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Review article

Oxygen and oxidative stress in the perinatal period

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ABSTRACT

Fetal life evolves in a hypoxic environment. Changes in the oxygen content *in utero* caused by conditions such as pre-eclampsia or type I diabetes or by oxygen supplementation to the mother lead to increased free radical production and correlate with perinatal outcomes.

In the fetal-to-neonatal transition asphyxia is characterized by intermittent periods of hypoxia ischemia that may evolve to hypoxic ischemic encephalopathy associated with neurocognitive, motor, and neurosensorial impairment. Free radicals generated upon reoxygenation may notably increase brain damage. Hence, clinical trials have shown that the use of 100% oxygen given with positive pressure in the airways of the newborn infant during resuscitation causes more oxidative stress than using air, and increases mortality.

Preterm infants are endowed with an immature lung and antioxidant system. Clinical stabilization of preterm infants after birth frequently requires positive pressure ventilation with a gas admixture that contains oxygen to achieve a normal heart rate and arterial oxygen saturation. In randomized controlled trials the use high oxygen concentrations (90% to 100%) has caused more oxidative stress and clinical complications that the use of lower oxygen concentrations (30–60%). A correlation between the amount of oxygen received during resuscitation and the level of biomarkers of oxidative stress and clinical outcomes was established. Thus, based on clinical outcomes and analytical results of oxidative stress biomarkers relevant changes were introduced in the resuscitation policies. However, it should be underscored that analysis of oxidative stress biomarkers in biofluids has only been used in experimental and clinical research but not in clinical routine. The complexity of the technical procedures, lack of automation, and cost of these determinations have hindered the routine use of biomarkers in the clinical setting. Overcoming these technical and economical difficulties constitutes a challenge for the immediate future since accurate evaluation of oxidative stress would contribute to improve the quality of care of our neonatal patients.

1. Introduction

1.1. Aerobic metabolism and oxidative stress

Oxygen is the final acceptor of highly energized electrons generated at different metabolic processes being the most relevant the oxidases' activity (xanthine oxido-reductase; NADPH oxidase), nitric oxide synthase (NOS), and the mitochondrial oxidative phosphorylation process. Under physiologic conditions, a small percentage of the total oxygen metabolized during aerobic metabolism is incompletely reduced leading to the formation reactive oxygen species (ROS). The most common ROS in human biology result for the reduction of oxygen

Abbreviations: AA, arachidonic acid; AF, amniotic fluid; BPD, bronchopulmonary dysplasia; CAT, catalase; CS, cesarean section; DHA, docosahexanoic acid; DR, delivery room; EPO, erythropoietin; FiO_2, oxygen inspiratory fraction 0.21–1.0); GC-MS/MS, gas chromatography coupled to tandem mass spectrometry; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; HIF, hypoxia inducible factor; HPLC-MS/MS, high-performance liquid chromatography coupled to tandem mass spectrometry; IsoFs, Isofurans; IsoPs, Isoprostanes; IUGR, intrauterine growth retardation; kPa, kilopascal; NAC, N-acetyl-cysteine; NFκB, nuclear factor kappa B; NADPH, phosphorylated nicotine adenine dinucleotide; NOS, nitric oxide synthase; NeuroPs, Neuroprostanes; NeuroFs, Neurofurans; P_aO_2/p_aO_2, partial pressure of oxygen in arterial blood (mmHg); $PIVO_2$, intervillous (placenta) partial pressure of oxygen (mmHg); PGF, placental growth factor; ppO, partial pressure of oxygen (mmHg); RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; SpO_2, arterial oxygen saturation (expressed in %); VEGF, vascular endothelial growth factor; XD, xanthine dehydrogenase; XO, xanthine oxidase

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with just one electron to form superoxide anion ($\bullet O_2^-$). In addition, the reduction of oxygen with 2 or 3 electrons leads to the formation of hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), respectively. In addition, nitric oxide (NO•) may combine with oxygen free radicals especially anion superoxide to conform peroxynitrite ($ONOO^-$) a reactive nitrogen species (RNS). ROS and RNS have short half-lives and react with nearby molecules such as proteins, DNA, RNA, glucids or free fatty acids begetting them as free radicals and altering their structure and/or function. In the presence of transition metals especially iron (Fe^{+2}) generation of hydroxyl radical is highly enhanced (Fenton chemistry). However, ROS and especially hydrogen peroxide can also act as cell-signaling molecules and regulate cell redox processes. Oxidative stress is broadly referred to as an imbalance between the generation of ROS and RNS and their clearance by the antioxidant defense system and has been associated with placental oxidative disorders and immune disturbances and newborn conditions [1–3].

Human life *in utero* elapses in an environment that is relatively hypoxic as compared to the *ex utero*. However, oxygen availability is provided by exquisite adaptive mechanisms that allow for an extraordinary growth and development that exceeds any other period of life. Maternal conditions during pregnancy may cause fetal hypoxia. Chronic hypoxia is caused by vascular or metabolic alterations in the mother such as preeclampsia, obesity or diabetes. Hypoxic fetuses are at higher risk of developing oxidative and nitrosative stress that may be determining for their *in utero* development and postnatal development [4].

Acute hypoxia leading to asphyxia is characterized by profound acidosis, base deficit and lactacidemia. Given this situation resuscitation maneuvers immediately after birth are indispensable for patient's survival; however, mechanisms inherent to ischemia-reoxygenation will inevitably increase initial damage [5]. Therefore, interventions to avoid reoxygenation damage such as reducing the inspiratory fraction of oxygen (FiO_2) in the delivery room (DR) have been advocated for resuscitation of asphyctic neonates and for preterm infants with immature lungs, surfactant production and antioxidant defense system [6,7].

1.2. Biomarkers of oxidative stress and clinical application

Biomarkers could be defined as metabolites that can be objectively measured in biofluids (plasma, urine, spinal fluid, etc.) in the laboratory and accurately reflect either normal biological or pathological processes or response to pharmacologic interventions. In the clinical setting they acquire relevance when capable of assessing response to specifically tailored treatments or are able to predict short and/or long term outcomes [8]. Sensitivity and specificity define the accuracy of a biomarker to identify and quantify changes in biomolecules available in easily accessible biofluids (plasma, urine, amniotic fluid, etc.). Furthermore, the ideal biomarker should remain stable during storing, require small volume, preferably non-invasively obtained (urine), easily determined by automatized methods, reproducible, quantitatively expressed results, and preferably inexpensive [9]. The newborn infant is at high risk for oxygen free radical derived conditions [10]. Many of the most relevant pathologies associated with prematurity such as birth hypoxia, retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD) or intraventricular hemorrhage (IVH) have been associated with the use of oxygen supplementation and the immaturity of the antioxidant defense system in the postnatal period [11,12]. However, the newborn infant and especially the very preterm have unique physical characteristics that limit the accessibility to blood vessels and the volume of blood that can be drawn for analytical purposes. Moreover, ethical considerations recommend limiting painful procedures. Both gas chromatography and high performance liquid chromatography coupled to tandem mass spectrometry (GC-MS/MS, HPLC-MS/MS) have been widely used to obtain snapshots of the oxidant status in plasma or serum of the newborn infant [13]. However, more recently repeated urine analysis have allowed to non-invasively monitor changes over time of the oxidant status after specific therapeutic interventions [14,15].

Although highly reliable methods have been put forward enabling multi-analyte detection in different biofluids the use of biomarkers of oxidative stress in neonatology has been almost confined to clinical research. The complex methodology requiring sophisticated equipment and highly specialized technicians, elevated cost, delay in providing results, unavailability round the clock, and the difficulty in interpreting data from a clinical point of view has limited the use of oxidative stress biomarkers to experimental and clinical research. However, the results of oxidative stress biomarkers has been crucial to support changes in the guidelines of newborn resuscitation that now recommend the use of use of room air instead of pure oxygen in asphyxiated term infants [16]. Moreover, the amount of oxygen provided during the stabilization of preterm infants correlates with the level of plasma and/or urinary biomarkers of oxidative stress and with the development of BPD [15,17–19]. These findings have notably influence the restriction of oxygen supplementation in the delivery room in preterm infants [17]. Further clinical application of oxidative stress biomarkers relates to the studies of Winterbourne et al. that have found increased levels of glutathione sulfonamide (GSA) in bronchoalveolar lavage fluid in preterm infants who later developed lung infection or BPD [20–22]. Using a similar analytical methodology, our group found increased

Table 1
Analytical biomarkers used for the assessment of oxidative stress in clinical research in the perinatal period and most reliable techniques employed for its measurement.

Oxidative biomarkers	Target biomolecule	Modification	Biological sampling	Analytical method
Glutathione (GSH/GSSG ratio)	Antioxidants	General Redox Status	Total Blood	LC-MS/MS
o-Tyrosine (o-Tyr/Phe ratio)	Proteins		Urine	HPLC-MS/MS
m-Tyrosine (m-Tyr/Phe ratio)	Proteins		Urine	HPLC-MS/MS
3N2-Tyrosine	Proteins	Tyrosine nitration	Urine	HPLC-MS/MS
8OHdG (8OHdG/2dG ratio)	DNA		Urine/Plasma/Serum/CSF/AF	HPLC-MS/MS
F2-IsoPs	DNA	DNA oxidation	Urine/Plasma/Serum/CSF/AF	GC-MS/MS; HPLC-MS/MS
D2/F2-ISoPs	DNA	DNA oxidation	Urine/Plasma/Serum/CSF/AF	GC-MS/MS; HPLC-MS/MS
IsoFs	DNA	DNA oxidation	Urine/Plasma/Serum/CSF/AF	GC-MS/MS; HPLC-MS/MS
NeuPs	DNA	DHA Peroxidation	Urine/Plasma/CSF/AF	GC-MS/MS; HPLC-MS/MS
NeuFs	DNA	DHA Peroxidation	Urine/Plasma/CSF/AF	GC-MS/MS; HPLC-MS/MS

Abbreviations: GSH: reduced glutathione; GSSG: oxidized glutathione; o-Tyr: ortho-tyrosine; m-Tyr: meta-tyrosine; 3N2-Tyrosine: 3-nitrotyrosine; 8OHdG: 8-hydroxi-2′-deoxiguanosine; 2dG: 2′-deoxiguanosine; IsoPs: isoprostanes; IsoFs: isofurans; NeuPs: Neuroprostanes; NeuFs: neurofurans; AA: arachidonic acid; DHA: docosahexanoic acid; CSF: cerebral spinal fluid; AF: amniotic fluid; LC: liquid chromatography: GC: gas chromatography; MS/MS: tandem mass spectrometry.

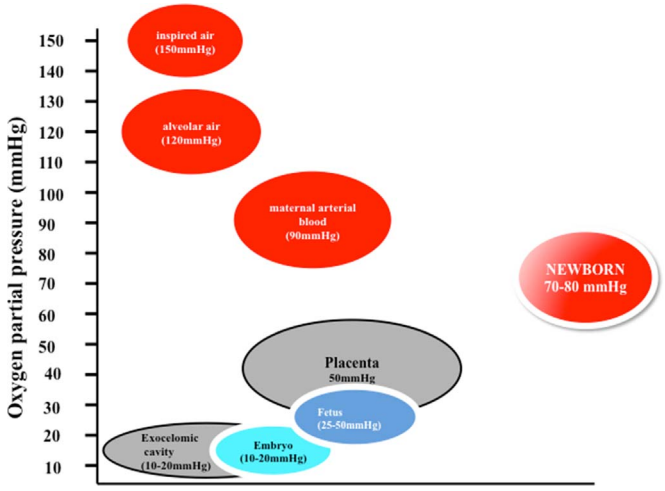

Fig. 1. Partial pressure of oxygen achieved by the embryo is strictly related to the oxygen content of inspired air, alveolar oxygen content, partial pressure of oxygen in the mother and placental fetal gas exchange. Changes at any of these stages will inevitably affect embryo/fetal/newborn oxygenation.

levels of 3-Chlor-tyrosine and GSA in ventilated preterm infants who developed ventilator-associated pneumonia [23,24]. Table 1 provides a summary of the most commonly employed biomarkers in neonatology.

The aim of the present review is to describe different perinatal circumstances leading to alterations of oxygenation and oxidative stress and to outline those biomarkers of oxidative stress that have evidenced clinical relevance.

1.3. The physiological hypoxic milieu in utero

The energy needed to sustain life in multicellular organism is provided by the aerobic combustion of nutritional metabolites in the mitochondrial oxidative phosphorylation system and accumulated as ATP [25]. However, in adult human tissues oxygen concentration lies substantially below conditions found in air. Hence, the range of oxygenation is between 25 mmHg and 55 mmHg partial pressure of oxygen (ppO) depending on the tissue and therefore it is known as physiological hypoxia. Oxygenation of the fetus is clearly dependent on the partial pressure gradients between maternal blood, placental tissue, fetal blood and fetal tissue (Fig. 1). The level of placental oxygen varies with gestation; thus, oxygen levels are extremely low in the first weeks after conception during embryonic development. Thus, before the 12th week of gestation, the intervillous partial pressure of oxygen ($PIVO_2$) has a median value around 18–20 mmHg, presumably to protect the embryo, which is highly sensitive to ROS [26]. Hypoxia in the embryonic phase triggers angiogenesis and is a prerequisite for the maintenance of stem cell pluripotency [27]. Notably, in the first trimester of pregnancy, embryonic stem cells depending on the oxygen provided by the placenta develop at around 10–15 mmHg ppO while in the endometrium levels are around 25 mmHg. Embryonic stem cells show more efficient growth and differentiation at lower oxygen pressures (10–15 mmHg) than at higher oxygen levels (25 mmHg) [25,28]. Prolonged hypoxia will stimulate angiogenesis through transcriptional and post-transcriptional regulation of growth factors such as vascular endothelial growth factors (VEGFs), erythropoietin (EPO), placental growth factor (PGF), and angiopoietins 1 and 1 [29]. The master regulator for the cell's adaptive responses to hypoxia is hypoxia inducible factor 1 (HIF-1), a heterodimeric transcription factor comprising HIF-1α and HIF-1β subunits. HIF-1α is stabilized when the concentrations of oxygen are below the specific critical oxygen threshold thus accumulating in the hypoxic cell. HIF-1β is constitutively present in the cell nucleus. Under low oxygen conditions it dimerizes with HIF-1α and binds to the hypoxia response elements in the regulatory region of a number of genes activating their transcription. Activated genes, and especially VEGF and EPO, enhance O_2 delivery to tissue [30,31]. Around the 14–16th week of gestation $PIVO_2$ steeply raises reaching stable values of 45–50 mmHg until the end of gestation. During fetal stage of development the rate of cell proliferation, division and vascularization occur at a slower rate coinciding with increased oxygenation [32,33].

P_aO_2 *in utero* is about 25–30 mmHg as compared to 80–90 mmHg in the mother. However, despite of this, adaptive mechanisms allow a similar oxygen delivery to tissue than provided after birth [34].

1.3.1. Oxidative stress at the maternal-fetal interface

Free radical generation is present in placenta and fetus from the beginning of pregnancy contributing to normal fetal development. At the end of the first trimester, physiologic oxidative stress prompts the regression of villi that were formed over the entire surface of the chorionic sac to leave the definitive discoid placenta. Placental detoxification activity protects the developing embryo from oxygen free radical mediated teratogenesis. However, at the end of the first trimester a three-fold rise in the oxygen concentration induced by placental maturation causes an exponential increase of ROS generation. Subsequently, activated apoptotic pathways in the peripheral villi finally will contribute to their regression. However, under stressful conditions oxidative stress will be a determinant factor leading to complications such as miscarriage, pre-eclampsia, intrauterine growth restriction (IUGR), and or premature rupture of membranes with the subsequent risk of preterm delivery and/or offspring infection [35].

1.3.2. Conditions associated with chronic fetal hypoxia and oxidative stress

Pre-eclampsia and type 1 diabetes are the two most frequent conditions that by different mechanisms lead to fetal hypoxia and neonatal morbidities and mortality. Pre-eclampsia is characterized by a low intermittent uterine blood flow to the placenta due to alterations of the lumen of spiral arteries [34,35]. Fluctuant perfusion causes a low-grade ischemia-reperfusion type of injury that provokes oxidative stress. ROS may cause pre-eclampsia by different mechanisms such as activating syncytiotrophoblast pro-apoptotic pathways during placentation thus impairing normal arteriolar remodeling, consolidate the inflammatory response, and/or alter the vascular endothelium response [35]. In a longitudinal clinical study analysis of plasma and urine biomarkers of oxidative stress and inflammation was sequentially performed along gestation. Hazard ratios of developing pre-eclampsia significantly correlated with increased urinary concentrations of 8-isoprostanes and plasma levels of c-reactive protein, and IL-1β, IL-6 and IL10 after the 18th week of gestation. Of note, while concentration of 8-isoprostanes consistently correlated with increased hazard ratios for pre-eclampsia levels of 8-OHdG did not [36]. Similarly, Longini et al. found increased F2-isoprostanes' levels in amniotic fluid that significantly correlated with pre-eclampsia and fetal growth restriction [37].

The precise mechanisms that cause fetal hypoxia in type 1 diabetic pregnancy have not been yet clearly established; however, epidemiological reports reveal that stillbirth and early neonatal mortality are significantly higher in diabetic pregnancies. Both hyperinsulinemia and hyperglycemia by independent mechanisms negatively influence fetal development resulting in fetal macrosomia and hypoxia. High glucose levels are accompanied by oxidative stress with alteration of antioxidant enzyme activities, impaired glutathione metabolism and decreased ascorbic acid levels [38]. Moreover, free radicals oxidize lipid membrane components and produce alterations of lipid bilayer of cell membranes altering their adaptability to capillary circulation, promoting platelet hyper-aggregability and shortening half-life especially in erythrocytes. These circumstances contribute to fetal hypoperfusion and growth retardation [38].

Fetal hypoxia has been assessed in the fetus determining EPO in

plasma and amniotic fluid (AF). EPO is not stored and does not cross the placenta. Thus, fetal plasmatic and AF EPO levels clearly reflect fetal synthesis and elimination and significantly correlate with the intensity and duration of hypoxia [39]. Both type 1 diabetes and insulin-treated gestational diabetes are characterized by hyperglycemia and oxidative stress, which consistently alter fetal metabolism even in euglycemic diabetic pregnancies [40]. Hyperglycemia causes an overproduction of advanced glycation end products and activates hexosamine biosynthesis. As a consequence, both NAD synthesis and rebuilding of GSH by GSH-reductase are reduced. Finally, activation of the polyol pathway, protein kinase C pathway, and oxidases activation also contribute to increased ROS production leading oxidative stress. Under a pro-oxidant status fetal NFκB, activator protein-1 and HIF-1α become activated and contribute to insulin resistance either acting directly acting upon insulin or indirectly negatively influencing insulin-signaling pathway [41,42]. In an observational study in pregnant women with type 1-diabetes or gestational diabetes treated with insulin EPO levels were determined in amniotic fluid (AF). Aliquots of AF were analyzed for DNA (8-OH-dG/2dG), meta-tyrosine/phenylalanine and nitro-tyrosine/phenylalanine ratios. A highly significant statistical correlation between these metabolite ratios and EPO in AF was established indicating that *in utero* hypoxia in insulin dependent diabetic pregnancies caused fetal oxidative stress and increased neonatal morbidities. [43].

1.3.3. Oxygen supplementation to the mother and effect upon the offspring

Oxygen has been supplemented to overcome high-risk situations during delivery in the mother and fetus. Oxygen supplementation to the mother during elective cesarean section (CS) under regional anesthesia increases oxygen transfer to the fetus but causes a simultaneous increase in lipid peroxidation byproducts such as isoprostanes both in the mother and fetus [44]. In a 2016 Cochrane review higher SpO_2 and p_aO_2 were obtained in pregnant women receiving supplementary oxygen during labor. In addition, offspring of mothers supplemented with oxygen had higher arterial and venous umbilical partial pressures of oxygen. Moreover, mothers receiving oxygen and their offspring had increased plasma lipid peroxidation byproducts. Of note, isoprostanes levels significantly correlated with maternal arterial partial pressure of oxygen (p_aO_2) [45]. Emergency CS is a different scenario where administration of oxygen to the mother could mitigate maternal brain de-oxygenation [46] and fetal hypoxia [47]. However, Thorp et al. reported a higher incidence of fetal acidosis when the mother was treated with supplementary oxygen for a longer period of time attributing this response to metabolic reactions associated with hypoxia-reoxygenation [48]. Nonetheless, Khaw et al. [49] studied the effect upon oxygenation and oxidative stress of the administration of a gas admixture containing 21% or 60% oxygen in pregnant women and offspring's submitted to emergency CS with regional anesthesia for sentinel signals of fetal compromise and didn't find any significant differences in mothers or newborn oxygenation status or in the levels of umbilical artery/vein total plasma free and esterified 8-isoprostanes as determined by GC-MS/MS. However, no clinical follow-up has been reported and information regarding any biological consequences upon the newborn infant is lacking.

1.4. Fetal to neonatal transition

Initiation of breathing immediately after birth triggers profound cardiorespiratory and metabolic changes. Pulmonary vascular resistance drops, intra-and-extra-cardiac shunting close and right ventricular output is redirected to the lungs where it gets oxygenated. PaO_2 rises abruptly from 40 to 50 mmHg to 70–80 mmHg in the first 5–10 min after birth [50]. Arterial oxygen saturation (SpO_2) reflects the percentage of hemoglobin that is saturated with oxygen. Under physiologic circumstances SpO_2 range oscillates between 95% and 100% in newborn infants. The SpO_2 ranges in normal newborn infants retrieved in the first minutes after birth show that term babies achieve oxygen saturations of 70%, 80%, 90% and 95% at 1, 3, 5, and 10 min after birth while preterm babies achieve oxygen saturations of 60%, 75%, 85% and 95% at 1, 3, 5 and 10 min after birth [51].

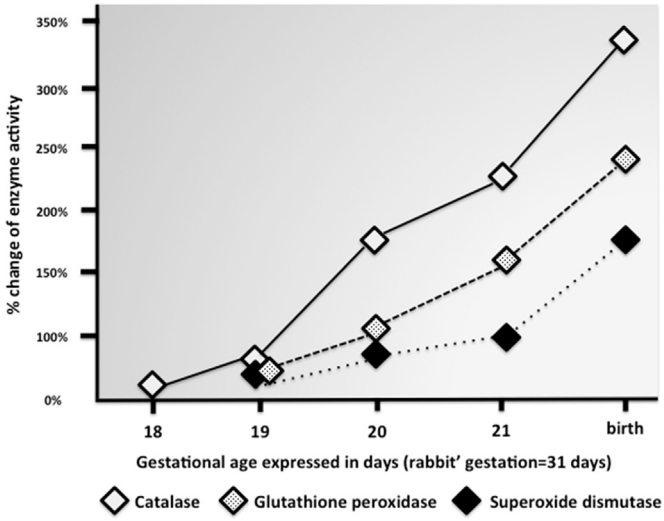

Fig. 2. Maturation of the antioxidant defense system occurs late in gestation in fetal lung coupled to lung surfactant adapting the respiratory and antioxidant system to face postnatal afflux of oxygen to tissue. The graph represents changes experimented by the antioxidant enzyme activity in fetal rabbits at the end of gestation. Modified from Frank et al. [84].

Healthy term newborn infants will usually require only positive pressure in the airways with air to achieve normal physiologic cardiorespiratory postnatal adaptation. However, preterm infants especially very preterm (< 32 weeks of gestation) will frequently need positive pressure in the airways using a gas admixture that may contain an oxygen concentration > 21% (air) to be successfully stabilized [7].

Postnatal increase in oxygen availability causes a burst of ROS and a physiologic oxidative stress. Remarkably, the expression of the antioxidant enzymes superoxide dismutases, catalase, and glutathione peroxidase changes dynamically during the last weeks of gestation preparing the fetus for lung respiration (Fig. 2). Similarly, availability of the most relevant non-enzymatic antioxidants such as reduced glutathione (GSH), thioredoxin (TRx), hem-oxygenases, vitamin C, E, β carotene, and transition metal chelators is not achieved until the end of gestation [11].

1.4.1. Perinatal asphyxia, newborn resuscitation and hypoxic ischemic encephalopathy (Fig. 3)

Perinatal asphyxia is a devastating disorder that affects roughly 2% of newborn babies in industrialized countries but constitutes one of the leading causes of early neonatal death in non-industrialized countries [52]. Ischemia and hypoxia reduce oxygen supply to neurons leading to ATP depletion and inactivation of ATP-dependent ion pumps causing both intracellular accumulation of Na^+(cell swelling), Ca^{2+}(increased free radical formation) and excitotoxicity (inhibition of neurotransmitters' recaptation at the synaptic cleft) that cause cell death [53]. Reperfusion injury, first described 4 decades ago, is a paradoxical tissue response that is manifested by blood perfusion-deprived and oxygen depleted organs following restoration of blood flow and oxygenation, and is caused by an excess of production of oxygen free radicals [54,55]. Upon resuscitation in asphyctic patients, reperfusion, restoring oxygen and glucose delivery to tissue may salvage neurons but will itself cause additional apoptotic brain damage amplifying the initial damaged areas of the brain. One of the most relevant sources of free radicals that enhance cellular damage upon reperfusion is related to the conversion of xanthine dehydrogenase (XD) to xanthine oxidase

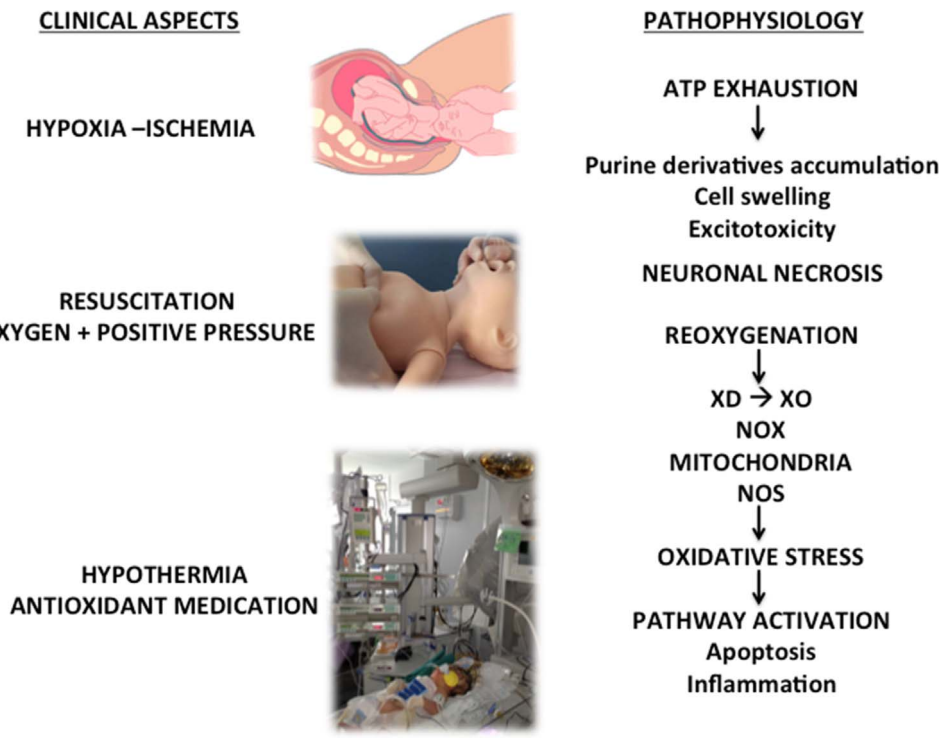

Fig. 3. Certain clinical conditions *in utero* cause hypoxia or hyperoxia in the fetus or during fetal to neonatal transition. Recovery of normality through resuscitation/reperfusion is going to generate a burst of oxygen free radicals by activation of oxidases, NO synthase and OXPHOS. Oxidative stress will induce pro-apoptotic and pro-inflammatory cascades thus contributing to the amplification of the initial damage. Abbreviations: XD=xanthine dehydrogenase; XO=xanthine oxidase; NOX=NADPH oxidases; NOS=nitric oxide synthase.

(XO). XD is unable to transfer electrons to molecular oxygen, although it uses NADPH as electron acceptor. The conversion of XD to XO involves either the oxidation of the thiol groups (R-SH→R-S-S-R) or proteolysis by activated proteases as an adaptive mechanism for Ca^{2+} transport in energy-deficient cells. In addition, ATP is converted into hypoxanthine a purine substrate for XO. Upon re-oxygenation XO converts hypoxanthine to uric acid and molecular oxygen to superoxide and hydrogen peroxide. In addition, transition metals (e.g., iron) precipitate the generation of hydroxyl radical via Fenton chemistry thus increasing exponentially oxidative damage [5].

In experimental studies Saugstad et al. showed that hypoxanthine accumulation in different animal models of hypoxia directly depended on the duration and intensity of hypoxia [56–58]. Remarkably, free radical production directly correlated with the amount of oxygen provided upon reoxygenation [59]. Urinary samples were analyzed for oxidative damage to DNA (8-hydroxy-2′-deoxyguanosine/2′deoxyguanosine ratio) and proteins (ortho-tyrosine/phenylalanine ratio) by HPLC-MS/MS. Analytical results showed that urinary levels of both biomarkers were significantly higher in piglets rescued with higher FiO_2 [59]. Additional experimental studies showed that reoxygenation with higher oxygen concentrations also induced dose-dependent increase in matrix metalloproteinase gelatinase activity and down-regulated the expression of VEGFR2 and TGFBR3 in liver [60]. Moreover, concentration of isoprostanes, isofurans, neuroprostanes, and neurofurans in brain tissue determined by GC-MS/MS were significantly increased in hypoxic piglets and clearly correlated with the amount of oxygen provided during reoxygenation [61]. Cheung et al. administered intravenous N-acetyl-cysteine (NAC) a well-known antioxidant to hypoxic piglets upon reoxygenation with 100% oxygen. NAC improved cardiac index, stroke volume and systemic oxygen delivery. In addition, changes in cardiac index directly correlated with tissue levels of myocardial lipid hydroperoxides, caspase-3 and lactate. The administration of NAC reduced myocardial oxidative stress and improved cardiac performance [62].

In a pilot study in severely asphyxiated newborn infants metabolomic analysis showed a significant increase of 8-iso-15 (R)-PGF2α that significantly correlated with the clinical and biochemical severity of the patients [63].

Based on a body of experimental evidence two decades ago Saugstad et al. hypothesized that the use of room air would be suitable for the resuscitation of asphyctic neonates and reduce damage caused by re-oxygenation using 100% oxygen. In two clinical studies they showed that the use of air was feasible for resuscitation of term infants, and it restored spontaneous respiration more rapidly, and showed a tendency towards reduction in mortality [64,65]. In a series of randomized control blinded studies, Vento et al. [66–69], showed that reduced-to-oxidized glutathione ratio (GSH/GSSG) was significantly diminished in asphyxiated babies receiving pure oxygen as compared to air. Intriguingly, GSH/GSSG ratio was significantly lower in babies receiving pure oxygen at four weeks after birth [66]. The use of 100% oxygen caused hyperoxemia, increased the activities of antioxidant enzymes (SOD, CAT, GPx), glutathione redox cycle enzymes (GSH-reductase, GSH-S-transferase). Furthermore, GSH/GSSG ratio and activities of the antioxidant enzymes correlated with p_aO_2 [67,68]. Finally, it was shown that plasma troponins and urinary levels of N-acetyl-glucosaminidase correlated with GSSG levels and were significantly higher in the pure oxygen group [69]. In 2008 a meta-analysis that included a total of 1082 newborn babies resuscitated with 21% and 1051 with 100% oxygen was published [55]. Neonatal mortality was significantly reduced in the 21% group compared to the 100% oxygen group [16]. In 2010 international guidelines for newborn resuscitation adopted air as the initial gas for newborn resuscitation [17]. Recently, clinical trials using antioxidant strategies such as XO inhibitors have been launched [70].

1.4.2. Postnatal stabilization of very preterm infants with oxygen

Premature birth interrupts lung, antioxidant and immune system maturation predisposing preterm infants to acute morbidities during the neonatal period and chronic impairment thereafter [71]. Supplementation with oxygen upon stabilization in the delivery room

is a prevalent intervention that may cause oxidative stress [72]. The percentage of oxygen in the gas admixture that is given to newborn infants is known as the oxygen inspiratory fraction (FiO_2) and ranges go from 0.21 (air) to 1.0 (pure oxygen). FiO_2 can be adjusted according to the response of the patients' heart rate and oxygen saturation. Tataranno et al., found a significant increase in plasma levels of advanced oxidative protein products and isoprostanes 12 h after birth in preterm babies receiving 100% oxygen [73]. Similarly, Vento et al. [18] compared resuscitation of extremely preterm infants randomly assigned to receive positive pressure ventilation of the airways with a gas admixture that contained a lower oxygen concentration of 30% (LowOx) vs. a higher oxygen concentration of 90% (HiOx). Neonates in the HiOx group exhibited significantly higher GSSG/GSH ratio in whole blood at days 1 and 3 and increased oxidative damage to lipids, proteins, and DNA measured by GC-MS/MS in urine. Moreover, patients with higher levels of oxidative stress had a significantly increase incidence of bronchopulmonary dysplasia (BPD) [18]. Ezaki et al. [74], also analyzed the correlation between FiO_2 and markers of oxidative stress and anti-oxidant capacity in preterm babies < 35 weeks gestation. Infants who received pure oxygen had significantly higher total peroxides and lower redox potential/total hydroperoxides ratios than neonates who received lower oxygen loads. Finally, Kapadia et al. [19], compared oxidative stress biomarkers in extremely preterm infants stabilized initially either with 100% oxygen or air. Total hydroperoxides (TH), biological antioxidant potential (BAP) and TH/BAP quotient were determined in cord blood and one hour after birth. At one hour BAP/TH was significantly higher in preterm infants ventilated initially with air. In addition, these babies needed less days of mechanical ventilation and had a lower incidence of bronchopulmonary dysplasia [19]. Remarkably, when the difference in the initial FiO_2 provided to preterm infants for stabilization in the DR was smaller (e.g. 0.3 vs. 0.6) no significant differences in oxidative stress biomarkers were found. Hence, in two randomized controlled trials performed by Aguar et al. [75] and Rook et al. [76] preterm babies were randomly assigned to be blindly resuscitated with initial FiO_2=0.3 vs. 0.6. GSH/GSSG ratio in total blood, and urinary 8-OHdG/2dG, Ortho-tyrosine/Phenylalanine ratios, and 3-Cl-Tyrosine, 3-N-Tyrosine, and non-protein bound iron values did not meet statistical significance. The capability of preterm infants to tolerate oxygen is limited even for brief periods of time. Oxidative stress is associated with relevant long-term conditions. Therefore, cautious supplementation with oxygen is recommended in the most recent resuscitation guidelines [17,77].

1.4.3. Lipid peroxidation biomarkers' nomogram in preterm infants

Prostaglandin-like derivatives such as isoprostanes (IsoPs), neuroprostanes (NeuroPs), isofurans (IsoFs) and neurofurans (NeuroFs) are the result of free radical interaction with arachidonic (AA) and docosahexanoic (DHA) acids respectively [78]. With the introduction of highly sensitive analytical techniques such as GC-MS/MS or UPLC-MS/MS accurately and reproducible measurements have been feasible (Table 1). At present these metabolites constitute one of the most reliable markers of *in* vivo free-radical induced oxidative stress. Plasma and tissue analysis have shown that these metabolites have the highest tissue and plasma concentrations during fetal and early neonatal life when they may play an important biological role and act as regulators of pulmonary vascular tone especially during the fetal-to-neonatal transition. [79]. Notably, IsoPs are highly reliable biomarkers of oxidative stress in newborn infants under normoxic conditions. However, hyperoxia limits the formation of IsoPs. Therefore IsoPs are poor prognostic predictive biomarkers for the development of oxygen-derived chronic lung disease in preterm infants [80]. On the contrary, formation of IsoFs becomes favored at higher oxygen tensions rendering these biomarkers especially relevant for clinical research in neonatology and predictive of BPD development [81] (Fig. 4). NeuroPs and NeuroFs are byproducts of DHA. DHA is an essential structural component of brain and therefore NeuroPs and NeuroFs are relevant

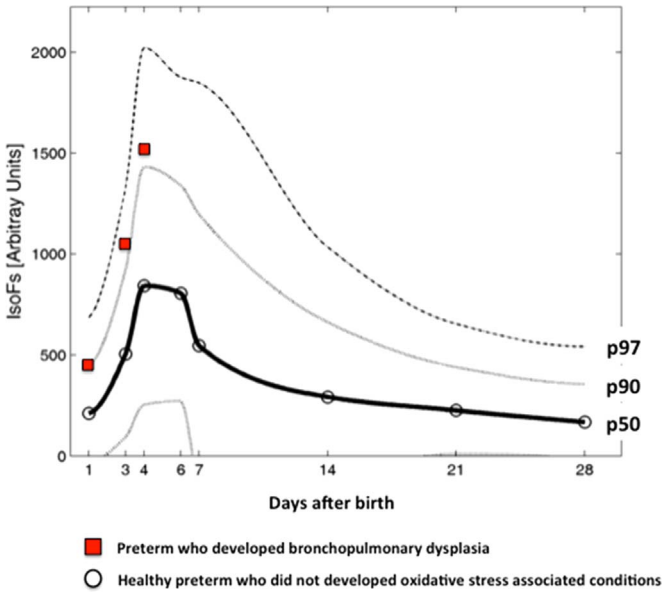

Fig. 4. Isofurans urinary levels are represented in the Y-axis and days after birth in the X-axis. The graph depicts mean (darker line) and standard deviations of the mean (dotted lines) for urinary isofurans (IsoFs) in healthy preterm infants < 32 weeks' gestation without oxidative stress-associated conditions (Refs. [75,76]). Preterm infants who developed bronchopulmonary dysplasia (red squares) exhibited significantly higher values for urinary IsoFs in the first 4 days after birth. Expressed in Intensity of Signal Arbitrary Units. Modified from Kuligowski et al. [15].

markers of brain damage under normoxic and hyperoxic conditions respectively [82]. The use AA and DHA peroxidation byproducts entail many difficulties because of the large number of metabolites, including isomers with highly similar molecular structures, physicochemical properties, and chromatographic behavior. Recently, using newly developed UPLC-MS/MS methods Kuligowski et al. [14,15], analyzed a total of 536 urine samples during the first 4 weeks of life in 184 preterm infants who did not develop any free-radical associated conditions during the neonatal period [75,76] and established a reference range for AA and DHA peroxidation byproducts (Table 2). The access to a large sample set of this especially vulnerable population has allowed establish a time frame during which lipid peroxidation byproducts could be quantified and compared to normality ranges [76].

2. Conclusions

An adequate availability of oxygen during the embryonic, fetal and postnatal periods is an essential prerequisite for normal metabolism, growth and development. Generation of ROS due to hypoxia or hyperoxia, inflammation or infection causes oxidative stress and alterations of cell structure and function. Research in the prenatal and postnatal period has underscored the role oxidative stress as causative agent of relevant perinatal conditions that will influence short and long-term development.

Despite the relevant findings in experimental research translation of perinatal oxidative stress into clinical research has been obscured by the lack of reliability and consistence of biomarkers and of lack of statistical power in most of the pilot studies. The recent incorporation of more precise, reliable, and reproducible analytical methods such as mass spectrometry coupled to gas chromatography or ultra-performance liquid chromatography, magnetic resonance or capillary electrophoresis has not overcome the previous difficulties, and its use is limited to research in highly supervised small clinical trials. The most relevant hindrance to the popularization of these techniques resides fundamentally in the complexity, cost and difficulty in driving of the laboratory equipment only available in academic setting with research facilities. Hopefully, the development of new machines with greater

Table 2
Analytical results for Isoprostanes (IsoPs), Isofurans (IsoFs), Neuroprostanes (NeuroPs) and Neurofurans (NeuroFs) determined in samples of urine of preterm infants ≤32 weeks' gestation without free radical associated conditions (Control) during the first 4 weeks after birth. Results are compared with preterm infants who developed bronchopulmonary dysplasia (BPD). Significant differences have been found for ISOFURANS (IsoFs) in the first 4 days after birth.

	Postnatal day 1		Postnatal day 3		Postnatal day 4		Postnatal day 7		Postnatal day 14		Postnatal day 21		Postnatal day 28	
	Control (95)	BPD (22)	Control (126)	BPD (24)	Control (93)	BPD (25)	Control (19)	BPD (18)	Control (33)	BPD (23)	Control (26)	BPD (15)	Control (48)	BPD (28)
IsoPs	494 (926)	534 (354)	1016 (1391)	955 (567)	1201 (1370)	1339 (1012)	154 (208)	194 (155)	81 (85)	102 (66)	75 (53)	96 (77)	152 (402)	177 (234)
IsoFs	211 (239)	445 (188)**	507 (413)	788 (374)**	844 (590)	1233 (667)*	547 (651)	612 (548)	292 (372)	334 (185)	225 (215)	198 (155)	167 (188)	198 (219)
NeuroPs	37 (61)	46 (51)	32 (34)	58 (42)	37 (65)	58 (44)	19 (18)	25 (14)	166 (291)	192 (144)	332 (356)	379 (224)	213 (290)	254 (310)
NeuroFs	14 (26)	20 (18)	24 (26)	39 (18)	28 (21)	38 (31)	44 (38)	54 (44)	39 (39)	51 (44)	50 (48)	43 (38)	23 (28)	34 (19)

Analyses were performed using UPLC-MS/MS.
Results are expressed as intensity of signal units/mL of urine, and expressed as mean (standard deviation). Modified from Kuligowski et al. [15]. Significance: *P < 0.05; **P < 0.01

clinical applicability and new techniques such as surface enhanced Raman spectroscopy (SERS) can render excellent results at the cot side and may perhaps in the coming future allow to perform serial determinations of oxidative stress biomarkers with very little volume of sample and immediate results [83].

Providing the tools for a prompt and accurate diagnosis of oxidative stress and response to treatment will undoubtedly contribute to improving the diagnosis and outcome of newborn patients.

Conflicts of interest

The authors of this manuscript declare no conflicts of interest or disclosures.

Authorship

All the authors contributed to updating and critically reviewing the literature, writing the manuscript and supervision and approval of the final version.

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References

[1] B. Kalyanaraman, Teaching the basis of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms, Redox Biol. (2013) 244–257.
[2] E. Maltepe, O.D. Saugstad, Oxygen in health and disease: regulation of oxygen homeostasis–clinical implications, Pedia. Res. 65 (2009) 261–268.
[3] F. Wu, F.J. Tian, Y. Lin, Oxidative stress in placenta: health and diseases, Biomed. Res. Int. 2015 (2015) 293271.
[4] M. Vento, K. Teramo, Evaluating the fetus at risk for cardiopulmonary compromise, Semin. Fetal Neonatal Med. 18 (2013) 324–329.
[5] D.N. Granger, P.R. Kvietys, Reperfusion injury and reactive oxygen species: the evolution of a concept, Redox Biol. 6 (2015) 524–551.
[6] M. Vento, Oxygen in the neonatal period: changing the paradigm, Neonatology 105 (2014) 323–331.
[7] I. Torres-Cuevas, M. Cernada, A. Nuñez, J. Escobar, J. Kuligowski, C. Chafer-Pericas, M. Vento, Oxygen supplementation to stabilize preterm infants in the fetal to neonatal transition: no satisfactory answer, Front. Pedia. 4 (2016) 29.
[8] M. Mussap, A. Noto, F. Cibecchini, V. Fanos, The importance of biomarkers in neonatology, Semin Fetal Neonatal Med. 18 (2013) 56–64.
[9] Biomarker Definitions Working Group, Biomarker and surrogate endpoints: preferred definitions and conceptual framework, Clin. Pharm. Ther. 69 (2001) 89e95.
[10] O.D. Saugstad, Y. Sejersted, R. Solberg, E.J. Wollen, M. Bjørås, Oxygenation of the newborn: a molecular approach, Neonatology 101 (2012) 315–325.
[11] M. Vento, H. Hummler, J.A. Dawson, J. Escobar, J. Kuligowski, Use of oxygen in the resuscitation of neonates, in: P.A. Dennery, G. Buonocore, O.D. Saugstad (Eds.), Perinatal and Prenatal Disorders1st edition, Springer Science+Business Media, New York, 2014, pp. 213–244.
[12] S. Perrone, C. Bracciali, N. Di Virgilio, G. Buonocore, Oxygen use in neonatal care: a two-edged sword, Front Pediatr. 4 (2017) 143.
[13] M.L. Tataranno, S. Perrone, G. Buonocore, Plasma biomarkers of oxidative stress in neonatal brain injury, Clin. Perinatol. 42 (2015) 529–539.
[14] J. Kuligowski, J. Escobar, G. Quintás, I. Lliso, I. Torres-Cuevas, A. Nuñez, E. Cubells, D. Rook, J.B. van Goudoever, M. Vento, Analysis of lipid peroxidation biomarkers in extremely low gestational age neonate urines by UPLC-MS/MS, Anal. Bioanal. Chem. 406 (2014) 4345–4356.
[15] J. Kuligowski, M. Aguar, D. Rook, I. Lliso, I. Torres-Cuevas, J. Escobar, G. Quintás, M. Brugada, A. Sánchez-Illana, J.B. van Goudoever, M. Vento, Urinary lipid peroxidation byproducts: are they relevant for predicting neonatal morbidity in preterm infants*, Antioxid. Redox Signal 23 (2015) 178–184.
[16] O.D. Saugstad, S. Ramji, R.F. Soll, M. Vento, Resuscitation of newborn infants with 21% or 100% oxygen: an updated systematic review and meta-analysis, Neonatology 94 (2008) 176–182.
[17] J.M. Perlman, J. Wyllie, J. Kattwinkel, D.L. Atkins, L. Chameides, J.P. Goldsmith, R. Guinsburg, M.F. Hazinski, C. Morley, S. Richmond, W.M. Simon, N. Singhal, E. Szyld, M. Tamura, S. Velaphi, Neonatal resuscitation chapter collaborators. Part 11: neonatal resuscitation: 2010 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations, Circulation 122 (suppl 2) (2010) S516–S538.
[18] M. Vento, M. Moro, R. Escrig, L. Arruza, G. Villar, I. Izquierdo, L.J. Roberts 2nd, A. Arduini, J.J. Escobar, J. Sastre, M.A. Asensi, Preterm resuscitation with low oxygen causes less oxidative stress, inflammation, and chronic lung disease, Pediatrics 124 (2009) e439–e449.
[19] V.S. Kapadia, L.F. Chalak, J.E. Sparks, J.R. Allen, R.C. Savani, M.H. Wyckoff, Resuscitation of preterm neonates with limited versus high oxygen strategy, Pediatrics 132 (2013) e1488–e1496.
[20] I.H. Buss, B.A. Darlow, C.C. Winterbourn, Elevated protein carbonyls and lipid peroxidation products correlating with myeloperoxidase in tracheal aspirates from premature infants, Pedia. Res. 47 (5) (2000) 640–645.
[21] F.C. Cheah, B.A. Darlow, C.C. Winterbourn, Association of hydrogen peroxide in exhaled breath condensates from infants with respiratory distress syndrome with the development of chronic lung disease, Arch. Dis. Child Fetal Neonatal Ed. 91 (2006) F155.
[22] D.T. Harold, B.A. Darlow, F.C. Cheah, N. McNeill, P. Graham, C.C. Winterbourn, Biomarkers of neutrophil-mediated glutathione and protein oxidation in tracheal aspirates from preterm infants: association with bacterial infection, Pedia. Res. 69 (2011) 28–33.
[23] J. Kuligowski, M. Cernada, J. Escobar, M. Brugada, E. Cubells, A. Nunez, M. Aguar, M. Vento, Glutathione sulfonamide as an early predictor of lung inflammation in the neonatal period, EPAS 2942 (2014) 572.
[24] M. Cernada, M. Brugada, S. Golombek, M. Vento, Ventilator-associated pneumonia in neonatal patients: an update, Neonatology 105 (2014) 98–107.
[25] F.Q.1 Schafer, G.R. Buettner, Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple, Free Radic. Biol. Med. 30 (2001) 1191–1212.
[26] M.C. Simon, B. Keith, The role of oxygen availability in embryonic development and stem cell function, Nat. Rev. Mol. Cell Bio 9 (4) (2008) 285–296.
[27] K. Stamati, V. Mudera, U. Cheema, Evolution of oxygen utilization in multicellular organisms and implications for cell signalling in tissue engineering, J. Tissue Eng. 2

(2011) (2041731411432365).
[28] Q. Lin, Y. Kim, R. Alarcon, Z. Yun, Oxygen and cell fate decisions, Gene Regul. Syst. Biol. 2 (2008) 43–51.
[29] G.J. Burton, Oxygen, the Janus gas; its effects on human placental development and function, J. Anat. 15 (2009) 27–35.
[30] G.L. Semenza, Hypoxia-inducible factors in physiology and medicine, Cell 148 (2012) 399–408.
[31] M.E. Hubbi, G.L. Semenza, Regulation of cell proliferation by hypoxia-inducible factors, Am. J. Physiol. Cell Physiol. 309 (2015) C775–C782.
[32] C.B. Martin, Normal fetal physiology and behavior, and adaptive responses with hypoxemia, Semin Perinatol. 32 (2008) 239–242.
[33] H. Schneider, Oxygenation of the placental-fetal unit in humans, Respir. Physiol. Neurobiol. 178 (2011) 51–58.
[34] P.J. Murphy, The fetal circulation, Cont. Educ. Anaesth. Crit. Care Pain. 5 (2005) 107–112.
[35] G.J. Burton, Oxidative Stress, Best. Pr. Res Clin. Obstet. Gynecol. 25 (2011) 287–299.
[36] K.K. Ferguson, J.D. Meeker, T.F. McElrath, B. Mukherjee, D.E. Cantonwine, Repeated measures of inflammation and oxidative stress biomarkers in preeclamptic and normotensive pregnancies, Am. J. Obstet. Gynecol. (2016). http://dx.doi.org/10.1016/j.ajog.2016.12.174 (Epub ahead of print).
[37] M. Longini, S. Perrone, A. Kenanidis, P. Vezzosi, B. Marzocchi, F. Petraglia, G. Centini, G. Buonocore, Isoprostanes in amniotic fluid: a predictive marker for fetal growth restriction in pregnancy, Free Radic. Biol. Med. 38 (2005) 1537–1541.
[38] A. Yessoufou, K. Moutairou, Maternal diabetes in pregnancy: early and long-term outcomes of offspring and the concept of “metabolic memory”, Exp. Diabetes Res 2011 (2011) 218598.
[39] K.A. Teramo, Obstetric problems in diabetic pregnancy- The role of fetal hypoxia, Best. Pr. Res Clin. Endocrinol. Metab. 24 (2010) 663–671.
[40] E. Taricco, T. Radaelli, G. Rossi, M.S. Nobile de Santis, G.P. Bulfamante, L. Avagliano, I. Cetin, Effects of gestational diabetes on fetal oxygen and glucose levels in vivo, BJOG 116 (2009) 1729–1735.
[41] R.A. Simmons, Developmental origins of diabetes: the role of oxidative stress, Free Radic. Biol. Med. (2006) 917–922.
[42] M. Lappas, U. Hiden, G. Desoye, J. Froehlich, S. Hauguel-de Mouzon, A. Jawerbaum, The role of oxidative stress in the pathophysiology of gestational diabetes, Antioxid. Redox Signal 15 (2011) 3061–3100.
[43] J. Escobar, K. Teramo, V. Stefanovic, S. Andersson, M.A. Asensi, A. Arduini, E. Cubells, J. Sastre, M. Vento, Amniotic fluid oxidative and nitrosative stress biomarkers correlate with fetal chronic hypoxia in diabetic pregnancies, Neonatology 103 (2013) 193–198.
[44] K.S. Khaw, C.C. Wang, W.D.N. Kee, C.P. Pang, M.S. Rogers, Effects of high inspired oxygen fraction during elective Caesarean section under spinal anaesthesia on maternal and fetal oxygenation and lipid peroxidation, Brit J. Anaesth. 88 (2002) 18–23.
[45] S. Chatmongkolchart, S. Prathep, Supplemental oxygen for caesarean section during regional anaesthesia, Cochrane Database Syst. Rev. 3 (2016) CD006161.
[46] N. Hirose, Y. Kondo, T. Maeda, T. Suzuki, A. Yoshino, Y. Katayama, Oxygen Supplementation is effective in attenuating maternal cerebral blood de-oxygenation after spinal anesthesia for cesarean section, Adv. Exp. Med Biol. 876 (2016) 471–477.
[47] K.S. Khaw, C.C. Wang, W.D.N. Kee, W.H. Tam, F.F. Ng, L.A.H. Critchley, M.S. Rogers, Supplementary oxygen for emergency Caesarean section under regional anaesthesia, Brit J. Anaesth. 102 (2009) 90–96.
[48] J.A. Thorp, T. Trobough, R. Evans, J. Hedrick, J.D. Yeats, The effect of maternal oxygen administration during the second stage of labor on umbilical cord gas values: a randomized controlled prospective trial, Am. J. Obstet. Gynecol. 172 (1995) 465–474.
[49] K.S. Khaw, W.D.N. Kee, C.Y. Chu, F.F. Ng, W.H. Tam, L.A.H. Critchley, M.S. Rogers, C.C. Wang, Effects of different inspired oxygen fractions on lipid peroxidation during general anaesthesia for elective Caesarean section, Brit J. Anaesth. 105 (2010) 355–360.
[50] Y. Gao, J.U. Raj, Regulation of the pulmonary circulation in the fetus and newborn, Physiol. Rev. 90 (2010) 1291–2010.
[51] J.A. Dawson, C.O.F. Kamlin, M. Vento, C. Wong, T.J. Cole, S.M. Donath, P.G. Davis, C.J. Morley, Defining the reference range for oxygen saturation for infants after birth, Pediatrics 125 (2010) e1340–e1347.
[52] L. Lehtonen, A. Gimeno, A. Parra-Llorca, M. Vento, Early neonatal death: a challenge worldwide, Semin Fetal Neonatal Med (2017) *in press*.
[53] M. Douglas-Escobar, M.D. Weiss, Hypoxic-ischemic encephalopathy: a review for the clinician, JAMA Pediatr. 169 (2015) 397–403.
[54] J.M. McCord, Oxygen-derived free radicals in post-ischemic tissue injury, N. Engl. J. Med. 312 (1985) 159–163.
[55] M. Odeh, The role of reperfusion-induced injury in the pathogenesis of the crush syndrome, N. Engl. J. Med. 324 (1991) 1417–1422.
[56] O.D. Saugstad, Hypoxanthine as a measurement of hypoxia, Pedia. Res. 9 (1975) 158–161.
[57] A.O. Aasen, O.D. Saugstad, Hypoxanthine in lethal canine endotoxic shock, Circ. Shock 6 (1979) 277–283.
[58] O.D. Saugstad, A.O. Aasen, Plasma hypoxanthine concentrations in pigs. A prognostic aid in hypoxia, Eur. Surg. Res. 12 (1980) 123–129.
[59] R. Solberg, J.H. Andresen, R. Escrig, M. Vento, O.D. Saugstad, Resuscitation of hypoxic newborn piglets with oxygen induces a dose-dependent increase in markers of oxidation, Pedia. Res. 62 (2007) 559–563.
[60] R. Solberg, J.H. Andresen, S. Pettersen, M.S. Wright, B.H. Munkeby, E. Charrat, M. Khrestchatisky, S. Rivera, O.D. Saugstad, Resuscitation of hypoxic newborn piglets with supplementary oxygen induces dose-dependent increase in matrix metallo-proteinase-activity and down-regulates vital genes, Pedia. Res. 67 (2010) 250–256.
[61] R. Solberg, M. Longini, F. Proietti, P. Vezzosi, O.D. Saugstad, G. Buonocore, Resuscitation with supplementary oxygen induces oxidative injury in the cerebral cortex, Free Radic. Biol. Med. 53 (2012) 1061–1067.
[62] J.Q.1 Liu, T.F. Lee, D.L. Bigam, P.Y. Cheung, Effects of post-resuscitation treatment with N-acetylcysteine on cardiac recovery in hypoxic newborn piglets, PLoS One 5 (2010) e15322.
[63] C. Chafer-Pericas, M. Cernada, L. Rahkonen, V. Stefanovic, S. Andersson, M. Vento, Preliminary case control study to establish the correlation between novel peroxidation biomarkers in cord serum and the severity of hypoxic ischemic encephalopathy, Free Radic. Biol. Med. 97 (2016) 244–249.
[64] S. Ramji, S. Ahuja, S. Thirupuram, T. Rootwelt, G. Rooth, O.D. Saugstad, Resuscitation of asphyxic newborn infants with room air or 100% oxygen, Pedia. Res. 34 (1993) 809–812.
[65] O.D. Saugstad, T. Rootwelt, O. Aalen, Resuscitation of asphyxiated newborn infants with room air or oxygen: an international controlled trial: the Resair 2 study, Pediatrics 102 (1998) e1.
[66] M. Vento, M. Asensi, J. Sastre, F. García-Sala, F.V. Pallardó, J. Viña, Resuscitation with room air instead of 100% oxygen prevents oxidative stress in moderately asphyxiated term neonates, Pediatrics 107 (2001) 642–647.
[67] M. Vento, M. Asensi, J. Sastre, A. Lloret, F. García-Sala, J.B. Miñana, J. Viña, Hyperoxemia caused by resuscitation with pure oxygen may alter intracellular redox status by increasing oxidized glutathione in asphyxiated newly born infants, Semin Perinatol. 26 (2002) 406–410.
[68] M. Vento, M. Asensi, J. Sastre, A. Lloret, F. García-Sala, J. Viña, Oxidative stress in asphyxiated term infants resuscitated with 100% oxygen, J. Pediatr. 142 (2003) 240–246.
[69] M. Vento, J. Sastre, M.A. Asensi, J. Viña, Room-air resuscitation causes less damage to heart and kidney than 100% oxygen, Am. J. Respir. Crit. Care Med. 172 (2005) 1393–1398.
[70] B.J. Dixon, C. Reis, W.M. Ho, J. Tang, J.H. Zhang, Neuroprotective strategies after neonatal hypoxic ischemic encephalopathy, Int J. Mol. Sci. 16 (2015) 22368–22401.
[71] S. Saigal, L.W. Doyle, An overview of mortality and sequelae of preterm birth from infancy to adulthood, Lancet 371 (2008) 261–269.
[72] M. Vento, G. Lista, Managing preterm infants in the first minutes of life, Paediatr. Respir. Rev. 16 (2015) 151–156.
[73] M.L. Tataranno, J.L. Oei, S. Perrone, I.M. Wright, J.P. Smyth, K. Lui, W.O. Tarnow-Mordi, M. Longini, F. Proietti, S. Negro, O.D. Saugstad, G. Buonocore, Resuscitating preterm infants with 100% oxygen is associated with higher oxidative stress than room air, Acta Paediatr. 104 (2015) 759–765.
[74] S. Ezaki, K. Suzuki, C. Kurishima, M. Miura, W. Weilin, R. Hoshi, S. Tanitsu, Y. Tomita, C. Takayama, M. Wada, T. Kondo, M. Tamura, Resuscitation of preterm infants with reduced oxygen results in less oxidative stress than resuscitation with 100% oxygen, J. Clin. Biochem. Nutr. 44 (2009) 111–118.
[75] M. Aguar, E. Cubells, J. Escobar, J. Kuligowski, M. Iriondo, M. Izquierdo, A. Nuñez, M. Cernada, M. Vento, Preterm babies randomly assigned to be blindly resuscitated with higher (60%) vs. lower (30%) initial FiO_2: effects on oxidative stress and mortality, EPAS 3843 (2014) 540.
[76] D. Rook, H. Schierbeek, M. Vento, H. Vlaardingerbroek, A.C. van der Eijk, M. Longini, G. Buonocore, J. Escobar, J.B. van Goudoever, M.J. Vermeulen, Resuscitation of preterm infants with different inspired oxygen fractions, J. Pediatr. 164 (2014) (1322-6.e3).
[77] Co-Chair* Jeffrey M. Perlman, Co-Chair* Jonathan Wyllie, Myra H. Wyckoff John Kattwinkel, Aziz Khalid, Guinsburg Ruth, Kim Han-Suk, Helen G. Liley, Lindsay Mildenhall, M. Simon Wendy, Szyld Edgardo, Tamura Masanori, Velaphi Sithembiso, On behalf of the neonatal resuscitation chapter collaborators part 7: neonatal resuscitation 2015 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations, Circulation 132 (Suppl 1) (2015) S204–S241.
[78] K.D. Hardy, B.E. Cox, G.L. Milne, H. Yin, L.J. Roberts 2nd, Nonenzymatic free radical-catalyzed generation of 15-deoxy-Δ(12,14)-prostaglandin J2-like compounds (deoxy-J2-isoprostanes) in vivo, J. Lipid Res. 52 (2011) 113–124.
[79] G.L. Milne, Q. Dai, L.J. Roberts 2nd, The isoprostanes--25 years later, Biochim Biophys. Acta 1851 (2015) 433–445.
[80] S.D. Reuter, D.J. O'Donovan, S.E. Hegemier, E.O. Smith, W.C. Heird, C.J. Fernandes, Urinary F2-isoprostanes are poor prognostic indicators for the development of bronchopulmonary dysplasia, J. Perinatol. 27 (2007) 303–306.
[81] J.P. Fessel, N.A. Porter, K.P. Moore, J.R. Sheller, L.J. Roberts 2nd, Discovery of lipid peroxidation products formed in vivo with a substituted tetrahydrofuran ring (isofurans) that are favored by increased oxygen tension, Proc. Natl. Acad. Sci. USA 99 (2002) 16713–16718.
[82] W.L. Song, J.A. Lawson, D. Reilly, J. Rokach, C.T. Chang, B. Giasson, G.A. Fitzgerald, Neurofurans, novel índices of oxidant stress derived from docosahexaenoic acid, J. Biol. Chem. 283 (2008) 6–16.
[83] J. Kuligowski, M.R. El-Zahry, Á. Sánchez-Illana, G. Quintás, M. Vento, B. Lendl, Surface enhanced Raman spectroscopic direct determination of low molecular weight biothiols in umbilical cord whole blood, Analyst 141 (2016) 2165–2174.
[84] L. Frank, I.R. Sosenko, Prenatal development of lung antioxidant enzymes in four species, J. Pedia. 110 (1987) 106–110.