula P. Issue in neonatal and pediatric oxygen therapy. *Respir Care Clin N Am* (2000) 6:675–91. doi:10.1016/S1078-5337(05)70094-0
76. Castillo A, Sola A, Baquero H, Neira F, Alvis R, Deulofeu R, et al. Pulse oxygen saturation levels and arterial oxygen tension values in newborns receiving oxygen therapy in the neonatal intensive care unit: is 85% to 93% an acceptable range? *Pediatrics* (2008) 121(5):882–9. doi:10.1542/peds.2007-0117
77. Sola A, Golombek SG, Montes Bueno MT, Lemus-Varela L, Zaluaga C, Dominguez F, et al. Safe oxygen saturation targeting and monitoring in preterm infants: can we avoid hypoxia and hyperoxia? *Acta Paediatr* (2014) 103(10):1009–18. doi:10.1111/apa.12692
78. Bancalari E,Claure N. Control of oxygenation during mechanical ventilation in the premature infant. *Clin Perinatol* (2012) 39:563–72. doi:10.1016/j.clp.2012.06.013
79. Zapata J, Gomez JJ, Araque Campo R, Matiz Rubio A, Sola A. A randomised controlled trial of an automated oxygen delivery algorithm for preterm neonates receiving supplemental oxygen without mechanical ventilation. *Acta Paediatr* (2014) 103(9):928–33. doi:10.1111/apa.12684

Conflict of Interest Statement: The authors declare that there is no commercial or financial relationship that could be constructed as a potential conflict of interest.

Copyright © 2017 Perrone, Bracciali, Di Virgilio and Buonocore. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.